University of Strathclyde

Faculty of Engineering

Department of Civil and Environmental Engineering

Nitrogen and phosphorous removal from groundwater using waste materials

BY

STYLIANOS GKIOUZEPAS, BSc, MAgr, MSc

A Thesis Presented to the University of Strathclyde in Fulfilment of the Requirements for the Degree of

Doctor of Philosophy (Ph.D.) in Civil and Environmental Engineering

August 2016

Glasgow, UK

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

The copyright of this thesis belongs to the author under the terms of the United Kingdom Copyright Acts as qualified by University of Strathclyde Regulation 3.50. Due acknowledgement must always be made of the use of any material contained in, or derived from, this thesis.

Signed:

Date:

ABSTRACT

The occurrence of high nitrate and phosphate levels in groundwater is a worldwide problem. The study of suitable methods to remove these compounds is crucial for the long-term environmental health of ecosystems. Nitrification and eutrophication occur in areas where there is extensive human activity for agricultural cultivations and industries. The study area for this project, Greece, is affected by nitrate and phosphate in groundwater. The extensive use of fertilizers and pesticides and the drilling of groundwater wells have reduced the quality of water on many Greek islands. A case study of Samos Island next to the borders between Greece and Turkey, support the problem in high nitrogen levels. The aim of this study was to determine if passive engineering solutions could be designed using cheap and easily available local materials that can remove nitrogen and phosphorous compounds from groundwater.

This study focus on laboratory based experiments with columns. The substrate materials were selected with specific criteria. In the first experiments materials that have already investigated and groundwater from the area that Nitrabar project took place in Northern Ireland was used, where known denitrifier bacteria already exist. The next experimental section used new materials for nitrate and phosphate reduction including perlite, tea waste materials and hazelnut husk wastes. In the this experiment these substrate materials were investigated in batch and column experiments, in short and long term time periods, and with two water sources, tap water and groundwater (Scotland, UK).

The investigation of denitrification process in all experiments proved successful. In all experiments removal of nitrate and phosphate compounds was observed. The best reduction was found in the last experiment with the new substrate materials showing a reduction between 90-99% for all nitrogen compounds and the reduction of phosphate levels was more than 80% at all cases. The degradation rates calculated were similar to the previous experiments showed efficiency with the new waste materials. It was interesting to note that each experiment showed an initial growth phase / adaptation lag phase followed by a stable biodegradation phase.

ACKNOWLEDGEMENTS

Firstly, and furthermost, I would like to thank my supervisors, Prof. Robert (Bob) Kalin and Prof Charles Knapp for all their support at all levels, motivation and guidance throughout my PhD research. Another person that I would like to thank especially is Ms Mara Knapp and her help to all the laboratory help that provide all times that I requested.

Another special thanks to my friend that we had the same issues and the same problems to solve in the lab, Neil, Andy, Fabrizio, Chris, Ann, Maura, Matteo and George.

I would also like to thank my Professor from Aristotle University of Thessaloniki, Professor Diamantis Karamouzis, for all the support and all the help with all the environmental and life issues, that his opinions and advices are really valuable.

A big thank you goes to my family for their emotional and financial support throughout my university studies and more specifically to my mum and to my dad that they really want to see me succeed in every step of my life.

Finally, I would like to thank my special one, Katerina, which is the person who with my family, makes me every day a better person and I want to give them back the most valuable present except from my love, and from the help in all difficult moments that exist.

PUBLICATION RESULTING FROM THIS WORK

- **Conference Papers**

Gkiouzepas, S.A., Knapp, C.W., Kalin R.M. (2014). Enhanced denitrification process using ready available materials: column studies. Proceedings of 12th International Conference on Protection and Restoration of the Environment, Skiathos Island, Greece, June 29 to July 3, 2014, pages 121-126.

Gkiouzepas, S.A., Knapp, C.W., Kalin R.M. (2014). A comparative denitrification process under anoxic conditions using waste materials. Proceedings of 10th International Hydrogeological Congress, 8-10 October 2014, Thessaloniki, Greece, pages 203-211.

Gkiouzepas, S.A., Knapp, C.W., Kalin R.M. (2015). Use of tea and hazelnut husk wastes on denitrification process to clear groundwater. Proceedings of 1st International Scientific Conference on Sustainable Solutions to Wastewater Management, 19-21 June 2015, Kavala, Greece. (Accepted)

Gkiouzepas, S.A., Knapp, C.W., Kalin R.M. (2015). Use of perlite, tea and hazelnut nut wastes on denitrification process to clear groundwater: column studies. Proceeding of 9th International PanHellenic Congress of EGME, Innovation and New Technologies in Agricultural Engineering and Management of Natural Resources, 8-9 October 2015, Thessaloniki, Greece, pages 241-247.

- **Journal Papers**

Burcu Özkaraova Güngör, E., Kalin R.M., Gkiouzepas G., Knapp C.W. Industrial and agricultural wastes as potential biofilter media for groundwater nitrate remediation. Ecological Engineering (Accepted November 2014)

- **Conference Posters**

Gkiouzepas S.A. (2013). An investigation of denitrification process in column experiments using different substrate media. Royal Geographical Society with the Institute of British Geographers Postgraduate Mid-Term Conference 2013, 25-27 March 2013.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

NOTATION BLOCK

Symbol	Explanation
PRB	Permeable Reactive Barrier
SBR	Sequence Batch Reactor
NVZ	Nitrate Vulnerable Zone
DNRA	Dissimilatory Nitrate Reduction to Ammonium
DO	Dissolved Oxygen
TOC	Total Organic Carbon
IC	Ion Chromatography
q-PCR	quantitative Polymerase Chain Reaction
K_w	the ionic product for water
ORP	Oxidation-Reduction Potential
v/v	Volume/Volume
HRT	Hydraulic Retention Time
TN	Total Nitrogen
λ	Saturation constant
$T_{1/2}$	Half life
RT	Retention Time
w/w	Weight/Weight
SD	Standard Deviation
TW	Tap Water
GW	Groundwater
EU	European Union
WHO	World Health Organisation
NI	Northern Ireland
UK	United Kingdom
DNA	Deoxyribonucleic acid
USEPA	United States Environmental Protection Agency
NAGREF	National Agricultural Research Foundation

NHL	non-Hodgkin lymphoma
ADI	Acceptable Daily Intake
MINAS	Minerals Accounting System
FAO	Food and Agriculture Organization
ATP	Adenosine triphosphate
DN	Denitrification
ha	Hectare
γ	Activity coefficient
LOD	Limit of detection
LOQ	Limit of quantification
TDS	Total Dissolved Solid
TC	Total Carbon
InC	Inorganic Carbon
IR	Infra-Red
HPLC	High-performance liquid chromatography
UV	Ultra Violet
SOP	Standard operating procedure
PV	Pore Volume

CHAPTER 1

INTRODUCTION

1.1 Introduction

Groundwater is widely disturbed in the Earth and is one of the most important resources for water. It contains 0.6% of the total water in earth and the 96% of all freshwater that is not frozen. Groundwater exists whenever the water penetrates the surface. It is the most important source of potable water and in many cases the only source of potable water. Groundwater is also heavily used for agricultural, municipal and industrial use (Pedredo et al., 2010).

Pollution of groundwater is a worldwide problem. Intensive agricultural cultivation and the demands of a rising population increases the need for food production and thus to use more and more fertilizers and pesticides. Fertilizer use is increasing geometrically during the last 20 years even though there is restrictive legislation from World Health Organization (WHO) and European Union (EU) with specific directives for those issues and more specifically for the nitrogen compounds pollution.

1.2 Aim of research

Mediterranean countries, and more specifically Greece, are facing groundwater pollution issues. The economic conditions and the increasing demand to use more agriculture fields means there is a need to investigate methods to remediate groundwater contaminated with nitrate and other agricultural impacts. The aim of this research is to determine if substrate materials that are readily and easily available from the area of the research can be used to reduce the concentration of nitrate and phosphate in groundwater. Greece is an agricultural country with many cultivations that depend on the intensive use of fertilizers and pesticides which end up concentrated in groundwater sources. The main fertilizers are nitrogen-based and phosphorous-based. Nitrate pollution is one of the main issues for groundwater and factors that contribute to this problem (Rivett et al., 2008), and high phosphate levels contribute to eutrophication.

The hypothesis investigated here is that substrate materials that are ready available in nature, (wastes or used for other purposes) can be used to enhance natural removal of N and P compounds. The simulation of ground conditions and the use of substrate material as interception filters to provide a treatment for groundwater can help us to address this problem. For that reason several experiments investigate the connection of substrate material with N removal firstly, and afterwards a combination of the removal of N and P compounds in the same approach. The removal of N compounds was expected from the initial hypothesis to achieve levels higher than 70% at all cases. The hypothesis investigated in details in two different types of experiment: Column experiment which are combined or not with initial pre-treatment tank and batch experiment. The hypothesis for P removal was not an initial approach but combined in the duration of the experiment with initial statement the removal of P compounds in the column system, higher than 70%.

1.3 Research objectives

At the start of project in October 2011 the following research objectives were identified:

- Investigate the efficiency of groundwater with known denitrifier bacteria from the Nitrabar project site. Combine groundwater properties with substrate materials in columns studies and to investigate the connection of them with denitrification activity.
- Start columns experiment studies that simulate environmental conditions. Use specific materials that already used (sand/straw) and to investigate if the results are in agreement with other researcher.
- Add new materials (perlite for phosphate removal) and to combine specific conditions with specific Hydraulic Retention Time (HRT) and flow rate to study the effect on nitrogen and phosphorous removal
- Investigate new materials of specific geographic relevance for nitrogen removal studies. Tea waste materials and Hazelnut husk wastes under not preferable conditions investigated in batch experiment and in columns experiment for short and long term period. Examine the results with two solutions tap water and groundwater. Finally investigate in these experiments the successful denitrification process and the phosphate removal simultaneously.
- Evaluate the field groundwater conditions for a geographically limited area in Greece.

- Propose engineering design options that may provide water remediation solutions (nitrate and phosphate) for the study area in Greece.

1.4 Scope of thesis

This thesis describes the development of an experimental approach to remove nitrogen and phosphorous compounds from groundwater using column studies and the potential application in Greece. The substrate materials that used are wheat straw, sand, mulch, perlite, tea waste materials and hazelnut nut wastes. The methodology that applied was similar at all experiments. The solution media used were tap water and groundwater that received from two different places (Ballymena, NI, and Largs, Scotland). The groundwater received from two different places because the approach in the initial and the next experiments changed. In the initial experiment the denitrifier bacteria received from the groundwater solution from an area that is used to remove N compounds and the second collection point selected to provide groundwater from an agricultural area that combines the cultivations and the livestock activity of the area. The results of experiments successfully demonstrated removal of nitrogen and phosphorous compounds.

Chapter 2 is a literature review of the theory. There is a description of the nitrogen problem worldwide and the source of pollution in the environment. Additionally, there is description of problems nitrogen creates to human health. There is also a description of methods that can remove nitrogen compounds. A brief description of legislation of EU for water and groundwater framework is provided. There is a discussion of the denitrification process that is applied in this research. Additionally, there is a description of riparian zones and the properties of common reed beds that are used in pre-treatment. There is also an intensive approach in the factors that can affect denitrification process. There is description of microbiology, biochemistry and stoichiometry of the denitrification process. Additionally, there is a discussion of phosphate problems in water and the methods that applied to reduce those issues. Finally, there is a detail approach for the microbial activity and the kinetics law that biological colonies and more specifically denitrification bacteria are following.

Chapter 3 is the investigation that took place in Samos Island in Greece in 2013, which was the evidence of the N and P pollution problem in the area. There are problems with groundwater pollution due to high levels of fertilizers that are used. There is a description of the field methodology used and the sampling method, and the results that showing the extent of the

nitrate problem. There is also a description of the substrate materials that will be used in the experiments and are connected with the area.

Chapter 4 is a description of the methodology of all the sampling and analysis of water samples for this research. There is a full description of colorimetric methods and Ion Chromatography methods and TOC analysis.

Chapter 5 has details of the first experiments. There is the description of the methodology used with characteristics about flow rate, the design and the substrate materials used. In this experiment, the substrate materials were sand and straw. There are detailed results about nitrate removal and degradation rates. This experiment was column studies that combined the pre-treatment tank, column part and groundwater from Ballymena, NI. The results were the expected to show that the experimental setup was working and to confirm previous results obtained by others.

Chapter 6 continues the experimental approach from the previous chapter. The new substrate materials were mulch, sand and perlite. Perlite is a material that for first time in this thesis was used in this kind of experiments. There is a details description of all substrate materials and the design of experiment. Additionally, there is an evaluation of results with different controls columns, sand columns and perlite columns. The results are exciting and the hypothesis for new materials is discussed.

Chapter 7 presents batch experiment of totally new materials from the geographical field area introduced in the experimental procedure. There is a supporting discussion for using hazelnut husk and tea waste materials and the experimental methodology applied. There is also a detailed approach of batch test with tap water in the first part and with groundwater in the second part. The differences of the new materials in experiments with different solution under not preferable in nature conditions and with low oxygen availability are discussed. The conditions are micro-aerophilic and anaerobic depending on the amount of nitrogen compounds that remain in the solution after denitrification process.

Chapter 8 is description of new columns experiment with tea waste materials, hazelnut husk and sand as substrate material. There is a separation in two flow rates one faster and one slower. The first part, tap water was used for the experiment and in the second part, upland surface water as an agricultural groundwater surrogate from Largs, Scotland was used. There is a detailed description of all results and the issues that existing discussed. The nitrogen levels were reduced but the concerning issue was the phosphate levels.

Chapter 9 is the logical sequence from chapter 8. The use of materials to reduce phosphate levels using perlite that absorbs phosphate compounds was studied again. The design of experiment is described and the results show in long time period, the effects of those materials. The solution as a groundwater surrogate as before was from Largs, Scotland and the duration of experiment was 98 days. In this chapter results confirmed the hypothesised use of perlite; there is a detailed discussion about the results in Chapter 8 and Chapter 9.

Chapter 10 presents the major findings of the research in this thesis and the recommendations for future work.

CHAPTER 2

LITERATURE REVIEW

2.1 Nitrogen compounds

Groundwater is one of the main sources of freshwater on our planet. During the last 30 years the excessive usage of fertilizers in crops, livestock, sewage waste and septic tanks have contributed nitrate contamination of groundwater bodies worldwide, which limits the prospective use of groundwater and harm the hydrological cycle (Rivett et al., 2008).

Increasing nitrate and phosphate levels lead to eutrophication of aquifers. Efforts are currently being made to control and reduce the amount of anthropogenic nitrogen compounds (NO_3, NO_2, and NH_4) going to groundwater (Marshall et al., 1995).

The element Nitrogen (N) is an essential element of protein for animal and plant life. Nitrogen is a crucial element for life cycle for many organisms and for human. The human body consists of nucleic acid and proteins which are part of Deoxy-Ribonucleic Acid (DNA). In DNA and at enzymes, nitrogen helps for all the processes in body. In the environment, nitrogen is present in various forms: as nitrogen gas (N_2) and gases (NO and N_2O), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and ammonia (NH_3). The concentration of each compound depends on redox conditions, pH and the activity of various bacteria. In the atmosphere nitrogen exist everywhere (78% of atmosphere is dinitrogen N_2 and it is in non-reactive formation).

Because N_2 is unreactive, most organisms cannot use it directly and nature relies on certain organisms that can convert it to usable forms. This process takes place under both aerobic and anaerobic conditions. The microorganisms that help this process are Rhizobium bacteria and are located on the roots nodules of legumes. N_2 compound is converted to different nitrogen compounds through a process, called nitrogen fixation (Rivett et al., 2008).

Plants and most of microorganisms assimilate only nitrate (NO_3^-) and ammonium-nitrogen (NH_4^+-N). These two forms of nitrogen are commonly found in considerable concentrations on the ground, whereas other forms such as nitrite-nitrogen (NO_2^--N), hyponitrite ($N_2O_2^{2-}$) or hydroxylamine (NH_2OH) occur only in specific cases and generally in trace quantities.

Nitrification is the process by which ammonium (NH_4^+) or ammonia (NH_3) is oxidized into nitrite (NO_2^-) by ammonia-oxidizing bacteria, often *Nitrosomonas spp*, and the NO_2^- further oxidized into nitrate (NO_3^-) by nitrite-oxidizing bacteria, often *Nitrobacter spp*. (Knapp and Graham, 2007)

The most common form of inorganic nitrogen in soil is nitrate-nitrogen (NO_3^--N), where nitrifying bacteria exist. These bacteria oxidize ammonia-nitrogen (NH_3-N) (genus *Nitrosomonas*) to nitrite and then to nitrate nitrogen (genus *Nitrobacter*) (Knapp and Graham, 2007).

Step 1: $NH_3 + O_2 + 2\ H^+ + 2\ e^- \Rightarrow NH_2OH + H_2O$ (Equation 2.1)

Step 2: $NH_2OH + H_2O \Rightarrow NO_2^- + 5H^+ + 4e^-$ (Equation 2.2)

Step 3: $½\ O_2 + 2\ H^+ + 2\ e^- \Rightarrow H_2O$ (Equation 2.3)

The total reaction is Σ: $NH_3 + 1.5\ O_2 \Rightarrow NO_2^- + H^+ + H_2O$ (Equation 2.4)

The ammonium-nitrogen (NH_4^+-N) is short-lived in aerobic environments (Rivett et al., 2008). Under anaerobic conditions, excessive moisture, low soil temperature, pH levels, ammonium can remain at high levels for extended periods, or when high doses of ammonium fertilizers have recently added in an area. Otherwise the compound is converted to NH_3 and oxidised by bacteria (Knapp and Graham, 2007).

Nitrate-nitrogen (NO_3^--N) can leach through soils by rainwater, as it does not readily adhere to soil. If nitrate is not absorbed by plants in surface layers of soil, it moves through the deeper layers of soil and ultimately enters into aquifers. The amount of nitrate leachate depends on the quantity of water which is moving through the soil, and the concentration of NO_3^- in the soil system. Large mobility of NO_3^- usually occurs in sandy soils areas with high precipitation levels or during excessive irrigation and high nitrogen fertilizer additions (Rodriguez et al., 2011).

Excess nitrate-nitrogen can be controlled by plants. Intensive absorption without further metabolism (conversion of organic nitrogen), results in accumulation of nitrate in plant tissues. The presence of nitrate in vegetable products and drinking water is contraindicated because it is potentially toxic for animals and for people (Rodriguez et al., 2011).

Natural nitrate levels in groundwater are generally low (typically < 10 mg/l NO_3^-). Nitrate concentrations higher than natural levels are often caused by human activities, such as agriculture, industry, domestic effluents, municipal use and emissions from combustion engines (EEA).

Nitrogen cycling has been investigated across terrestrial and aquatic environments (Figure 2.1) (Payne et al., 2014). This knowledge can be applied to nitrogen removal pathways in all systems and more specifically in biofilters and column studies.

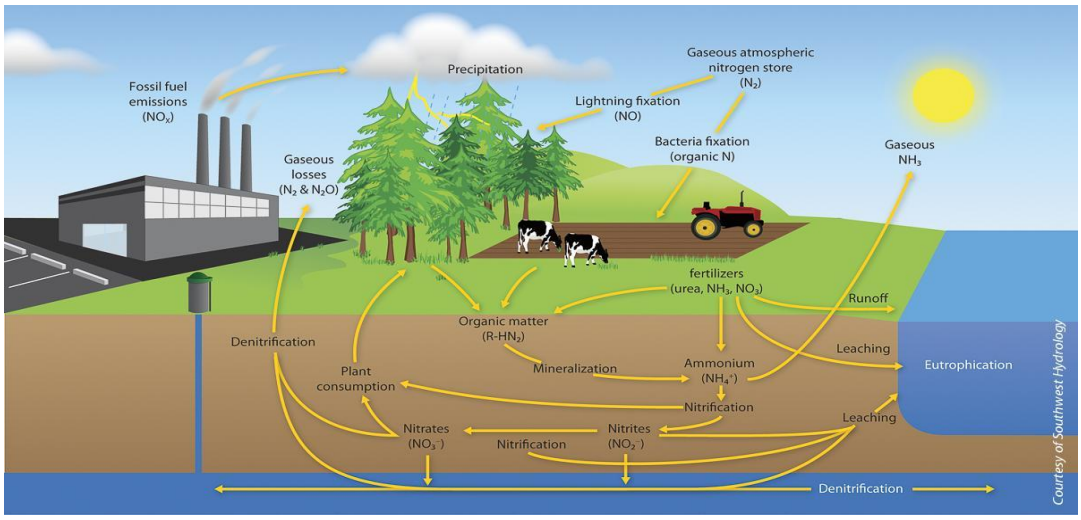


Figure 2.1: Nitrogen cycle (californiaagriculture.ucanr.edu)

Nitrogen pollution has a variety of possible fates, including assimilation, transformation by microbial processes (which includes nitrification, denitrification, dissimilatory nitrate reduction to ammonium (DNRA) processes), abiotic processes (including filtration and adsorption), or leaching from the system (Payne et al., 2014).

Dissimilatory nitrate reduction to ammonia (DNRA) and assimilatory nitrate reduction to ammonia retain the nitrogen in a fixed form, ammonia, keeping it available for further biological processes (Zumft, 1997). In contrast, denitrification (Zumft, 1997) ultimately transforms nitrate to dinitrogen (N_2), a gas that removes the nitrogen from the habitat, unless/until nitrogen fixation once again fixes the nitrogen. In the presence of nitrite, the anaerobic ammonium oxidation (anammox) process also produces N_2, by effectively combining nitrite with ammonia (Keunen, 2008).

DNRA, denitrification, and anammox processes result in energy conservation and provide an electron sink. DNRA also functions to remove excess fixed nitrogen from an organism. Assimilatory nitrate reduction provides ammonia for biosynthesis of nitrogen-containing compounds (Zumft, 1997; Kraft et al., 2011).

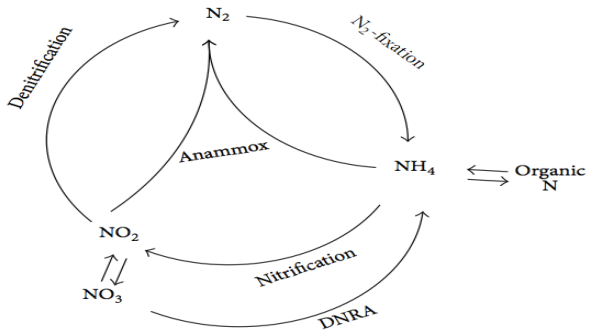


Figure 2.2: The biological N cycle. DNRA, dissimilatory nitrate reduction to ammonium (Ni and Zhang, 2013)

Based in different surroundings studies (Vymazal, 2007; Rivett et al., 2008) the key processes for nitrate removal are the biotic assimilation (uptake by plants, bacteria, fungi or other microbes) and denitrification (conversion into gaseous forms either N_2 or N_2O) process.

Assimilated nitrate is consequently transformed into a selection of organic compounds and deposited for time period, before returning to environment upon cell death or exudation.

The nitrogen which remains within the soil organic matter is available for uptake, transformation or leaching via the decomposition process. In several cases, temporary storage from assimilation can retain nitrogen for days, years, decades and beyond (Powlson, 1993; Reddy et al., 2008).

Denitrification is the process that is investigated in thesis. The definition of denitrification: is a microbially facilitated process of nitrate reduction (performed by a large group of heterotrophic facultative anaerobic bacteria) that may ultimately produce molecular nitrogen (N_2) through a series of intermediate gaseous nitrogen oxide products (Bernard et al., 2015).

2.1.1 Source of nitrogen compounds

Water pollution by nitrates is an important environmental problem. Most nitrogenous materials in aerobic surface waters tend to be biologically converted to nitrate ions. All sources of nitrogen, organic nitrogen and particularly ammonia could be considered as possible sources of nitrates.

The most important sources of nitrogen pollution are fertilizers and waste products of animals that are used in crop cultivation. In areas with intensive agricultural activity, the concentration of nitrates in soil can be increased more than 5-10 times over neighbouring clear non-cultivated areas (Horvartha et al., 2010, Pena Haro et al., 2010, Petersen et al., 2012)

More than 30% of nitrogen added as chemical fertilizer is not used by plants (Castaldi et al., 2011). The problem of nitrate pollution has become more intense the last 40 years due to the excessive use of nitrogenous fertilizers in agricultural crops (EEA). From 1970 till 2005, there was an increase in the usage of nitrogen fertilizer more than 200%. In 1970, more than 32 million tonnes of inorganic nitrogen as fertilizer were applied in fields, of which 23 million in Europe and America (FAO, Maia et al., 2012). The global nitrogen consumption reached 91 million tonnes of inorganic nitrogen in 2005, of which 56 million tonnes was in Asia (IFA, FAO).

The presence of nitrates in waters is not only caused by the use of agricultural fertilizers, but also by the decomposition of animal and plant organisms, plant debris and organic substances in the soil, waste resulting from the breeding of animals, industrial waste, municipal waste water and the underground disposal of domestic waste water in septic tanks (Rivett et al, 2008).

Industrial wastes can provide also another type of wastes nuclear wastes. Nuclear wastes come from the enrichment process for nuclear fuel and characterized by low levels of radioactivity. These nuclear wastes have huge volume and high concentration of nitrate and nitrite ions (Misaelides, 2011)

2.1.2 Nitrogen compounds and environmental pollution

Pollution of surface water and groundwater by nitrates is one of the most visible and persistent signs of human impact on natural environment. The high solubility of nitrate in water results in transport of these compounds through the flow in underground aquifers and water sources. In many aquatic ecosystems there was a dramatic increase in nitrate levels with an increase to more than 200 mg /l (Costelo and Laberti, 2009)

Pollution by nitrates is currently ranked among the world's major environmental problems. According to USEPA (Environmental Protection Agency) nitrate is one of the 100 main contaminants, and it is evaluated to be fourth in order of importance of water pollution problems.

The problem of water pollution by nitrates is predicted to become stronger in the near future, with severe impact and adequacy of drinking water (EU).

The most important effects of nitrates in the environment relate to:

a) deterioration in quality of surface waters,(e.g. lakes and rivers), because of the expansion of eutrophication (eutrophication limit for calm lakes: 15 mg/l NO_3);

b) pollution of groundwater aquifers, which are using for drinking water; and
c) contribution to greenhouse effect, through the conversion of nitrogen oxides from soil bacteria or by chemical reduction. As such, nitrogen removal is crucial to avoid eutrophication in nature, and the increased concentration of nitrogen fertilizers can disturb the biological balance of ecosystems. The presence of high concentrations of nitrogen and phosphorus in surface water recipients, such as lakes and rivers, has a result to grown up the aquatic vegetation and biomass in water. There is also a reduction of dissolved oxygen to beneficiaries and the generation of toxic and harmful gases, which convert water to a dead zone. It becomes difficult for aerobic aquatic life to survive (Chen et al., 2011)

Additionally, the growth of phytoplankton causes significant problems in water purity (colour, turbidity, odour, etc.), and it could be declared unhealthy or unsafe to drinking purposes and for home use (Voutsa et al., 2001).

2.2 Nitrate pollution problem in Greece

The connection of the whole research with the Mediterranean area was the initial aim of the program and more specifically the connection with Greece and the East Mediterranean countries. Greece is geographically in a position that connects Europe with Asia and Africa, so it can simulate the environmental problems that exist there. The Mediterranean climate is the best climate for human life and cultivation with wide variety of flora and fauna. The country is an agricultural country with emphasis in fruits, vegetables and crop production.

The water resources management in these areas is a major issue due to the climate of the area and the factors that are connected and are increasing every year (Daskalakis and Voudouris, 2007). The average temperature is increasing the last decades and the semi-arid climate is visible in that area. The warm dust aerial movement from Africa to Greece creates in many places dessert and arid conditions, and for that reason the water management is crucial for that area. Greece as a country is characterised agricultural and touristic country that there are water sources and the water stress is visible (Nikolaidis et al., 2008; Gikas et al., 2009). It is characterised in the top 35 country worldwide that face water stress problems (Stamatis et al., 2011). The water management is crucial. The majority of fresh water except from drinking purposes is used for irrigation in agriculture (86% of total volume). Due to the increasing levels of water demand the amount of wells and boreholes has increased the last years (300.000) (Dimitriou and Mossoulis, 2010).

The last decades the agriculture and more specifically the systematic agriculture was a way to increase the income salary of the farmers and the people in areas outside of the big city centres (Skoulikidis et al., 2000). The combination of intensive agriculture and the European money that promote as a bonus the land use for increasing the production in all sectors, force the people to stress the water levels and to use in intensive way fertilizers to increase the production. The fertilizers are nitrogen and phosphorus based. The application of these fertilizers in combination with the reuse of industrial and municipal wastes and pesticides create problems of nitrification and eutrophication in several areas around Greece (Gikas et al., 2011; Daskalakis and Voudouris, 2007). The nitrate pollution (nitrification problem) is visible from the ministry of Agriculture at all mainland areas and especially in areas that intensive agricultural activities exist. The same is happening with eutrophication in small streams and bigger rivers and in lakes, where the results are visible in everyday life (Stamatis et al., 2011).

Except from mainland that is the area that face the main nitrate pollution problem there are several island in Aegean and Ionian Sea that the problem is visible. The area of east and south Aegean are characteristic because there are green islands with water sources and demand an orthologistic water management to avoid the problems that exist in mainland the last 2 decades.

Greece is an EU member and due to the directives that exist has to face these problems. The monitoring of the areas is based due to the economical situation that the countries face since 2009 only from international programs that are funded by EU through the universities and NAGREF (National Agriculture Research Foundation). The equipment and the research that is ongoing try to create as much as possible actions to face those problems. The economic levels and the budget is decreasing year by year and focus more on the economic crisis and the survival of the people rather than the pollution of the environment. For those reasons the application of new cheap and easily applied methods to face those problems is crucial for the future. The ministry of Agriculture and more specifically NAGREF focus on that field and try to face nitrate and phosphate pollution problems using cheap materials that are probably wastes from other activities. The use of waste materials from agricultural cultivations except for heating and fertilizers sectors must be applied also in the pollution sector and more specification in the water protection.

Due to the EU legislation and the markets that demand products with standards (especially visible), there are many products that are not achieving (the visible not the quality) standards. Additionally there is the demand of the people and the markets. In Greece that attitude is not exist in the consumers and the home market is that products. The issues are increasing when the producers choose to destroy the production and not to provide it to the poor people due to EU

legislation. Except from that, from all cultivations (fruits, vegetables, crop) the amount of waste materials that is produced is huge. It is better to reuse the waste materials than burning them creating more and more problem the environment (CO_2 emissions).

The methods that are used till now in the country to face those problems are the well-known and worldwide applied. There are reed bed projects, artificial wetlands, use of chemical, physicochemical and biological methods (Milovanovic, 2007; Nikolaidis et al., 2008; Karavoltsos et al., 2008; Stamatis et al., 2011). The main problem is the budget and the cost that must be reduced year by year and parallel to catch the EU demands for lower pollution limits. For those reasons the application of waste materials and new cheap materials that are produced in the country are important to face the nitrification and eutrophication problems.

The NO_3 pollution is visible at all the country. The policies of EU and the demands of the market to increase the quantity of products force all the farmers/producers to increase the amount of fertilizers. The majority of fertilizers in N and P based. The effect of them the last years create problems now. The EU legislation becomes more strict the last years also, but the combination of the production and the whole layout of a destroy from economic crisis Greece enlarge the problems and that is visible from 2009 and afterwards. The government is reducing the budget year by year for the protection measurements for the environment issued without facing the problem. The demand to face the problem with different approach is crucial. And for that reason the introduction of waste from other activities materials and materials that come from Greece is the main purpose. The new approach must be and can be economical and the design of the project and the cost must remain very low. The point is to combine low cost with the effective removal of pollution. In that sector with nitrogen pollution that is possible. There are several studies that try to reuse waste materials to provide the technology to face the pollution issues. The positive point is that the low cost waste materials till now can provide that results not only in the lab but also in the field with long term positive results. That is the strategy that Greece should move on.

2.3 Nitrogen compounds and human health

The main source of nitrate intake by humans is drinking water and food (cereals, vegetables and processed meat, which is used as a preservative and to add artificial colour as $NaNO_3$).

The presence of increased amounts of nitrate (>70 mg/kg) and nitrite (>20 mg/kg) ions in human body can be hazardous to health. The consumption of drinking water with a high content of nitrates can cause a type of anaemia in infants, methaemoglobinemia, which can be fatal (known

as blue-baby syndrome) (Johnson et al., 1990, Dusdieker et al., 1996). In adults, it is a possible contribution to cancer (gastric cancer) (Fan et al., 1996).

In the body, nitrate ions are converted to ammonia. Partial reduction of nitrate leads to the formation of nitrite ion as an intermediate. Nitrates are not toxic to the human body (Hill, 1999). The risk of nitrates depends not only on the intake, but also by the amount and the type of enteric bacteria.

However, microorganisms, such as *E.Coli* and *Clostridium spp*. found in the upper digestive system, reduce nitrates to highly toxic nitrite ions according to the following reaction (Eq 2.5) (Bryan, 2006):

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + 2H_2O \qquad \text{(Equation 2.5)}$$

Nitrite ions are absorbed by blood and react with haemoglobin to oxidize divalent iron (Fe^{2+}) to trivalent (Fe^{3+}), thereby methaemoglobin is produced. The generated methaemoglobin cannot transfer either oxygen or carbon dioxide (Curry, 1982).

When a large part of haemoglobin converted to methaemoglobin, observed symptoms include headaches, weakness, fatigue, nausea, dizziness, chest pain, shortness of breath and loss of consciousness (Bradberry, 2007). Methaemoglobinemia is a very rare disease in developed countries. All the cases that reported in Europe are due to consumption of contaminated water with nitrate concentrations greater than 100 mg/l (Lecloux et al., 1999, Pintar, 2003a, Centi et al., 2003).

Infants are more vulnerable to methaemoglobinemia (Blue Baby syndrome), since the gastric pH levels of infants (pH=5-7) are higher than the pH levels of adults (pH <4). Bacterial conversion of nitrate to nitrite ions and subsequent oxidation of haemoglobin from nitrites can more frequently occur in infants than in adults (Lecloux et al., 1999, Pintar, 2003b, Centi et al., 2003). Additionally infants, in contrast to adults, have about 50% lower levels of enzyme *5b erythrocyte cytochrome reductase* which acts as a protective, catalyst in the conversion of methaemoglobin to haemoglobin (Sicolo et al., 2009, Venkateswari et al., 2007).

At that level methaemoglobin is normally less than 2% of total amount of haemoglobin. When methaemoglobin levels exceed 10%, it prevents the flow of oxygen to tissues. In non-anaemic patients, the lethal concentration of methaemoglobins is 60-70% of total haemoglobin (Cantor, 1997). The lack of oxygen causes bruising in infants (methaemoglobin levels >25%) and generates asphyxiation and even cause death (methaemoglobin levels >60-85%). Symptoms of

methaemoglobinemia are bruising of lips and skin, weak and rapid pulse. There is also evidence that infants with gastrointestinal problems are more sensitive to methaemoglobinemia (Pintar, 2003a, Centi et al., 2003).

In adults, nitrates are responsible for cancer which is caused by nitrosamines (R_2N-N≡O) and nitro-amides. This happens due to the conversion of nitrate ions to them that exist in stomach (Walters, 1985). These two compounds are associated with the occurrence of various cancers, such as oral, nasal cavity, bronchus, lung, oesophagus, stomach, bladder, kidneys, nervous system and the skin (Cuello et al., 1976). Stomach cancer is the second cause of death worldwide and is connected with high nitrate levels in drinking water (Addiscott et al., 1991).

Besides carcinogenesis, nitrates have been associated with other diseases, such as the occurrence of genetic defects (van Loon et al., 1997), the appearance of cancer of lymphatic system, a non-Hodgkin lymphoma (NHL) (Ward et al., 1996) and *type I* childhood diabetes (Parslow et al., 1997, Kross et al., 1995). The limits (mg/l) according to World Health Organisation (WHO) and European Union (EU) (98/83/EEC) for human (Table 2.1)

Table 2.1: Limits of nitrogen compounds in water (mg/l)

mg/l	WHO	EU
NO_3^-	50	50
NO_2^-	0.03	0.1
NH_4^+	0.4	0.5

The proposed concentration of nitrates in drinking water is 50 mg/L (Horold et al., 1993, Turner et al., 1981) and with the review in EU legislation the limits are review every 5-10 years to reduce the higher limit levels. In the USA the maximum levels for nitrates in drinking water set at 50 mg/L (WHO, EEC, Fan et al., 1996) and reviewed in 2003 between 10-15 mg/l (USEPA). In UK, the concentration limit of nitrates in drinking water in urban areas is between 10-20 mg/l that is also following the EU directive (50 mg/l).

Higher concentrations >200 mg/L have been detected in drinking water around the world. The contact authorities to ensure the quality of water used the following equation (WHO, 1993):

$$\frac{[NO_3^-]}{50} + \frac{[NO_2^-]}{3} < 1 \qquad \text{(Equation 2.6)}$$

(The square brackets denote the concentration in mg/L for NO_3^- and NO_2^-).

The highest amount of nitrate which is absorbed by humans comes mainly from vegetables. Vegetables are the main source of nitrate influx for adults (Walker, 1990). It is estimated that more than 80% of the average daily input nitrates derived from vegetables. The accumulation of nitrate in foods, and more specifically in leafy vegetables, is particularly high. Maximum limits

for nitrates have been established for some vegetables (leafy vegetables such as lettuce and spinach range from 2.500 to 4.500 mg/kg fresh weight, depending on the season) in several countries and European Union (Walker, 1990). Based on Regulation 466/2001 of the EU "Every country should be active to aware the presence of nitrate contaminants in food". In EU countries there are reported high levels of nitrate in certain vegetables.

For the total amount of nitrates that can be absorbed by human daily, European Union and more specifically the proficient Committee on Food recommended in 1995 the following Acceptable Daily Intake (ADI) levels: ADI=3.65 mg NO_3^-/kg body weight, while the corresponding amount for the USA is ADI=3.2 mg NO_3^-/kg body weight (EEA).

2.4 EU Legislation

Clean water is a vital element for health and well-being human and natural ecosystems. Protecting the water quality is main concern of EU environmental policy and the problem of nitrate pollution is not just located in Europe but it is serious globally. In EU countries the regulation of nitrates was sorted relatively early (1970s). European Guidelines for the protection of surface and groundwater by nitrates and deterioration are part of a broader regulatory framework that was developed in 1990s. The aims of Directives were to promote a sustainable water management (Bouraoui et al., 2009).

The water protection against nitrates was described by individual Directives (91/676/EEC and 91/271/EEC), and it is now fully integrated into the basic standards of the Framework Water Directive (2000/60/EC) and the Groundwater Directive (2006/118/EC). The Framework Water Directive is part of measurement and Directives that should achieve the objective of ‘good environmental status’ by the end of 2015 and will be reviewed again. The ultimate goal is to prevent or limit inputs of pollutants into waters.

2.4.1 The Nitrates Directive (91/676/EEC)

In 1991, European Union introduced the legislation to reduce nitrate pollution from agricultural use of fertiliser, and to prevent this type of pollution from affecting future generations. The Nitrates Directive (91/676/EEC) objective is to protect waters against pollution caused directly or indirectly by nitrates from diffuse agricultural sources through a number of actions that must be implemented by EU Members: water monitoring (with regard to nitrate concentration and

trophic status); identification of waters affected by pollution (>50 mg/L NO_3^-) and those areas that may be polluted, by characterizing vulnerable zones (all known land areas which drain into water bodies affected by pollution or could be affected in the future); establishment of codes of good agricultural practice and action programs (a set of measures to prevent and to reduce nitrate pollution); and review at least once every four years, for the design of vulnerable zones and action that happened to reduce the pollution.

The Directives' main objective is to label Nitrate Vulnerable Zones (NVZ). A NVZ is an area of land which either already has polluted groundwater by nitrate pollution or will become polluted if preventative steps are not taken. The secondary objective is to establish a policy of good agricultural practice which should be implemented and followed by all agricultural workers within EU. The third objective is to create a series of directives to eliminate nitrate levels on agriculture. These steps should be followed in NVZ to decrease nitrate contamination. These plans include time limits on the storage of manure and livestock slurry and plans for fertilizer use on areas which are NVZ (Ghiglieri et al., 2009). The final objective is extension of NVZ and the effectiveness to minimise nitrate contamination in these areas (Martin, 2009).

Many EU Members have already adopted limits, which are lower than the directive in order to achieve better results. For example the Netherlands since 1998 has operated 'MINAS' system (Minerals Accounting System) which monitors the quantities N and P that used in agriculture. The project objective is to reduce NVZ from 40 % (1985) to 21% by 2015, and 12% by 2037.

2.4.2 Urban Waste Water Directive (91/271/EEC)

The Urban Waste Water Directive (91/271/EEC) aim is to protect the environment from the negative effects of the discharge of urban wastewater and industrial wastewater discharge. In this context, the definition of 'sensitive areas' is mainly referred to: freshwater, estuaries or coastal waters which are eutrophic; lakes and streams which discharge in lakes/reservoirs with low levels of water exchange; and surface freshwater which are intended for drinking water purposes, and could contain nitrate levels higher than 50 mg/L. The Urban Waste Water Directive 91/271/EEC sets the maximum total nitrogen concentration limits (mg/L nitrogen) in treated municipal wastewater effluent between 10-15 mg/l nitrogen depending on the water body recipient.

2.4.3 Water Framework Directive (2000/60/EC)

The Water Framework Directive (2000/60/EC) is legislation that is obligatory for all EU members. The primary focus is to standardise the management and maintenance of water bodies in every country. The directive was designed to protect groundwater sources, groundwater dependent ecosystems, surface freshwaters and coastal waters up to one kilometre from the shore (SEPA, 2005).

The primary objective from the directive is to improve the quality of aquatic ecosystems and protect them from possible pollution that might occur in future. The secondary objective is to ensure that water pollution is reduced and remained at the minimum levels everywhere as possible. The directive also suggests that member states should implement an action plan, which will help to enforce the idea of sustainable water use.

The successful implementation will help to protect all parts of water cycle and improve the quality of water bodies. This directive also replaces previous legislation regarding water bodies and their maintenance and protection. Examples of previous directives are the Surface Water Abstraction Measurement/Analysis Directive (79/869/EEC), which was replaced at the end of 2007, and the Groundwater Directive (80/68/EEC) which was replaced at end of 2013 (Bell and McGillivray, 2008).

2.4.4 Groundwater Directive (2006/118/EC)

In 2006, European Union introduced legislation designed to bring about provisions to protect groundwater quality within its member states. This legislation was termed the Groundwater Directive (2006/118/EC) and is derived from the previous EU legislation and Water Framework Directive (2000/60/EC). Groundwater is a valuable resource within Europe and feeds many natural water bodies in the continent, and represents the main source of drinking water for a high percentage of population. Furthermore, many ecosystems are also depended on high quality groundwater levels (DEFRA, 2008).

The primary objective of this Directive is for member states to follow a set of actions for monitoring groundwater. This is to keep nitrate and pesticide levels low. These findings should be reported, and any increasing trends should be monitored and reversed as quickly as possible. The secondary objective of Directive is to ensure that all groundwaters are meeting the

environmental standards that set by European Union. In some cases, the environmental standards are set by the member states and are lower than the EU standards (Quevauviller, 2006).

2.5 Nitrogen removal methods

Despite the considerable efforts made in technology, denitrification effective removal of nitrogen from wastewater and groundwater remains extremely important. The estimated cost is about € 70-320 billion/year only for Europe. The cost of nitrates removal by drinking water remains high (Strebel et al., 1989) although recent methods have reduced significantly the costs (Trois et al., 2010)

The disposal of treated wastewater to sensitive natural water sources requires prior the removal of nitrogen. 40% of Total Nitrogen in the effluent is organic and 60% is ammonia. In conventional wastewater (pH=6-7), ammonia is encountered as ammonium ions (NH_4^+). The concentration of nitrates in various types waste varies (IFA, FAO).

High standards for the quality of drinking water are set by the European Union and World Health Organization. Continuous incidents of eutrophication, massive fish deaths and eradication of plant species in waters bordering mainly industrial cities demonstrates both a lack of environmental awareness and the absence of appropriate and applicable technology (Rivett et al., 2008). There is a compelling need to develop alternative technologies for nitrate removal from aqueous media.

Water purification for drinking purposes combines a series of physical and chemical processes to reduce organic matter and chemical pollutants and disinfections. Most processes involve a step of strong oxidation using chlorine, sodium hypochlorite, ozone or hydrogen peroxide to reduce the microbial load, which affect the chemical composition of water. However, various undesirable ions such as nitrate remain unaffected by oxidation process (Sa et al., 2007).

2.6 Methods to remediate environmental nitrate (denitrification)

The denitrification process is most commonly used process to face the worldwide problem of high nitrogen levels; in particular, enhanced in situ denitrification. In-situ denitrification involves indigenous microorganisms and the addition of an external electron donor. Nitrate is converted to harmless nitrogen gas with this biological process (Calderer et al., 2014).

In nitrate-contaminated aquifers, several environmental issues could affect the internal denitrification potential. These factors are: microbial populations, pH levels, temperature on site, oxygen levels and the availability of electron donors (Vymazal, 2007).

The measurement of in-situ denitrification potential requires knowledge of the location and water flow conditions to identify the limiting factors that exist. Microbial growth can be influenced by hydraulic properties of saturated porous media in bioremediation. This process can reduce bioremediation success because contaminated parts of aquifer can clog due to microbial biofilms and the degradation rates can consequently decline (Thullner et al., 2002, Lee et al., 2009, Long et al., 2011).

The effects of bacterial growth on hydrodynamic conditions based on soil column experiments have investigated by several researchers (Soares et al., 1991, 2000, Vandevivere and Baveye, 1992, Mattison et al., 2002). The importance of using and investigating both biochemical and hydraulic parameters, when studying the biological denitrification was noticed by several researchers (Zhong et al., 2013, Calderer et al., 2014).

At laboratory scale, the investigation of denitrification process under dynamic conditions to calculate the potential of in situ treatments was intensive (Schnobrich et al., 2007, Gibert et al., 2008, Martin et al., 2009). These studies focus on the feasibility of using different substrate materials to promote denitrification process in biofilters (Martin et al., 2009) and Permeable Reactive Barriers (Gibert et al., 2008).

There are also denitrification investigations that focused on the community structure. These are based on the availability and diversity of denitrifiers in nature (Henry et al., 2006, Kandeler et al., 2006). There are few studies that have been completed during enhanced groundwater denitrification treatments (Dandie et al., 2007, Saleh-Lakha et al., 2008, Read-Daily et al., 2011), but majority of these studies focused on batch experiments instead of soil column systems (Torrentó et al., 2011, Tran et al., 2011).

Nitrogen is the mineral nutrient most often in demand from microorganisms and plants. The nitrogen cycle is the one of the best-studied and most complex elemental cycles (Maier et al., 2009). Nitrogen biogeochemistry is complex involving numerous catalysed transformations, including nitrogen fixation, ammonium oxidation, assimilatory and dissimilatory nitrate reduction, ammonification, and ammonium assimilation (Lin et al., 2010).

Dissimilatory nitrate reduction to ammonia (DNRA) is the process where ammonium is the end product, and denitrification is the process where mixtures of gaseous products are formed. These

are two separate pathways for the dissimilatory nitrate loss from soils and waters. Nitrite results from nitrate reduction. Nitrite can be converted to N_2 via anammox pathway, involving the simultaneous conversion of nitrite and ammonium to N_2 (Koh et al., 2010).

Denitrification, the stepwise microbial reduction of dissolved nitrogen (N) oxides, nitrate (NO_3^-) and nitrite (NO_2^-), to the gases nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2), is the primary type of dissimilatory NO_3^- reduction found in soil (Groffman et al., 2006). It assists to remove NO_3^- from the soil and aquatic ecosystems.

The most common approach to denitrification, until now, is a two-step process:

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2 \qquad \text{(Equation 2.7)}$$

although since 1973, Payne had proposed the following sequence:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{(Equation 2.8)}$$

In agricultural fields, denitrification can cause nitrogen loss from soils (Yoshida et al. 2009, Philippot et al., 2007). In aquatic ecosystems, denitrification helps to relieve eutrophication and promote water purification process. Denitrification is the principal process in wastewater treatment wetlands (Vymazal, 2007, Lee et al., 2009), wetlands treating high nitrate groundwater (Lin et al., 2010) and riparian soils receiving agricultural runoff (Mathenson et al., 2002).

It is also significant for atmosphere. Some of gaseous intermediates (N_2O) formed and it can cause depletion of ozone layer or assist as a greenhouse gas that can affect the climate change (Maier et al. 2009).

2.6.1 Autotrophic and heterotrophic denitrification

Autotrophs are organisms that can produce their own food from the substances available in their surroundings using light (photosynthesis) or chemical energy (chemosynthesis) (Huang et al., 2012).

Heterotrophs cannot synthesize their own food and rely on other organisms both plants and animals for nutrition. Technically, the definition is that autotrophs obtain carbon from inorganic sources like carbon dioxide (CO_2) while heterotrophs get their reduced carbon from other organisms (Zhou et al., 2011).

Biological denitrification can be achieved in an autotrophic or a heterotrophic way. The heterotrophic denitrification process is applied most extensively because of its high efficiency and the simplicity of the reactors required (Zhao et al., 2011). Autotrophic denitrification has the advantage of low microbial output and activated sludge, and no secondary pollution.

Several researchers used wheat straw, mulch as organic carbon sources for heterotrophic denitrification (Aslan, 2005; Zhou et al., 2011). The method was cost-effective but the pre-treatment process was complicated and extensive. The CO_2 levels which produced during heterotrophic denitrification were high in all of the studies creating more problems (Zhao et al., 2011).

2.6.2 Denitrification and nitrous oxide (N_2O) emissions

Denitrification is important for groundwater purification, but it is also leads to N_2O emissions. N_2O is a gas responsible for global warming and the destruction of the protective ozone layer in the atmosphere.

N_2O, as a trace gas, has long residence time and is highly efficient in absorbing long-wave radiation. As a greenhouse gas, nitrous oxide is about 200 times more effective than carbon dioxide (CO_2) (Maier et al., 2009). N_2O is a natural catalyst of stratospheric ozone degradation (Bange, 2000). N_2O is photolytically converted into NO, which reacts with ozone (O_3) to produce NO_2 and O_2. Based on the sequence of reactions in atmosphere, NO is regenerated and a large number of ozone molecules can be destroyed for every molecule of N_2O released to atmosphere. Soil is the major source of nitrous oxide. The production of N_2O is connected with two processes, nitrification and denitrification. N_2O production from denitrification is favoured in wet soils with low aeration levels (Maier et al., 2009). The effect of temperature and soil moisture on N_2O emission from denitrification was investigated (Maag and Vinther, 1996). N_2O production in sandy loam soil is noticed significantly to both increased soil moisture and temperature; however the coarse sandy soil only reacted to increased temperature.

2.6.3 Denitrification and nitrate removal

Denitrification is a significant process for dissolved NO_3^- removal in ecosystems (Willems et al., 1997). NO_3^- is a soluble anion. It can be removed though biological process and it is not commonly adsorbed onto transferable sites on clay and organic matter.

Plant uptake, microbial immobilization, and denitrification are three main pathways for biological nitrate removal. Denitrification is performed by groups of bacteria that consume NO_3^- by respiration in the absence of O_2, and convert it into nitrogen gas (N_2O, N_2). Denitrification is a desirable nitrate removal mechanism, as there is a complete removal of nitrogen from the systems. Unfortunately, plant uptake and microbial immobilization through the mineralization process will rerelease nitrate back to soil solution (Groffman et al. 1991).

2.6.3.1 Denitrification removal methods

A considerable number of processes have been proposed for the removal of nitrate ions from aqueous solutions. The existing methods of denitrification are divided into: biological, physicochemical, chemical and electrochemical (Walker, 1990, Kapoor et al., 1997, Schmit-Hieber et al., 2004, Rivett et al., 2008). The electrochemical method, biological treatment and catalytic reduction have the ability to convert NO_3^- into N_2.

2.6.3.1.1 Biological methods

Biological methods are used in secondary treatment of wastewater based on the application of denitrifying microbial communities. These microbes exist in activated sludge systems, biofilters, PRB and several other engineer systems. The biological denitrification converts nitrates into nitrogen and it is the method that is preferred and chosen for investigation in details in thesis. Biological treatment is very effective for the removal of most contaminants (Wu et al., 2012, Rodriguez et al., 2011, Trois et al., 2010)

However, there are disadvantages. The process that requires continuous maintenance, supply of microorganisms and organic substrate maintain the process. It is not applicable to all types of liquid wastes. In concentrated solutions with nitrate levels greater than 1000 mg/L, or high levels of other salts, the biological process was not function due to osmotic pressure on cell membrane. These methods are also not effective in very acidic (pH<4) or very basic (pH>11) solutions or in

solutions containing heavy metals, radioactive or other toxic components (Trois et al., 2010). The use of microorganisms in those conditions can lead to bacterial contamination of treated water. These processes are slow and do not allow high removal rates in sludge and fast in PBR, SBR and column studies (Canter, 1997, Pintar, 2003b, Vidal et al., 2002, Rivett et al., 2008).

2.6.3.1.2 Physicochemical methods

Physicochemical treatment is the technique that involves construction of barriers and physical adsorption/absorption. Generally with these treatment techniques, more than one process is used in combination. Physicochemical methods include ion exchange, reverse osmosis and electrodialysis. In ion exchange, the solution that is treated, passes through a column where nitrate ions selectively retained by ion exchange resin (Johir et al., 2011, Minet et al., 2011, Christianson, 2011). In reverse osmosis, the solution is separated to a pure solvent through a semipermeable membrane. Pressure greater than osmotic is applied on solution and the solvent water penetrates the membrane from a pure solution (Della Rocca et al., 2006, Qambrani et al., 2013). Electrolysis is based on the separation of ions under the application of an external electric field using semipermeable membranes, in which nitrates, as negatively charged ions penetrate the membrane and are directed toward the positively charged electrode (Della Rocca et al., 2007, Zhao et al., 2009).

These processes have the advantage that they almost completely remove contaminants without the addition of other materials. In physicochemical methods nitrate and nitrite ions are not converted to harmless products (e.g. N_2), but are concentrated in secondary waste (brine) requiring further processing before discarding them. This is not a permanent solution to the problem but can help in the transportation of it. Reverse osmosis and electrodialysis are not selective to anions (e.g. NO_3^-). Besides nitrate removal these processes remove other anions, which are necessary in drinking water. This happens in films that are selectively semipermeable to nitrate compounds (Canter, 1997, Pintar, 2003b, Rivett et al., 2008).

2.6.3.1.3 Electrochemical methods

Electrochemical methods are used to convert NO_3^- into N_2, by applying electricity in an electrolytic cell. It has been demonstrated by other researchers (Saleem et al., 2011, Lacasa et al., 2011) where different metals and alloys for the reduction of nitrate are used, but the problems of

nitrogen low selectivity and the high energy cost remain. Currently several studies (Della Rocca et al., 2005, 2007) published about direct or indirect electrochemical reduction of nitrate ions. The problem is that the method cannot be applied commercially. This is because the selectivity to nitrogen is usually low (<40%); the high selectivity to toxic by-products such as nitrates and ammonium ions and N_2O (greenhouse gas); the speed of reaction is slow; and the energy cost is high (Polatides et al., 2000, Dem et al., 2004, Bockris et al., 1997).

2.6.3.1.4 Chemical methods

Chemical methods can be used to remove nitrate using appropriate reductants or creating conditions conducive for its reduction. The removal of nitrate ions can be achieved by chemical reduction using alumina powder and iron (Parslow et al., 1997b).

Nitrate removal by alumina powder equation (Fanning, 2000)

$3NO_3^- + 2Al + 3H_2O \rightarrow 3NO_2^- + 2Al(OH)_3$ (Equation 2.9)

$NO_2^- + 2Al + 3H_2O \rightarrow NH_3 + 2Al(OH)_3 + OH^-$ (Equation 2.10)

$2NO_2^- + 2Al + 4H_2O \rightarrow N_2 + 2Al(OH)_3 + 2OH^-$ (Equation 2.11)

Nitrate removal by iron equation (Fanning, 2000)

$10Fe + 6NO_3^- + 3H_2O \rightarrow 5Fe_2O_3 + 6OH^- + 3N_2$ (Equation 2.12)

$Fe^+NO_3^- + 2H^+ \rightarrow Fe^{3+} + H_2O + NO_2^-$ (Equation 2.13)

The main products are nitrite and ammonia, which are much more toxic than nitrates. This method requires large amount of metals and produces wastes with high levels of metallic ions. Complete removal of nitrate can be achieved at high temperature (>350^{o}C) and pressure (170-200 atm), accompanied by nitrogen selectivity to 99.9% (Turner et al., 1981). The operating cost of the process is high due to high temperature and pressure that are required. Under these conditions, corrosion levels are really high and that is disadvantage for the whole method. Successful reduction of nitrate ions with metal catalysts has been achieved using formic acid (hydrogen source for the reduction of nitrate). However, the concentration of ammonium ions remains high (Horold et al, 1993).

Finally, the selective catalytic reduction (H_2-SCR) nitrate to N_2 in the presence of H_2 as a reducing agent is an effective and efficient method (Costa and Efstathiou, 2007). Since the 1980s, this method was proposed by scientific community as the best solution (not time-consuming and low-

cost method) for removal of nitrate from drinking water and wastewater (Li et al., 2011). It has been demonstrated that the use of H_2, O_2 and CO_2 in the gaseous reaction mixture enables the achievement of a high conversion rate of NO_3^-, reduced selectivity NH_4^+ and satisfactory pH respectively. Although significant progress has been observed in recent years in field of catalytic denitrification, significant problems remain unsolved. The development of catalysts to reduce nitrates (nitrate high activity) was not effective solution for practical application due to its by-products (e.g. NH_4^+) (Hekmatzadeh et al., 2012, Li et al., 2011).

2.7 Stoichiometry of denitrification

Denitrification is considered as a strictly anoxic process because denitrifying bacteria are facultative aerobic microorganisms and prefer the use of oxygen even at low concentrations, in contrast to use of nitrates and nitrites as final electron acceptors. The correlation between oxygen, nitrate and nitrite nitrogen in terms of their function as final electron acceptors, is understood from the following half-reactions (van Haandel et al., 1981):

Oxygen: $e^- + {}^1/_4 O_2 + H^+ \rightarrow {}^1/_2 H_2O$ (Equation 2.14)

Nitrate: $e^- + {}^1/_5 NO_3^- + {}^6/_5 H^+ \rightarrow {}^1/_{10} N_2 + {}^3/_5 H_2O$ (Equation 2.15)

Nitrite: $e^- + {}^1/_3 NO_2^- + {}^4/_3 H^+ \rightarrow {}^1/_6 N_2 + {}^2/_3 H_2O$ (Equation 2.16)

The use of oxygen as a final electron acceptor energy is preferable than the use of nitrates, because the energy efficiency of aerobic metabolism of organic carbon is higher than the anoxic catabolism nitrate.

It is investigated and improved that the oxidation of glucose in presence of oxygen yields 686 kcal/mole glucose and anoxic conditions; in the presence of nitrate, it yields 570 kcal/mole glucose (Delwiche, 1970).

The heterotrophic denitrifying bacteria require an organic carbon source for the cell synthesis and the production of electrons, which are necessary for the reduction of nitrate and nitrite.

Methanol is widely used as a carbon source and an electron donor in the various denitrifier systems, more than any other organic compound, due to the low cost. Ignoring temporarily the cell composition, the denitrification process using methanol can be described as a two-step process with the following reactions (USEPA, Metcalf and Eddy, 2003):

Step 1: $6NO_3^- + 2CH_3OH \rightarrow 6NO_2^- + 2CO_2 + 4H_2O$ (Equation 2.17)

Step 2: $6NO_2^- + 3CH_3OH \rightarrow 3N_2 + 3CO_2 + 3H_2O + 6OH^-$ (Equation 2.18)

Overall: $6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$ (Equation 2.19)

The overall reaction methanol functions as an electron donor (oxidized to CO_2), while the nitrate as an electron acceptor (reduced to nitrogen gas).

As in nitrification process, the equations take place in aqueous solutions participating to the system of carbonic acid. The obtained OH^- reacts with carbonic acid (H_2CO_3) increasing the alkalinity. Taking into account this reaction and cell composition (empirical formula cell recommendation: $C_5H_7O_2N$):

$3NO_3^- + 14CH_3OH + CO_2 + 3H^+ \rightarrow 3C_5H_7O_2N + 19H_2O$ (Equation 2.20)

The nitrate reduction reaction given by (Metcalf and Eddy, 2003):

$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7O_2N + 0.47N_2 + HCO_3^- + 1.68H_2O$ (Eq. 2.21)

Similar expressions can be developed for any organic carbon source, if it is known the return rate of denitrifier organisms.

In contrast to nitrification process in which consumed alkalinity, during denitrification process there is production of alkalinity. Along denitrification process there is a rising trend in pH values.

The choice of carbon source that will be used for denitrification is very important. The speed of the process is depending from the availability of the carbon. The most readily available are the biodegradable organic compounds, the higher the rate of denitrification is. The origin of the carbon can be from external addition from different substrate material that are high in carbon levels, sewage or endogenous.

2.8 Biochemistry of denitrification

The first step of denitrification is catalysed by a molybdenum (Mo) containing enzyme, nitrate reductase (Vivian et al., 1999). Generally, nitrate reductase is involved in nitrate assimilation, during which a soluble protein is sourced from ammonia. Instead, the nitrate reductase which is involved in the catabolism of nitrates is membrane-bound protein and it is synthesized only under anoxic conditions. Therefore, in most of bacteria, denitrification is strictly anoxic process, while

the uptake of nitrates can equally take place and under fully aerobic conditions. The assimilation of nitrate found in all plants, in most of fungi and in many prokaryotes. In contrast, catabolism of nitrate confined between prokaryotic cells, although a wide variety of such organisms can carry out this process (Madigan, 1997).

Another enzyme, nitrite reductase is responsible for the second step of process. In catabolic processes, two paths are possible. The first one leads to the production of ammonia and the second one to production of nitrogen gas. Ammonia process is performed by numerous bacteria, but there is little practical importance. There are also some bacteria that do not reduce nitrate compounds but nitrite compounds to ammonia. This is a mechanism for the protection of cells from the toxicity that is caused by nitrite compounds (Madigan, 1997).

There are two basic types of enzyme nitrite reductase (Knowles, 1982, Hochstein et al., 1984). One enzyme is a *haemoprotein cytochrome cd* and it is found in bacteria *Ps. Denitrificans* (Vignais et al., 1981), *Ps. aeruginosa* (Shimada and Orii, 1975) and *Ps. perfectomarinus* (Zumft and Vega, 1979). The second enzyme is a copper containing metaflavinoprotein enzyme and it is found in bacteria *Ps. denitrificans* (Iwasaki et al., 1963) and *R. sphaeroides f. sp. denitrificans* (Sawada and Satoh, 1980). Almost all studies show that nitrite reductase is a soluble enzyme (Cox and Payne, 1973, Iwasaki et al., 1963).

The reduction of N_2O to N_2, also relates to production of ATP after observed that proton accompanied by displacement (Boogerd et al., 1981, Urata and Satoh, 1985) and production capacity in the cell membrane (McEwan et al., 1985). Nitrous oxide reductase is the enzyme which is responsible for the reduction of N_2O to N_2 and it is bound to the cell membrane (Payne, 1981).

2.9 Genes responsible for denitrification

Denitrification is the microbial process by which dissolved nitrogen oxides serve as terminal electron acceptors for respiratory electron transport resulting in the reduction of nitrate to gaseous products (Wallenstein et al., 2006). The successful process depends on the microbial activity and the genes that create suitable conditions. Especially in biological denitrification that is investigated in that research is the most important sector, to find out which genes provide that process.

Most bacteria with this functional trait belong to a wide range of various subclasses of *Proteobacteria*. However, the ability to promote denitrification is widely distributed in the microorganisms, not only in large group of phylogenetically unrelated bacteria (Zumft, 1997), but also can be found in mitochondria of certain fungi (Shoun et al., 1992) and some *Archaea* (Philippot et al., 2002). Lateral gene transfer is the most likely explanation for this widespread ability to denitrify (Braker et al., 2001).

With developments of molecular biology towards analysis of functional genes for those organisms, *Archaea*, bacteria, and fungi, various denitrifying genes have been sequenced and exploited as biomarkers to discriminate between closely related but ecologically different populations (Throback et al., 2004).

Although denitrification process can be found within more than 50 genes (Zumft, 1997), the functional genes for denitrification pathways are common. Denitrification involves four enzymatically catalysed reductive steps: NO_3^- reduction, nitrite reduction, nitric oxide reduction, and N_2O reduction (Philippot, 2002).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{(Equation 2.22)}$$

2.10 Microbiology of denitrification

Denitrification carried out by microorganisms belonging to the following genes: *Bacillus, Pseudomonas, Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Chromobacterium, Corynebacterium, Flavobacterium, Hyphomicrodium, Moraxella, Neisseria, Paracoccus, Propionibacterium, Rhizobium, Rhodopseudomonas, Spirillum,* and *Vibrio* (Payne, 1981, Essandoh et al., 2013).

The genus *Bacillus* includes species which produce nitrate nitrogen and nitric oxide (NO), but not nitrite nitrogen and nitrous oxide. *The type B. Azotoformans* may use any of the three electron acceptors, nitrate nitrogen, nitrite nitrogen and nitrous oxide, which is possible in other denitrifier bacteria. These bacteria use wide variety of organic substrates.

The genus *Pseudomonas* contains the best known and most prevalent denitrifier bacteria (*Ps. aeruginosa, Ps. denitrificans, Ps. fiuorescens*). Some species do not use nitrate nitrogen but start denitrification process from nitrite nitrogen. In other bacteria, nitrous oxide is the final product instead of nitrogen gas. *Pseudomonas spp*. uses a wide variety of organic compounds which include: methanol, carbohydrates, organic acids, alcohols and aromatic compounds.

2.11 Kinetics-Degradation rates

The relation between the specific growth rate of a population of microorganisms and the substrate concentration is a valuable indicator. This association is represented by a number of empirically derived rate laws referred to as theoretical models. These models are mathematical expressions that generated to describe the behaviour of a given system.

A number that relates the rate of a chemical reaction with the concentrations of the reacting substances provides the order of the reaction: the sum of all the exponents of the terms expressing concentrations of the molecules or atoms defining the rate of the reaction.

Chemical kinetics is that branch of chemistry which deals with the study of the speeds or the rates of chemical reactions, the factors affecting the rates of the reactions and the mechanism by which the reactions proceed. It concerns itself with the measurement of rates of reactions proceeding under the given conditions of temperature, pressure and concentration (Cheyns et al., 2010; Tang et al., 2010).

2.11.1 First order reaction

A first-order reaction is a reaction that proceeds at a rate that depends linearly only on one reactant concentration. The rate at which a reactant is consumed in a first-order process is proportional to its concentration at that time.

The rate of a first-order reaction is proportional to the concentration of one reactant:

$$\text{Rate} = k [A]^1 = k [A] \qquad \text{(Equation 2.23)}$$

2.11.2 Second order reaction

A second-order reaction depends on the concentrations of one second-order reactant, or two first-order reactants. The rate of a second order reaction has a rate proportional to the square of the concentration of a single reactant or else the product of the concentration of two reactants.

$$\text{Rate} = k [A]^2 \text{ or } \text{Rate} = k [A] [B] \qquad \text{(Equation 2.24)}$$

2.11.3 Zero order reaction

In some reactions, the rate is apparently independent of the reactant concentration. The rates of these zero-order reactions do not vary with increasing nor decreasing reactants concentrations. This means that the rate of the reaction is equal to the rate constant of that reaction. The rate of a zero-order reaction is constant and independent of the concentration of reactants.

Rate = $k [A]^0 = k$ (Equation 2.25)

2.11.4 Microbial kinetics

Microbial populations increase until nutrients are exhausted. Analysis is aided by focus on the limiting nutrient. To model microbial degradation rates of chemicals, the knowledge of substrate unlimited growth, substrate limited growth and the substrate disappearance due to both growing and non-growing organisms.

In biochemistry, Michaelis–Menten kinetics is one of the best-known models of enzyme kinetics. The model serves to explain how an enzyme can cause kinetic rate enhancement of a reaction and explains how reaction rates depend on the concentration of enzyme and substrate (Ayyasamy et al., 2009).

The Monod equation relates limiting nutrient concentration to a population's growth rate. The Monod equation is empirical but fits actual data quite well. The Monod equation is a mathematical model for the growth of microorganisms. The Monod equation has the same form as the Michaelis–Menten equation, but differs in that it is empirical while the latter is based on theoretical considerations.

The Monod equation is commonly used in environmental engineering. It is used in the activated sludge model for sewage treatment and the denitrification process (Lee et al., 2008; Kaelin et al., 2009).

2.11.4.1 Monod kinetics

The Monod model which suggested in 1942 has been one of the most commonly used models in microbiology (Monod, 1949, Pirt, 1975, Koch, 1997, Kovarova-Kovar and Egli, 1998). The majority of the models in chemical analysis are based on the Monod equations (Pirt, 1975), and

numerous models of microbial ecology incorporate Monod growth kinetics (Koch, 1997, Stanescu and Chen-Charpentier, 2009). One of the very important practical applications of this model is the evaluation of the biodegradation kinetics of organic pollutants in environmental systems (Blok, 1994). The Monod model describes microbial growth with three parameters:

1) Maximal specific growth rate;
2) A saturation constant;
3) A yield coefficient.

In a simple homogeneous batch culture it is assumed that the growth conditions are similar for all cells (Pirt 1975). A typical growth curve is divided into six phases: 1) lag, 2) accelerating, 3) exponential, 4) decelerating, 5) stationary, and 6) declining growth (Monod, 1949).

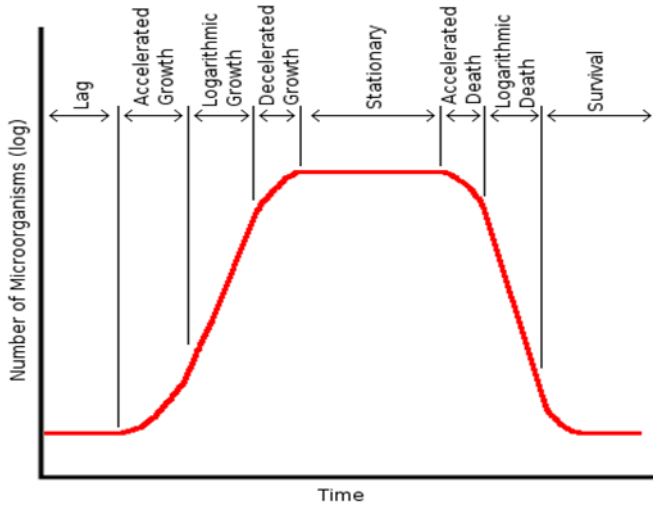


Figure 2.3: The growth curve in microbial organisms (Naturaleza.Eldietista.es)

Monod was the first one that described empirical models for microbial growth kinetics. The Monod model introduced the concept of a growth limiting substrate.

$$\mu = \mu_{max} \frac{S}{K_s + S} \qquad \text{(Equation 2.26)}$$

Where μ = specific growth rate, μ_{max} = maximum specific growth rate, S = substrate concentration, K_s = substrate saturation constant (i.e. substrate concentration at half μ_{max}).

In Monod's model, the growth rate is related to the concentration of a single growth-limiting substrate through the parameters μ_{max} and K_s. In addition to this, Monod also related the yield

coefficient ($Y_{x/s}$) to the specific rate of biomass growth (μ) and the specific rate of substrate utilization (q).

$$Y_{x/s} = \frac{dx}{ds} \qquad \text{(Equation 2.27)}$$

$$\mu = \frac{Yx/s}{X} * \frac{ds}{dt} = Y_{\frac{x}{s}} * q \qquad \text{(Equation 2.28)}$$

The kinetics of many biological systems is either zero or first order or a combination of them called Michaelis-Menten kinetics for enzyme catalytic reactions and Monod equation for cell growths.

The reaction is zero order when S is significantly greater than Ks (S >>Ks), transforming the equation into μ ≈ μmax.

When S is considerably less than Ks (S << Ks), the equation may be approximated by

$$\mu = \frac{\mu_{max}}{Ks} * S \qquad \text{(Equation 2.29)}$$

which represents a first-order reaction rate.

As a result, zero-order kinetics prevail for high substrate concentrations while first-order kinetics govern for low substrate concentrations, with regard to the growth rate limiting substrate (Grady and Daigger, 1998).

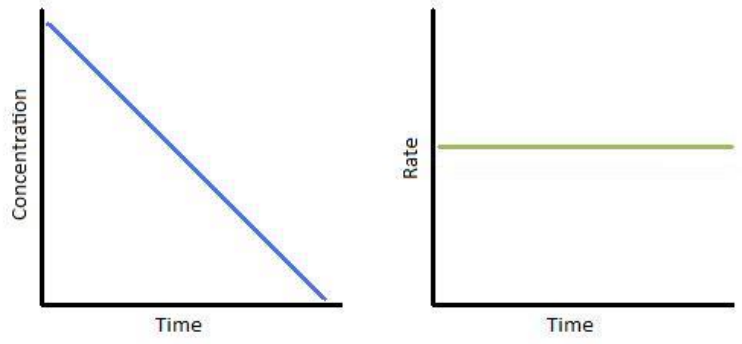


Figure 2.4: Concentration vs. time and Rate vs. time of a zero-order reaction (chemwiki.ucdavis.edu)

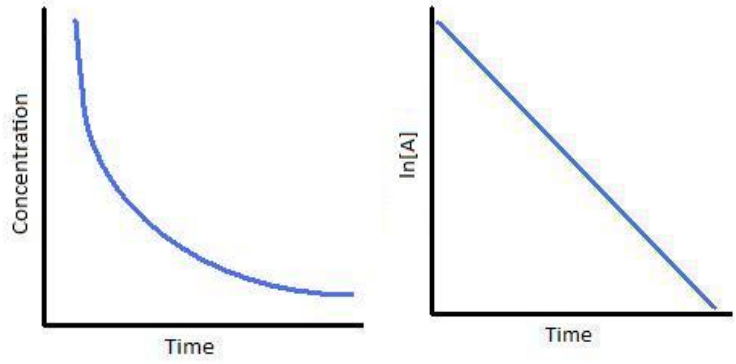


Figure 2.5: Concentration of reactants versus time of a first-order reaction (chemwiki.ucdavis.edu)

The kinetics in the columns and in the experiments have been characterized using zero, first, and second equations with respect to the limiting substrate (Okpokwasili and Nweke, 2005), as represented by the following equation:

General equation:

$$r = \frac{dC}{dT} = -k\,C^n \qquad \text{(Equation 2.30)}$$

where C = substrate concentration, k = denitrification reaction coefficient, and n = order of reaction. The reaction order within a filter is greatly affected by the degree to which the reaction rate limiting substrate penetrates the biofilm.

The field of nitrate removal is based on several studies depending on the substrate materials, HRT, porosity, flowrate and several other parameters that affect the denitrification process. There are several studies that investigate the kinetics of the systems even in PBR (Gibert et al., 2008) even in column studies (Robertson et al., 2010; Jing et al., 2010) even in open flow systems (Schipper et al., 2010). The most detailed approach of kinetics focused on two order kinetics. It is recognised that the denitrification process is connected with first and zero order kinetics (Qambani et al., 2013).

The first order kinetics met by researchers in SBR (Trois et al., 2010), reactors (Vassiliadou et al., 2009), PRB (Su and Puls, 2007) and column studies (Lee et al., 2004; Chabani et al., 2007). In these studies there is a connection between the carbon source and the denitrification rates. The half-life depends on the systems, with smaller half-life in column studies (1.45-46.51 hours) and with higher duration in reactors (8.6-313 hours).

The zero order kinetics is connected more with column studies than any other denitrification system. There are several column studies (Bratieres et al., 2008; Gibert et al., 2008; Robertson et al., 2010; von Rohr et al., 2014; Jing et al., 2010) that the kinetics described by zero order kinetics. That approach is logical due to the duration of the experiments that is longer than the 1st order kinetics experiments. The combination of two kinetics is also described (Qambani et al., 2013), showing that the first part is 1st order kinetics to achieve the best reduction levels and then in the second part there is the stable phase that the description of kinetics is following the zero order kinetics.

Except from the column studies, the zero order kinetics are mentioned in biofilters (Bratieres et al., 2008) and bioreactors (Schipper et al., 2010; Christianson et al., 2011) with the same successful removal rates as it is mentioned in column studies. The half-life in the columns studies has a wide range (10 hours – 126 days) (Aslam and Turkman, 2005; Robertson et al., 2010; Jing et al., 2010). In larger scale bioreactors the half-life is connected with substrate materials and the durations is increased from several days (5-30 days) (Hamersley and Howes, 2002) till many years (36.6 years) (Schipper et al., 2010).

2.12 Limiting factors in remediation

Availability of carbon substrates is usually a restrictive factor for heterotrophic microbial processes in soil and ground water, and may control complete microbial activity (Starr and Gillham, 1993).

In a previous study (Sgouridis et al., 2011); denitrification was higher in surface soils than in subsurface soil, indicating the potential influence of soil organic carbon. Comparison between the soil organic carbon in surface and subsurface soils in forest and agricultural soil profiles showed that concentrations of both water-extractable organic carbon and bioavailable dissolved organic carbon were significantly higher in surface layer than those in subsurface layer (Boyer and Groffman, 1996). In subsurface layers, amount of total organic carbon dropped considerably with depth which heads to a lower rate of microbial activity. This conclusion is also consistent with the research conducted in aquifers where denitrification potential is related to water table depth (Sgouridis et al., 2011). Denitrification is faster in aquifers with a very shallow water table (Starr and Gillham, 1993), because organic carbon availability decreases with depth below the ground surface. The differences in bioavailability lead to dissimilar influence on these microbial processes. The high molecular weight organics are not as easily integrated as low molecular

weight organics. One possible explanation indicates that the C: N ratio of organic matter is a factor effecting bioavailability of soil organic carbon (Boyer and Groffman, 1996).

The nitrogen removal depends on the method that is used to remove all the nitrogen compounds. Generally sand filter and filters that contain inert material can provide to the system a removal rate between 30-50% (Aslan, 2005; Della Rocca et al., 2007). The addition of carbon materials like wheat straw can increase the removal rate in higher levels between 60-90% (Aslan and Turkman, 2005; Xing et al., 2011) and finally the addition of substrate materials rich in carbon levels like mulch, woodchips can increase the removal rates in levels higher than 90% (Ray et al., 2006; Su and Puls, 2007). The removal is not only depending on substrate materials but also on the characteristics of each experiment (treatment way, temperature, flow rate, oxygen levels) (Saeed and Sun, 2012).

2.13 Effects of hydrology on denitrification

There are differences between column experiments and field experiments. The nitrate removal capacity could be affected by other important factors such as local hydrology and groundwater flow patterns in the field (Hill et al., 2000).

The subsurface denitrification in lower levels of groundwater is not as high as that in shallower groundwater region under riparian buffers. At deeper levels below riparian buffers the interaction of groundwater flow, and electron acceptor and donors, could be effectively used to remove NO_3^- levels. The location of denitrification is connected to the stock of oxidised organic carbon (Hill, 1996).

Effective removal of NO_3^- happens worldwide in different areas, but there is a related hydrogeological background at most of locations. The effective hydrological setting usually includes numerous factors, some of which are characteristic: long residence times along groundwater flow paths, dilution of NO_3-N rich waters by less concentrated older groundwater, the bypassing of riparian zones by tile drains or ditches, and the movement of groundwater along deep flow paths below shallower, organic rich reducing zones. (Hill, 1996; Puckett, 2004). Those riparian zones have permeable surface soils and an impermeable layer under them at a depth of 1 to 4 m. Such impermeable layers create shallow subsurface flow of groundwater under the riparian area.

The flow rate depends on the solution that is used and the characteristics of the substrate materials (porosity, temperature). There are experiments with several solution media like tap water (Della Rocca et al., 2006; Gustafsson et al., 2008), wastewater (Tanaka et al., 2007; Zhao et al., 2009), artificial wastewater (Essandoh et al., 2011), groundwater (Liu et al., 2013). The flow rate at all the column experiments, PBR, bio barriers are up side flow opposite from the gravity (Della Rocca et al., 2007; Robinson Lora et al., 2009; Jing et al., 2010, Liu et al., 2013; Calderer et al., 2014). The upside flow is used to achieve the suitable conditions of saturation for the experimental process and to minimize all the loses of other parameters.

2.14 Effects of other factors (pH, T, HRT, Porosity) on denitrification

Denitrification rates are connected with several chemical and environmental factors. Meta-analysis was used to categorize the tendencies that exist between nitrogen removal efficiency and buffer width, hydrological flow path, and vegetative cover (Mayer et al., 2009).

pH factor influencing denitrification with the best range between 6.0 and 8.5. The effluent nitrate concentrations could be described by a linear combination of temperature, flow rate and influent NO_3^- concentrations. Nitrate removal was reduced with increasing flow rate and increased with increasing temperatures (Willems et al., 1997). Dawson and Murphy (1972), running experiments in cultures without acclimation period, observed that for pH values between 7.0 and 7.5 achieved the highest denitrification rates, while 50% of the denitrification rate achieved at pH 6.0 and 8.5. Other researchers such as Nomnik (1965), Wiljer and Delwiche (1954) and Bremner and Shaw (1958) showed that there is no influence of pH on the rate of denitrification rates between 7.0 and 8.0, while the rate decreases linearly for values pH from 8.0 to 9.5 and from 4.0 to 7.0. It was observed that denitrification is inhibited in acidic conditions. Specifically, for pH values below 5.0 there was no denitrification activity (Rivett et al., 2008). It has been detected that the optimum pH value is between 7.0 and 8.0, depending on the populations of denitrifier bacteria (Henze et. al., 2008).

The pH is another indicator for denitrification process. The most suitable limits in between the denitrification process achieves the best results are pH 6.5-8.5 (Tanaka et al., 2007; Huang et al., 2011). The more neutral the environment is the higher removal rates achieved. High carbon materials provide to the system pH levels between 7.5-8.5 (Trois et al., 2010; Nakatani et al., 2011).

Because of low dissolved organic carbon in groundwater, denitrification in buffer areas and in all nitrate removal processes is carbon limited (King, 2005, Knies, 2009).

Denitrification is a strictly anoxic process (Payne, 1981, Knowles, 1982). Denitrifier bacteria are facultative aerobic microorganisms. They prefer the use of dissolved oxygen, even at quite low concentrations, thereby preventing the use of nitrates and nitrites as the final electron acceptor. Oxygen prevents the process of denitrification, and suppresses the synthesis of enzymes which are necessary for nitrate reduction and inhibits the activity of the already formed enzymes. Thermodynamic data shows that the energy efficiency of aerobic metabolism of organic carbon is higher compared to the performance of anoxic denitrification. For this reason, denitrification must be carried out in an anoxic environment to ensure the use of nitrates and nitrites, and not the oxygen, as final electron acceptor. Considering, all factors are related with the synthesis and activity of enzymes which are responsible for denitrification, the levels of dissolved oxygen should be almost zero to achieve the best performance.

Denitrification occurs in a wider temperature range of 0-50^{o}C (Henze and Harremoes, 1977, Rivett et al., 2008). The best range for denitrification process is between 15 and 35 ^{o}C (Ge et al., 2012, Metcalf and Eddy, 2003).

The ideal temperature is mentioned by several researchers (Chu and Wang, 2013, Nordstrom, 2014) for denitrification process is between 20-35 ^{o}C. There are several also researches that mention successful denitrification process with high removal rates even in higher temperatures (Cameron and Schipper, 2010) even in lower (Christianson, 2011). Generally the higher the temperature is, the higher removal rates achieved.

Porosity or void fraction is a measure of the void spaces in a material, and is a fraction of the volume of voids over the total volume. The porosity is connected with the design of the experiment. The mulch according to the mixture have porosity between 0.40-0.60 (Saeed and Sun, 2011), the wheat straw between 0.45-0.65 (Aslan, 2005), the sand in columns 0.30-0.45 (Payne et al., 2014), perlite 0.60-0.90 (Ilstedt et al., 2007), and woodchips 0.40-0.82 (Warneke et al., 2011). There are several ways to measure the porosity. Along the chapters the porosity measured with water saturation method (pore volume = total volume of water − volume of water left after soaking).

Another characteristic that is important along the experiments and it is connected with the porosity, the substrate materials and the solution is the hydraulic retention time (HRT). HRT is the amount of time in time units (hours/days) for solution to pass through a storage unit. HRT is

measured versus time, that is hours in chapters 5, 6 and in days in chapter 7,8,9. Several researchers with different approaches on column experiment mentioned HRT between 0.5 hours till 8 days (Robertson, 2010; Jing et al., 2010; Saeed and Sun, 2011). In larger scale, in denitrification walls, layers, and artificial wetlands the HRT was between 2 hours till 15 days (Schipper et al., 2010; Cameron and Schipper, 2011). The detailed description of the measurements of porosity and HRT is given in Chapter 4 with methodology.

2.15 Biological denitrification in Bio-barriers

Biological treatment is based on processes in which plants and microorganisms cooperate in the remediation of metals in soil and in groundwater. Denitrifying bioreactors can be used to reduce nitrate from water (Schipper et al., 2005). These bioreactors are divided into three categories: denitrification walls, denitrification beds, and denitrification layers.

Denitrification walls are used in shallow groundwater that contain large amounts of carbon in a solid state, and there are designed to sustain elevated hydraulic conductivity. The design of walls can be either 100% woodchips or mixture of soil and sawdust, or any other substrate material. The removal rate for nitrates is between 0.014-3.6 N g/m^3 per day. The amount of nitrate removal is depending on the mixture of the media and the hydraulic retention time of treated water in walls (Schipper et al., 2010a). Denitrification beds are larger than the walls, and have larger treatment area. Beds have a rectangular shape and consist of woodchips or other substrate materials that can be used separately or in combination. The amount of nitrates that can accept is bigger. The sources of nitrate levels can be wastewater or tile/drain discharges. The removal rate of nitrates at beds is between 2-22 N g/m^3 per day, and the optimal temperatures range is between 2 and 20 ^oC (Warneke et al., 2011). Denitrification layers are the largest design of bioreactors. They are installed under septic tanks or effluent irrigated soils. (Schipper et al., 2010b, Long et al., 2011)

The disadvantage of bioreactors is the supply of source carbon. The half-life of bioreactors is between 4-37 years, and problems are not visible for long time (Schipper et al., 2010b). But, if there is a problem the addition of substrate media (generally cheap media) can be proposed. The advantages of bioreactors also includes removal at nitrites, phosphates and the amount of pathogens, pesticides and the amounts of *Escherichia coli* (<10 cfu/100 ml) are also reduced (Schipper et al., 2010b). Additionally, it is a reliable system after 5-7 years the rates at nitrate removal stay more than 75% of initial removal rate (Schipper et al., 2010a).

The typical amount that can be removed in a year from a typical bioreactor with dimensions (13m long x 1.2m wide x 1.1m depth) is 11.3 N kg/year (Schipper et al., 2010a). Significant role at denitrification processes in bioreactors are playing the denitrification organisms (bacteria and microorganisms). In biofiltration systems concentrations of organic matter are the eliminated point for sandy substrate (Payne et al., 2014), and it is unidentified if a source could provide significant longstanding nitrogen storage.

Another important point is that nitrogen processes includes nitrification and denitrification, which are both mediated by microbes, but require dissimilar redox environments. Many biofilter designs incorporate an upper drained layer underlain by a saturated layer, maintained by using a raised outlet, which may theoretically provide zones for nitrification and denitrification respectively. An additional carbon source (e.g. wood chips, straw, mulch, softwood) is often mixed in the saturated zone to offer an electron source for denitrification (Kim et al., 2003, Robertson, 2010).

2.15.1 Denitrification in Batch and Column experiments

In denitrification process there are several studies that used batch and column experiment (Grau-Martínez et al., 2015, Hekmatzadeh et al., 2012). The batch test used to analyse non preferable (micro-aerophilic and anaerobic) for microbial colonies conditions for denitrification process with lack of oxygen, lack of light and lack of carbon source. Batch tests used as it is happened in column studies several waste materials (wood chips, straw, mulch, softwood). The column experiments examine the reaction of these materials in long term experiments in contrast to batch test and with a continuous flow rate of the solution that is used (water, groundwater). The waste materials that used at all cases are the same in both type of experiments and there is a main characteristic, they are used to provide in the system the organic carbon to accelerate denitrification process. The waste materials as already mentioned are several cheap materials (cotton, woodchip, softwood, sawdust, mulch, wheat straw, pine bar) that can be found everywhere and the common characteristic is that are not expensive.

The design of columns experiments cannot be characterized that is following only one general trend. There are several researches (majority of them) that the waste materials are mixed with inert material (sand) and used in column studies homogenized (Martinez et al., 2005). That is the approach that is followed in chapters 5,7,8,9. The percentage of mixture was chosen by each research and the results targets that need to achieve. There are also several researches that columns used as lab scale barrier with different substrate materials in a row to examine the effectiveness

of the selected design in denitrification process (Viggi et al., 2010, Christianson 2011). At all the designs of column experiments there is a standard approach of the characteristics. The solution that is pumped in the columns with peristaltic pump to ensure the stable flow rate moves opposite with gravity. For other characteristic of columns like length and diameter there are several approaches depending in the research. Finally it is important to analyse the conditions that the experiment is running (Stable temperature, light effect, humidity).

2.16 Phosphate levels in water

Phosphorus exists in soil in organic form or as assimilable inorganic salt (phosphate). These two forms can be absorbed by plants. Inorganic phosphorous is rapidly absorbed by plants. A source of organic phosphorous is the dead plants and animals that release biologically bound of phosphorus of their tissues, through decomposition by microorganisms. The incoming amount phosphorus fertilizers are very important.

In natural waters and wastewaters, phosphorus exists mainly as dissolved orthophosphate and polyphosphates and organically bound phosphates can be observed continuous changes in the forms of phosphorus due to the decomposition and composition in the organic frozen forms and oxidized inorganic forms (Vanek, 1993, Rivett et al., 2008).

Natural sources of phosphorus are mainly erosion of phosphorus rocks and the decomposition of organic matter. Other sources can be considered household wastes, especially those containing detergents, industrial wastes and runoff fertilizers. Phosphorus associated with organic and inorganic components of sediments can be mobilized by bacteria and released into the water. Often phosphate is the main limiting nutrient in aquatic ecosystems. Phosphorus is rarely found in high concentrations in fresh water as it is absorbed by plants (Connolly et al., 2009).

As a result it is possible to observe significant seasonal variations of concentrations in surface waters. In most natural surface waters, phosphorus has range from 0.005 to 0.02 mg/l PO_4^{2-}-P. Concentrations low as 0.001 mg/l PO_4^{2-}-P can be found in clear water and as high as 200 mg/l PO_4^{2-}-P in some polluted water.

High concentrations of phosphate pollution cause eutrophic conditions. The management of a pond or a reservoir, especially when the water used for drinking, requires knowledge of phosphate levels in order to control the growth of algae. Concentrations of phosphorus are usually identified as orthophosphate, total inorganic phosphate or total phosphorus (Gustafsson et al., 2008).

The increase of inorganic phosphate and biomass in the sediment is the main mechanism of phosphorus removal in an artificial wetland treatment system with surface flow.

In contrast to nitrogen which is returned to atmosphere in gaseous form after denitrification in riparian zones, the negative point for phosphorus in soil is the fact that it accumulates in soil and biotic uptake from plants. There is a possibility for the sustained phosphorus to be released from the vegetation and soil and given again in the system in soluble form (Vought et al., 1994; Muscutt et al., 1993).

Phosphate investigated because it is connected with denitrification process. All the pollution issues that investigated combine P-pollution with N-pollution and eutrophication that is the result of high phosphate levels is connected with nitrification. Denitrification is connected with P-removal due to the waste materials and methods that are used. The introduction of new materials in that process must be careful. The combination of N and P removal is the best proposed solution and it finally the method that is proposed in that research. The investigation of P levels was not an initial target of this research but receiving the concerning results along the experimental procedure, the combination of all removal process was the final target without loosing the initial hypothesis of nitrate removal.

2.16.1 Physical and chemical separation

The role of riparian zones at the transport of phosphorus is very important, due to the excessive loads in these areas that primary cause of eutrophication in lakes and rivers. Phosphate particles can be deposited on the bottom of wetland through sedimentation; adhere on the surface of vegetation, or to be absorbed by microorganisms.

The exchange of dissolved phosphate between pore water and in the water column through diffusion or sorption/desorption is the main source of dissolved phosphate. In trapped water, phosphates can be precipitated as insoluble ferrous, calcareous and aluminous phosphates or adsorbed by sludge particles, organic peat and ferrous and aluminous oxides and hydroxides (Gustafsson et al., 2008).

Phosphates can be released from the metal complexes depending on redox potential of the system. Phosphate released from ferrous and aluminous complexes by hydrolysis which takes place under anoxic conditions.

The adsorbed phosphate sludge particles and hydrous oxides can also be returned to water by ion exchange. If pH of system decreased as a result of biological formation of organic acids, nitrate or sulphate, and amount of phosphate can be released.

2.17 Problem with groundwater pollution in Greece

Greece is a country that is based on the agricultural activity. The Greek government as part of European Union has accepted the legislation for the water pollution and the problem that exist in the area with high nitrate levels and the reuse of wastewater. Vulnerable zones have been designated along the country and 'Codes for a good practice' have been developed (Konstantinou et al., 2006).

The main problem with groundwater pollution is focused on the fertilizers from agricultural activities, the disposal of wastewater and the seawater intrusion due to overexploitation of coastal aquifers.

The main problem is the extensive use of nitrogen fertilizers, nitrates, phosphorous and organic discharges from urban and agricultural wastewater. The effect of all these factors are visible in the rivers and lakes with high levels of nitrate pollution, eutrophication (phosphorous pollution), agricultural pollution (wastes of livestock farming, fertilizers and pesticides). Additionally household, industrial and solid waste, heavy metals, organic matter and untreated sewage are also affect the groundwater quality and nitrate pollution.

Groundwater quality is threatened by uncontrolled wastewater disposal and seawater intrusion at coastal aquifers. High nitrates concertation have source nitrogenous fertilizers, livestock manure and pesticides and that has already noticed in several areas in Greece (Voutsa et al., 2001).

According to chemical analysis of groundwater in several areas of Greece, groundwater contains except from high nitrates and phosphate levels due to applied fertilizers. High sulphate ($SO4^{2-}$) concentrations in western Greece can be associated with the dissolution of gypsum. High potassium concentration is related to mixed-type fertilizers and to the presence of K-feldspar. High concentrations of Fe and Mn are attributed to lithological

conditions. High concentrations of heavy metals (Zn, Cu, Ni, Pb) are recorded in areas with mining activities (Daskalaki and Voudouris, 2006).

2.18 Research that is needed

This research focused on biological treatment and specifically at permeable reactive barriers (PRB) and bioreactors, which is used for groundwater remediation. The method of PBR is cheaper than a traditional ex situ remediation (Hashim et al., 2011). A PBR consists of a narrow trench below the ground downstream of a contaminated groundwater plume. There are also substrate media which are mixture of natural or reactive substances (mulch, chip wood, straw). PBR is easily established and it uses a small amount of chemicals. Additionally, the system does not need high maintenance and energy. The process that is taking place through PBR is denitrification process due to the help of microbes (enzymes activity) that exist in the mixture of substrate materials.

Several other researchers have investigated the removal of nitrogen through denitrification process and continuously the phosphorous compounds using different techniques. The artificial wetland for larger scale experiments is mentioned and according to the substrate materials that are used in combination with the plants that exist (sand, woodchips, sawdust, Phragmites australis, etc.) (Aslan, 2005; Della Rocca et al., 2007; Gibert et al., 2008; Huett et al., 2005). The removal rates started from 30% in environments that exist only sand and inert materials that there are low carbon materials and reach levels between 80-99% in substrate materials that can provide high carbon levels (Lee et al., 2004; Yang et al., 2008; Zhou et al, 2011; Liu et al., 2013).

The next technique that is used by many other researchers was the column studies (Tanaka et al., 2007; Alcala et al., 2009; Robinson Lora et al., 2009).The advantage of these studies was the environmental conditions, that are totally controlled and the substrate materials. The materials depending on the initial carbon level can provide to the system the suitable amount of energy to succeed the nitrogen removal till 99.9%. It is also important that part of the columns experiment are the permeable reactive barriers (PBR) and sequence batch reactors (SBR) depending on the design and the characteristics that are used (Gibert et al., 2008; Su and Puls, 2007;Trois et al., 2010; Rodriguez et al., 2011; Calderer et al., 2014).

2.19 Proposed research

The proposed research focus on was column studies that supported heterotrophic denitrification, in which only substrate materials affect the denitrification process. Several materials were investigated and the performance results were compared. In these experimental systems, the only carbon source in the system is the substrate material. The research was focused on the engineering parameters from column studies that can be used to develop engineering solutions to groundwater nitrate and phosphate contamination in Greece, and did not focus on the detailed microbiology or biogeochemistry of these reactions.

My research contributes to fill a knowledge gap in the utility of waste substrate materials available in Greece and the regional geography that could be a carbon source for denitrification/nutrient removal. There are several previous studies about the substrate materials that are used for denitrification process: cotton burr compost, cotton, liquorice wastes materials, seaweed, woodchips, cardboards, cornstalks, softwood, hardwood, coniferous waste materials, mulch, compost soil, willow, leaves, newspaper, wheat straw, pine bark, alfalfa, soil, sand and much more.(De Catanzaro et al., 1987, Gilbert et al., 2008)

The experiments focus on two main columns experiments in which new substrate materials were added. The first part was a combination between already used materials (sand, mulch, wheat straw) and the new substrate material that used was perlite. The second part of experiments was with totally new substrate materials (waste tea materials, hazelnut husk wastes) and there was combination of them with perlite.

Denitrification process is chosen to that research for several reasons. The most important part of the research was to remove the Nitrogen compounds and to find out methods that can keep the total cost of the process very low. According to this denitrification process can provide all the results that expected. The choice was strategically due to the waste and low cost materials that can enhance denitrification process. All that materials that are chosen in this research can create the suitable conditions and the activity to achieve those results.

The choice to focus on biological denitrification was critical. With that option the combination of microbiology of the waste materials and the condition that denitrification process take place is important. The denitrifier bacteria can easily increase their colonies even in the presence of organic carbon that is provided by the waste materials (heterotrophic denitrification bacteria) or can create carbon alone (autotrophic denitrification bacteria). The waste materials that chosen can provide to the system huge amount of organic carbon. The application of biological denitrification

is also important because the main aim of the research was to keep the cost as low as it is possible. Except from that in biological denitrification and the methodology that is chosen a lot of environmental and design conditions are under control and can change with the way that is proposed.

Denitrification process has been investigated in environments that the flowrate was stable with wetland, PBR, columns experiments. In this research there are column experiments that the flowrate remain stable at all the duration of the experiments and there are several HRT which are in the limits that other researchers proposed in the first experiment. In the last experiments the HRT are much higher than the providing literature, giving in details the approach to all the characteristics that investigated.

The proposed research focused on heterotrophic denitrification on column studies. The application of denitrification on columns was important because the environmental conditions of the research were under control and all the parameters can change in the way that the research demands. With that research there is an approach on denitrification (DN) process with stable inlet flowrate in the columns.

The design of the experiments was separated in two parts. In the first part (Chapters 5 and 6); there is an initial artificial wetland that was used to provide to the system the suitable environmental conditions for groundwater and the microbial activity that exist there. In the first part there was investigation in the columns with already used materials in columns studies (wheat straw, sand, mulch) and the only new substrate material that insert in that research was perlite in Chapter 6. The groundwater received by Northern Ireland in the area that NITRABAR project (EU program for nitrate removal in agricultural area took place). The HRT time that investigated was in Chapter 5, 7 hours and 50 hours in a system that columns contain only wheat straw and sand in different percentages in each column. In Chapter 6, the columns combine all the substrate material in layer, creating a reactive layered barrier. The groundwater that used was again from Northern Ireland, and there was pre-treatment in artificial wetland. The HRT in that experiment was 16 hours.

In the second part of experiment there is a total approach in two new investigated materials in denitrification process. There are two waste materials from the east Mediterranean area (Greece and Turkey). The first one was tea waste materials and the second one was hazelnut husk wastes. The research was original and the design of the experiments started with batch experiments and different percentages of substrate materials mixed with sand to find out the reaction of those materials under anoxic and without light connection conditions that are met in the ground of earth.

The next part was the long term experiments with these two new substrate materials in columns studies. The substrate materials used in different percentages in columns and two HRT time investigate. The HRT was for short term period columns 3.25 days and for the long term experiment 6.10 days. The groundwater that used in those experiments received from the suburban area outside from Glasgow near Largs, Scotland. The collection time of groundwater was firstly in October after summer and the second collection was in March after the winter, so there are some differences in the results due to the application of different groundwater. The batch and column experiments contain a part that the solution that used was tap water. Tap water used to find out if denitrification process can provide the expected results even with clear water that does not contain any microbiological activity. The duration of experiment with tap water was 51 days. In the second part the solution that used was groundwater. The first experiment with groundwater had duration of 31 days. The results that received ensure that denitrification process took place even in tap water solution even in groundwater. The concerning point that noticed in both experiments was the high levels of phosphate that received. Finally the last experiment combined all the knowledge from Chapter 5 that perlite was with the promising results of Chapter 8. Finally it was introduced the last experiment with the same columns as Chapter 8 adding additional columns that the only substrate material was perlite. The HRT remain the same and the perlite column adding exactly after the columns with tea and nut waste materials. With that design the initial hypothesis about nitrate removal achieved and additionally the secondary hypothesis that inserted in the duration of the experiments with the phosphate removal also achieved.

CHAPTER 3

SAMOS ISLAND AND NITROGEN PROBLEMS

3.1 Samos Island

Samos was chosen as a characteristic island located between Greece and Turkey to represent the environmental conditions that exist in the area and an example of what is really happening. It is an island that is strongly agricultural with many water resources. The island contains the characteristics of both countries even in cultivation, the climate of area, and in problems that exist with water. The island can be simulated as an example of all islands in the eastern Aegean Sea. Samos was chosen in cooperation with the Archipelagos Institute, which is focuses on water resources and the water pollution in combination with cultivation problems. The island is very characteristic example due the water issues noticed in the last years and due to geographical location next to the Africa dessert and as a crossroad for flora and fauna between Europe, Asia and Africa. Temperatures are high and biodiversity activity is high and important for island.

Samos Island (Figure 3.1) is located in the heart of Central Aegean. Its area is 477,395 km^2 and geographically located between the parallels 37.49 and 37.37 to the North and the meridians 26.33 and 27.04 to the East. Samos is a Greek island in the eastern Aegean Sea, south of Chios, north of Patmos and the Dodecanese, from which it is separated by the 1.2 kilometre wide Mycale Strait which is called ‘Eptastadio’ channel (Stamatakos, 2010).

The geography of island is dominated by two large mountains, Ampelos and Kerkis (anc. Kerketeus). The Ampelos mountain range with 1095 metres high (known as ‘Karvounis’) is the larger of two and occupies the center of island. Mount Kerkis is the highest with 1434 metres high at the west side of island. The mountains are an extension of Mycale range on the Anatolian mainland at Turkey (Vassilopoulos et al., 2008).

The plain areas of island are: next to the capital of the island, at Vathy in northeast part of island. The next plain area is in northwest at Karlovasi. In south part, there are two more plain areas one in southwest part at Marathokampos and finally in southeast at Pythagoreio. The island's population is 34,000 people, which is the 9^{th} most populated of Greek islands. The climate is typically Mediterranean, with mild rainy winters, and warm rainless summers.

According to ancient Greek philosopher Strabo, the name Samos is from Phoenician meaning ‘rise by the shore’ (Stavrianou, 2009).

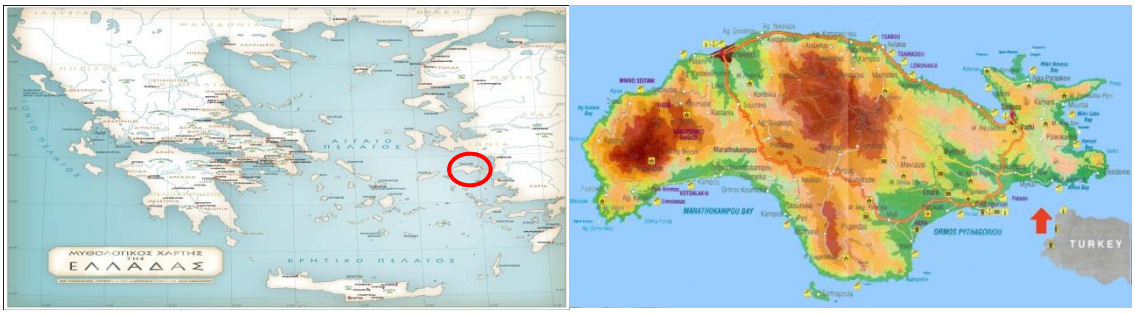


Figure 3.1: Samos maps (Samos Municipality)

3.2 Ancient and agricultural history of Samos

In ancient times Samos was a rich and powerful city-state and known for its vineyards and wine production. It is home to Pythagoreion and Heraion of Samos, UNESCO World Heritage Site that includes the Eupalinian aqueduct, a marvel of ancient engineering. Samos is the birthplace of Greek philosopher and mathematician Pythagoras, philosopher Epicurus, and astronomer Aristarchus. Samos wine was famous in ancient times, and is still produced on the island (Stavrianou, 2009).

The ancient Greeks who loved to observe and study nature, often praised the beneficial climate and fertile soil of Samos. Although many things have changed since the first known cultivation on island in 900 B.C., the quality of vines remains the same due to Samos' favourable climate. Viniculture and wine production permanently marked island's economy and history (Stavrianou, 2009).

Samos wine (moschato variety) was famous for its sweet taste. It was frequently referred in many ancient myths and it was exported to all places in Mediterranean Sea. After the ancient times, the first mention of Samian wine was in 12th century. The Samian Moschato originally came from Asia. Samian Moschato became well known after phylloxera destroyed many European vineyards in France and Italy in 16th century. Phylloxera is a nearly microscopic root insect, similar to an aphid, which primarily attacks the roots of grape vines. That was the time when French and Italian and west European winemakers ‘discovered’ the Samian wine (EOSS). Due to the demand of wine, prices increased. It also enlarged the vineyards area in Samos to 4,700 square meters. French enologists brought the most modern equipment to Samos in order to produce sweet wines. These French enologists were the first that practice the method of

stopping the fermentation of must by adding alcohol. Even today this method is used in island. Samian Moschato wine became famous and started to win awards from 18th century till now (Samos Municipality).

3.3 Geology of Samos

Samos Island belongs to Aegean crystallo-schistosive zone. It is between the Attica-Cycladic complex and the crystalloschistosive massif of Menderes (West Turkey). The geological structure of Samos can be separated in two main pre-Neogene geotectonical units (with individual subunits for each one) and the Neogene and Quarternary formations that fill the island's basins (Riedl, 1989, Kammas, 1998).

The bedrock in the research area is consisted of metamorphic rocks, marbles, phyllites and serpentinites, conglomerates, calciferous formations of lacustrine phase, tuffs, white limestone and dolomite and biogenic siliceous sediments (Stamatakis, 1989).

3.3.1 Hydrology of Samos

Samos (Figures 3.2) does not have any major rivers or lakes but only creeks that retain water during winters (Vavlianakis, 2002; Mourtzios, 2008; Stamatakos, 2010). In contrast, there are many karstic springs. The high amount of rainfalls all the year and the dense vegetation contribute to the abundance of underground waters. Most settlements water supply comes from springs and small depth drillings. The general direction of groundwater flow is NW-SE heading to sea and the recharge areas are the two main mountainous volumes in the west part and in the centre of the island (Kammas, 1998, Tziritis et al., 2008). The prevailing winds in Samos are north-western, mainly during summer period when rains are rare. That separates the island in three climatic areas. The northern part is influenced by winds from sea which are humid and cool. In the southern part, winds descend from the mountains dry and turbulent (downwards winds). Lastly, there is the mountainous part where lower temperatures and high humidity prevail.

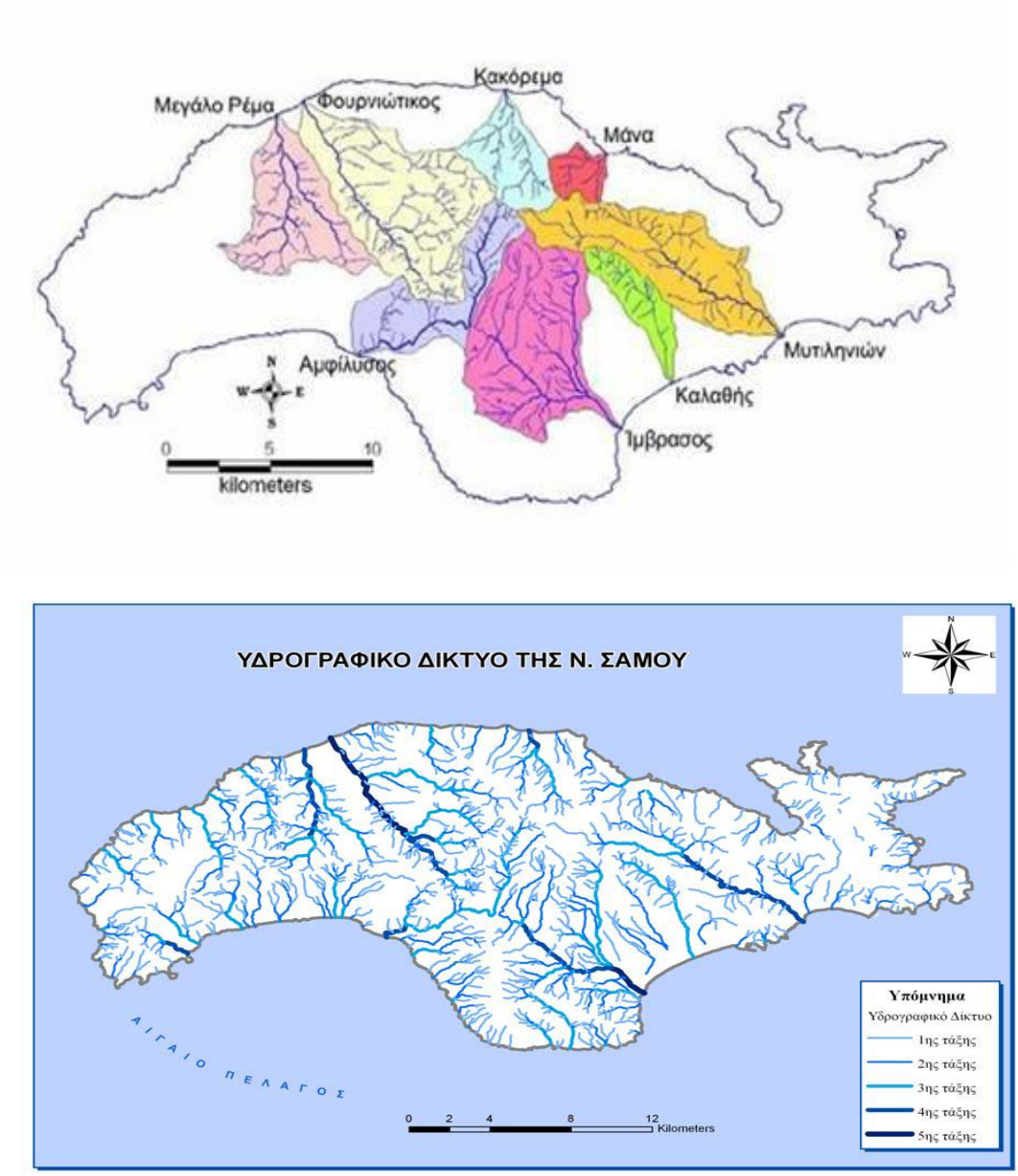


Figure 3.2: Samos rivers and hydrology map (After: Vavlianakis, 2002; Mourtzios, 2008; Stamatakos, 2010)

Environmental degradation in combination with the increasing needs of local people, and the growing numbers of tourists have negatively influenced water potential (Mourtzios, 2008). Additionally, surface water has decreased considerably after extended forest fires the last years. In the last 20 years, since 1993, more than 300 fires where noticed. Areas covered mainly by olive trees, vineyards, forests of black pine and citrus trees were incinerated (Fotiadis and Siasios, 2007, Kammas, 1998). As a result of fires the land use of island changed (Myteletsi and Theodorou, 2000, Tziritis et al., 2008). All these changes affect also the hydrologic map. Dense

drainage pattern is characteristic for the area and ensures the run-off of the region (Pe-piper et al., 1991).

3.3.2 Meteorology and climate of Samos

There are meteorological data from three places in island for years 2006-2007 (Ministry of Agriculture). The first two are at the south part next to airport; the one in Pythagoreio (Airport 1, (37.691908, 26.938251)) and the second one in Heraion (Airport 2, (37.669352, 26.877195)). The last station is in Vathy (37.759588, 26.969887) at the north east part (Appendix VII).

The average hyper-annual value of precipitation is higher in south part of island with 715 mm per year in the south centre and 918 mm per year in south east island. The precipitation in north part is much lower (306 mm). The average monthly air temperature presents simple fluctuation in island. The lowest temperature observed in February (6.5^{o}C) and the highest in July (32.5^{o}C). The differences of average temperature between summer and winter months are very small, while bigger fluctuations occur between spring and fall. At the south part, it is observed the lowest and the highest temperature. The average annual temperature was 18.4^{o}C.

Relative Humidity follows opposite trend of temperature. When temperature increases relative humidity decreases and vice versa. A minimum (43.7%) is detected in July and a maximum (79.1%) in October. The average annual value for relative humidity was 63.1%.

The dry period on the island lasts 5 months, from the end of May till the end of October. This happens due to northern dry continental winds (meltemia) that overcome this season on the island. In Samos the yearly prevailing winds are of north-western direction and reach an average of 1.4-3.5 on Beaufort scale. Meltemia are most frequent in August. The wind's force in island is stronger during winter months.

Samos is a region with high sunlight levels in Greece. The average annual sunlight was 2885 hours. July had the most sunlight (378 hours on average), while December the minimum (122 hours on average). Extreme climatic conditions as frost, hail, snow, dew, fog and storms occur only few days per year (Appendix VII).

3.3.3 Hydrogeology of Samos

The soils in estuaries of torrents are alluvial and colluvial on the hill slopes, with good penetrability. The soils on the slopes are formed in terraces with dry walls, fertile and without excessive salt. The hydrolithology of the area has the following characteristics (Figure 3.3):

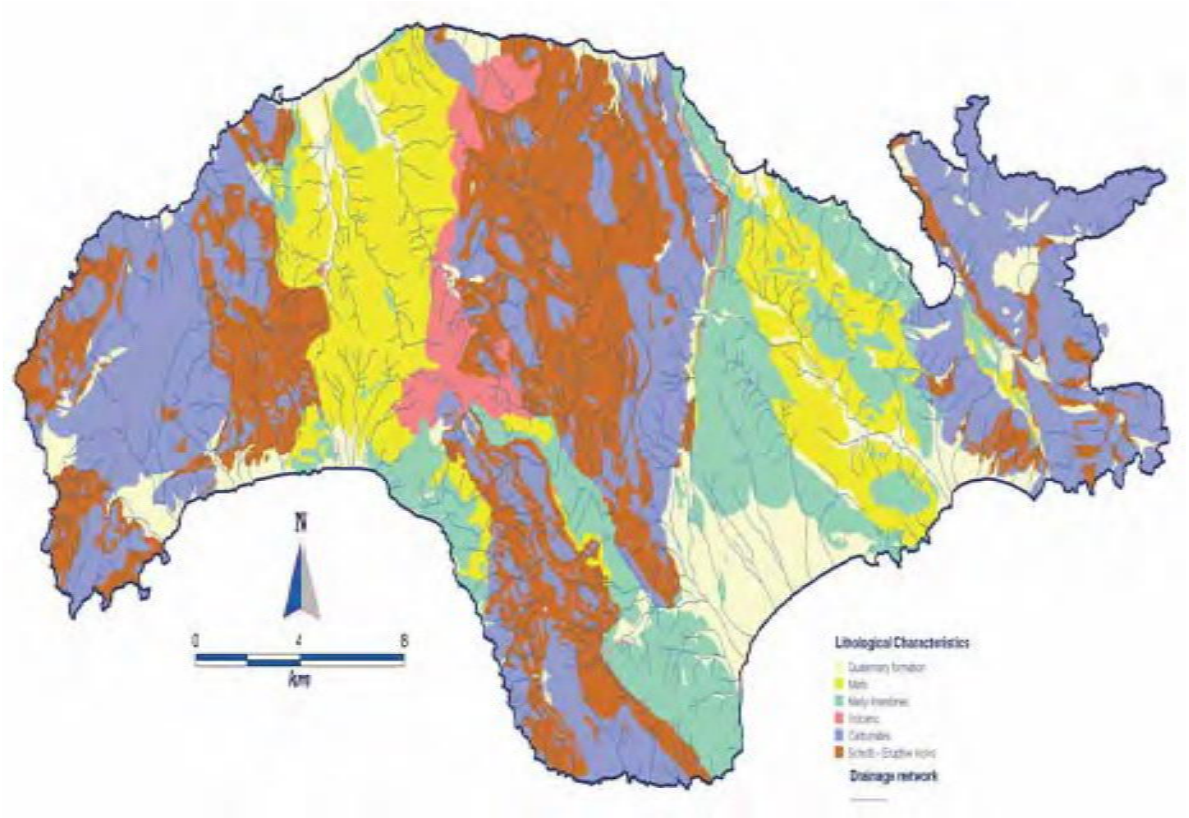


Figure 3.3: Lithology map Samos Island (After Vassilopoulos, 2008)

The low regions alluvial depositions are consisted of clayey silts, sands and gravel formations. They form in general shallow aquifers with variable permeability depending on the particle size distribution of the various layers. Lateral (mainly limestone) talus is generally composed of non-cohesive coarse materials (sand, gravels, shingle and locally blocks of stone) of small thickness and quite satisfactory permeability. The depositions from the torrential terraces are consisted of sand, gravel, breccias, clay and silt. They generally present a water table that depends on depth of the underlying impermeable formation. Calciferous formations (limestones, marbles, and dolomites) are characterised by intense karstic phenomena (Pe-piper et al., 1991; Kammas, 1998; Tziritis et al., 2008).

3.4 Land use of Samos

Samos has more forested regions, than it had the last two hundred years. The high reforestation speed permits the fast coverage of significant agricultural areas which have been abandoned by farmers. These areas are depending on the climate and flora of each region. Samos surface is 69.5% mountainous, 22% semi-mountainous and 8.5% flat (Stamatakos, 2010). According to Ministry of Agriculture and the Directorate of Forests of Samos this extent is distributed (Table 3.1 and Figure 3.4):

Table 3.1: Area distribution (Ministry of Agriculture and Directorate of forests of Samos)

Type	Regions(acres)	Percentage
Agricultural region, Meadows, Pasturage	206450	43.20%
Forests	136400	28.60%
Forestall bushy regions	88700	18.50%
Settlements, Roads	18150	3.80%
Arid, Rocky	14050	2.90%
Alpine areas	10150	2.10%
Lakes, Rivers	4300	0.90%

The main agricultural product of Samos is olives. At about 1,567,000 olive trees are regularly cultivated and according to Ministry of Agriculture and Samos Municipality. Trees occupy about 90,000 acres. The majority of olive trees (77%) are in the southern part, where the duration of dry period is extended.

The second agricultural products are vines, which are cultivated in 15,000 acres (Moschato variety). The wine of this variety made the island famous worldwide. Its cultivation is gathered in northern and central part where there is specific Moschato cultivation zone.

Citrus fruits flourish in low altitudes and in irrigated regions of low winds. Scattered trees exist abundantly and systematic cultivation exists in the southern part. All kinds of fruit-bearing trees like orange, lemon and clementine trees prosper in Samos, without systematic cultivation and the sporadic trees abound, mainly in northern part and in mountainous regions.

Systematic horticultural cultivations are located in northern part of island, in villages next to Karlovasi where flat, irrigated regions exist. Additionally, smaller areas are cultivated in the entire island. In the southern part, these cultivations present difficulties, because of winds and high temperatures. Few areas with cereals and legumes are cultivated, exclusively in the southern part (Papanikolaou, 1979). Additionally new ways of agriculture exist in south part of Island and focused on vegetable production. The ultimate agriculture with greenhouses has risen

up the last ten years. The main way of production is totally controlled systems of hydroponic cultivation and exist in the south east part of the island next to Pythagoreio.

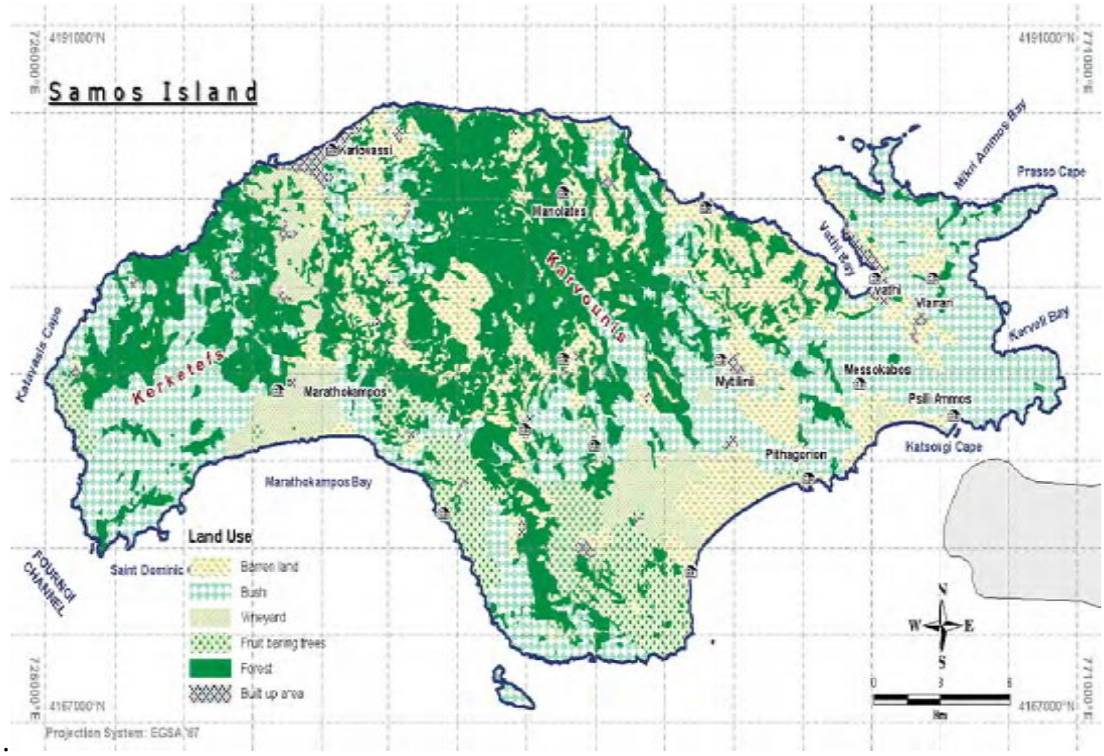


Figure 3.4: Samos land use map (After Vassilopoulos, 2008)

Pine forests in Samos cover 136,400 acres or 28.5% of island's surface, mainly mountainous areas in northern Samos. In southern part, these forests are few. *Pinus brutia* is the species that is met next to sea and in low high areas. In areas over 700m *Pinus nigra* is the domain species. This type of Pine is common in central and meridian Europe. Till the beginning of 20th century extensive forests of chestnut, hazelnut and oaks existed and disappeared by forest fires.

Scrub land is estimated in 88,700 acres. The area covered by various species of plants, whose height exceeds five metres. These regions exist everywhere around Samos. This type of vegetation covers usually rocky and barren surfaces, with main characteristics, the small, hard and usually thorny leaves covered by waxy substances, fat rind and deep radical system. The main species are *Quercus coccifera, Pistacia lendiscus, Olea oleaster, Ceratonia siligua, Juniperus sp., Pinus brutia, Spartium sp., Kalycotomus sp., Robus sp., Lonicera sp.* and many others (Vassilopoulos, 2008).

At higher altitudes, where the soil is decalcified, apart from previously plants and trees, which do not depend on calcium, *Arbotus sp., Myrtus, Pistacia terebinthus, Erica verticilata and*

E.arborea exist. These bio-systems regenerated fast after fires and in a few years the landscape returns to its primary conditions (Vassilopoulos, 2008).

The regeneration of the environment in the pines forest areas becomes with 2 ways. The first one is with burnt pines that spread new seeds and the second one is with new roots that should be planted in these areas. In rocky, gravel and barren dry grounds, thorny, usually brushwood semi bushes, like *Poterium spinosum, Satureia thymbra, Origanon sp., Cistus incanus, Sarcopoterium spinosum, Thimus capitatus, Genista acanthoclada, Salbia sp., Sideritis sp.* and many others species exist. These regions exist mainly in southern and western slopes of Kerketeas, where the soil has developed from gravels.

In local rivers and regions with springs or high territorial humidity, hydric trees grow up. There are *Platanus orientalis* and *Laurus nobilis* that are located in certain regions, such as the basin of Mitilinioi. *Salix cinerea* is met also in that area. The bushes that exist in these areas are *Nerium oleander, Vitex agnus-castus,* and many others, and from creepers, *Rubus sanctus, Hedera helix,* and *Smilax aspera* (Vassilopoulos, 2008).

Annual plants abound, with sovereign (*Graminae* and *Leguminae*), which due to the island's climate, develop too much. Their biological cycle begins with the start of autumn rains and finishes by the end of June.

In contrast to Aegean islands, the biggest part of Samos (23%) is occupied by forest pines, while one third of the island's surface is covered by bushes and olives trees. The half surface of island is cultivable (areas with olive trees, ranches, horticultural and vegetables).

3.5 Waste materials

The hypothesis that initial investigated in that research was to use waste materials that can easily found in the East Mediterranean area, and more specifically in Samos Island. The land use of Samos allows choosing from a wide variety of waste materials.

3.5.1 Wheat straw

Wheat straw is an abundant agricultural residue with low commercial value. Wheat (*Triticum aestivum L.*) is the world's most widely grown crop, cultivated in over 115 nations under a wide range of environmental conditions (Talebnia et al., 2010). Wheat straw, like any other biomass of lignocellulosic composition, is a complex mixture of cellulose, hemicellulose, lignin, and a

small amount of soluble substrates, which are known as extractives and ash. The overall chemical composition of wheat straw could slightly differ depending on wheat species of each area, the soil, and the climate conditions of the area. Cellulose, hemicellulose and lignin content of wheat straw are in the range of 33–40, 20–25, and 15–20 (%w/w), respectively (Prasad et al., 2007). Lignocellulosic waste materials obtained from energy crops, wood and agricultural residues represent the most abundant global source of renewable biomass (Lin and Tanaka, 2006). Wheat straw is an important substrate material that contains high levels of carbon and can force the system to denitrification process.

3.5.2 Hazelnut husk wastes

The hazelnut (Figure 3.5; also known as filbert or cob nut), is a species widespread over North America, Europe and Asia. European hazels generally, are larger plants, 3-4 m tall, with large thin shelled nuts (Erdogan et al., 2010).

Hazelnuts have not been commercially successful in the UK. This is largely due to a disease called eastern filbert blight, a fungus disease which invades the twigs and eventually kills the plant. The Turkish tree hazel however is resistant to eastern filbert blight. *Corylus colurna* (Turkish hazel) is a deciduous tree native to southeast Europe (Greece and Turkey) and southwest Asia. It is commonly found in Balkan countries (especially in Greece), Turkey and Iran. The history of hazelnuts in Turkey, and mainly in the Black Sea region started more than 700 years ago (Dede et al., 2012). Trees have intermediate size and they have European nut size. *Corylus colurna* is occasionally drought tolerant and alkaline soil tolerant. It prefers moist, well-drained soil and long sunny periods.

Turkish hazelnut production is 650,000 tonnes per year and produce approximately 78-80% of worldwide production, 35% of that production creates husk. Turkey is the first producer country and export 75% of the total world production of hazelnuts (FAO, 2012). The production countries of hazelnut worldwide are the following countries: Turkey (79%), Italy (11%), Spain (6.5%) and United States (2.5%) (Demir, 2014).

Figure 3.5: Hazelnut husk wastes

Anatolia is the source of hazelnut and the area of the most valuable wild species and of cultivated varieties (Köksal et al., 2006). The Black Sea Region has the appropriate climatic conditions for the cultivation of hazelnuts and it is the most important hazelnut production center. Hazelnut plantations spread in 20-30 miles lanes off the shores of Black Sea from the province of Duzce on the West through Turkey/Georgia border on the East. According to productivity levels, two zones can be distinguished: i) First Standard Production Area (Ordu, Giresun, Rize, Trabzon, and Artvin) and ii) Second Standard Production Area (Samsun, Sinop, Kastamonu, Bolu, Düzce, Sakarya, Zonguldak, and Kocaeli). The first area has a comparative advantage due to location, weather and soil quality (Yavuz et al., 2005, Demir and Beyhan, 2000, Çalışkan, 1995).

In general, hazelnut husk used to be considered as waste garbage. Hazelnut husk is an important chemical raw material. Hazelnut husks are consumed as combustibles in the Black Sea region (Boubaker et al., 2014).

Hazelnut husks for all the experiments were obtained from local farms in Ordu (Middle Black Sea Region) after harvesting. The husk was cleaned of non-husk impurities, washed and dried at 105° C for 3 hours.

3.5.3 Tea waste materials

Tea (*Camellia sinensis*) is one of the most spread beverages in the world. The global production in 2014 was 4 million tonnes. Due to the great production and consumption, large quantities of tea wastes are discarded (Nandal et al., 2014).

Tea (Figure 3.6) grown in the Balkan area, and more specifically in Greece and Turkey's East Black Sea region. After harvest time, tea manufacturing wastes include high value of carbon and nitrogen chemicals and an insufficient amount of phosphorus (Donmez et al., 2011).

Tea production in Turkey has been produced in 76,000 ha in Black Sea Region. With about 780,000 tonnes productions of tea is the sixth grade in agriculture products of Turkey, and it is one of the most important agricultural waste in Turkey (Topuz et al., 2014).

Figure 3.6: Tea waste materials

High quality tea is harvested the three top leaves of the shoot on tea plant in tea garden. While tea producer cut the top tea leaves with special tea shears, some overgrown woody shoots, which may include six-seven top leaves, mixed in tea harvest. During the tea production procedure, this woody overgrown shoots are not treated by tea factory and form tea factory waste.

In general tea manufacturing waste is a dry material (93% of total waste volume). The amount of tea mill waste depends on manufacturing techniques and physical properties of raw tea leaf. The waste amount varies from 7% to 15% of dried tea leaf, which is about 30,000 tons annually from state-owned companies. Tea mill waste is a reusable lignocellulosic material.

There are many tea factories in the Eastern Black Sea region and its produce about 30,000 tonnes of tea factory wastes. Tea factory wastes are not used for any purpose. They are deposit in depository area or occasionally discharge in small bays in Black Sea.

With such a great production and consumption large quantities of tea wastes are usually discarded into environment without any treatment (Cavdar et al., 2011). Like other biomass residues, tea wastes represent an unused resource and create a disposal problem (Arvanitoyannis and Varzakas, 2008, Ho et al., 2005).

After process of combustion, ash rate is just 2-5%. This ash is very rich of potassium for cultivation areas. Ash is very good potassium source for tea gardens and basic potassium value has a positive effect on soil pH value.

Currently tea wastes used as a cost-effective adsorbent to remove various types of contaminants from aqueous solution (Amarasinghe and Williams, 2007, Hameed, 2009, Weng et al., 2013, Ng et al., 2013; Akar et al., 2013). Since the tea leaves contains insoluble cell walls with some specific functional groups which are able to uptake the contaminants, thus the tea leaves can potentially use as pollutant scavengers from aqueous solutions. The functional groups which contribute in contaminant removal process may include carboxylate, aromatic carboxylate, phenolic hydroxyl, and oxyl groups of tea leaves (Wasewar et al.,2008, 2009).

These tea wastes could cause environmental problems during their degradation process and contaminate water environment by releasing organic matter. The cell wall of waste tea consists of cellulose, lignin, and carbohydrate which have hydroxyl groups in their structures (Aikpokpodian et al., 2010).Waste tea has been used to produce mushroom, organic fertilizer and particleboard, to resist biological resistance of wood, and in other applications.

Tea wastes used as an adsorbent for the removal of heavy metals (Cd, Pb, Ni) from industrial waste (Mahavi et al., 2005). About 94-100% removal of lead, 86% for Ni and 77% for Cd were achieved using tea waste. It is also mentioned that tea wastes are cheap materials for industrial waste water treatment plants (Ajmal et al., 1998).

Tea wastes have good potential also for arsenic removal (Shaikh et al., 2011). It was noticed that arsenic adsorption onto tea waste adsorbent is highly dependent on pH. The optimum arsenic removal was noted as 92.5% at pH=7 (Weng et al., 2014).

The tea factory wastes were obtained from the Caykur tea factory located in Cayeli Rize, the East Black Sea Region of Turkey.

3.5.4 Waste materials Samos

Samos is an island that is focused on agriculture. Due to the green background and the water resources that exist in the island the amount of cultivated area is increased year by year. According to the Ministry of Agriculture and Table (3.1) the agricultural activity and the forest dominate the surface of Samos in almost 90% of total surface. The cultivation of the area

except from the viniculture and the oil trees contains high percentages of wheat and barley for the local activities of the island.

Additionally there are wild plants of tea that appeared every year in the mountainous area of the island. The varieties are worldwide known and except from the rehearsal properties create huge amount of waste materials. *Sideritis* (also known as mountain tea) is a genus of flowering plants well known for their use as herbal medicine, commonly as an herbal tea. Additionally there are also plants of the main tea production, *Camellia sinensis*. There are more than 319 distinct species from which more than 10 are met in Samos Island.

Finally, in the forest area of Samos, except from the *Pine* variety of trees, there are also wild trees of Nut family (includes species of the genus *Corylus, Quercus Pistacia*). The waste materials that produced from those trees can be used as waste materials.

Finally the sources of waste materials in Samos are numerous and the amount that can provide is huge. So according to the design and the initial hypothesis, the research focus on the next 3 materials; wheat straws (Chapter 5), tea waste materials and hazelnut husk wastes (Chapter 7, 8, 9).

3.6 Water quality of Samos

The central and east part of Samos is the area where the majority of population lives. Additionally, the majority of plain and cultivated areas are there. Land use is dominated by agricultural activities such as cultivation of grapes and greenhouse vegetables. The main mountainous area in the middle of the island creates numerous small rivers around it. During winter period the amount of water resulting from mountain runoff is high. All villages around the island receive water for drinking and agricultural purposes from local springs. The increasing amount of cultivated areas and the increasing amount of tourists every year stress the water horizon causing problems in quality and quantity.

The research took place from May 2013 till August 2013. From 15 different points at the central east part of island water samples collected and analysed for the water quality. It is also investigated the relationship between water pollution and fertilizers and pesticides to cultivation areas.

The areas separated in three different categories: the springs (water directly from the groundwater basin of island), the cultivated areas (areas next to rivers with vegetables and fruits cultivation and next to total new hydroponic factories that use huge amount of water and discard

wastes in local rivers where other farmer use this water in their fields) and finally springs in small villages (transfer water from the spring with inhabitant pollution).

3.6.1 Survey in Samos Island

The sample points are visible to map below (Figure 3.7) and contain all important hydrological basins of island and the places that are more cultivated.

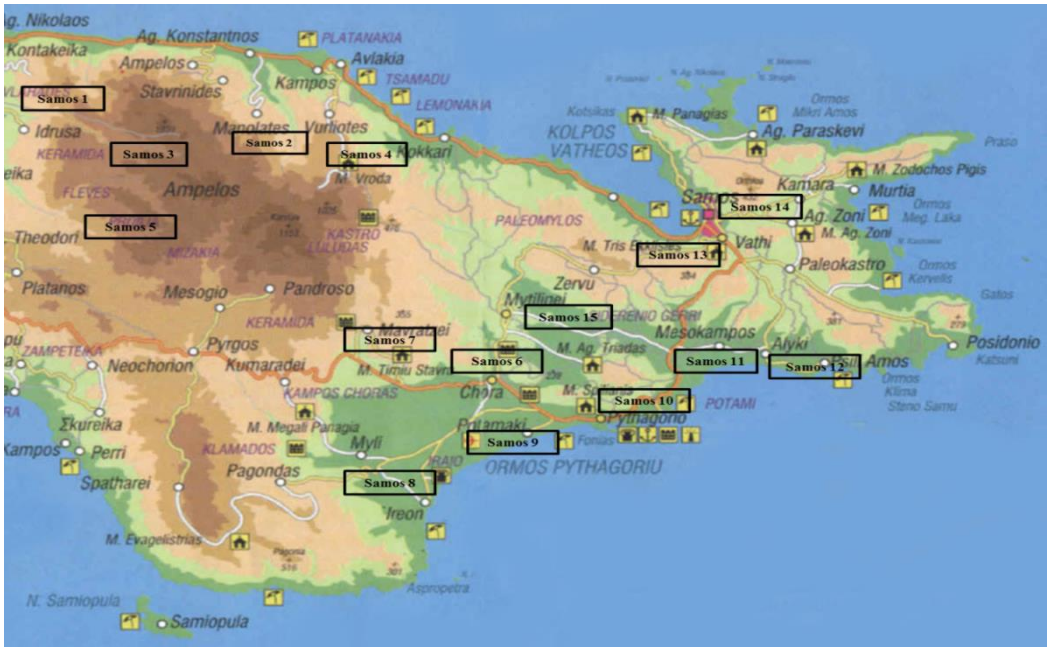


Figure 3.7: Survey map (Samos Municipality)

A huge number of water samples of 15 water points were collected from available boreholes, wells and springs, and local rivers covering an area of 100 km^2 (Figure 3.7). During sampling all necessary protections were taken in order to avoid any possible contamination. Samples were collected in polyethylene sterile bottles 50 ml and stored at (4^{o}C) till analysis.

The samples were analysed (initial measurements of pH and Nitrogen compounds, As measurements) at the research laboratory of Archipelagos Institute, at Vathy in Samos and the total analysis of water samples (IC chromatography) finished in Strathclyde University laboratories. The sample points where selected in combination with rivers and the activities that exist in the area. Samples received almost from all the main streams. Research focused on southeast part of island that more plain areas with agricultural activities exist there and the majority of population lives.

The most important problem that exists in the area is the recirculation of waste water from agricultural field, and from local industrial areas and houses. It is noticed that liquid wastes of companies are deposited in local streams without any protection or any treatment. Additionally from these streams, local farmers irrigate water for their fields. The whole ecosystem of the area is influenced by pollution.

Research focused on nitrogen and phosphate compounds of water samples but in the past it was noticed amount of arsenate in water distribution system. Arsenate analysis was completed in Vathy laboratory without finding any dangerous levels.

The 2013 cultivations were more intensive. The water quality of the area was downgraded and the levels of chemicals in water sources increased. In combination with fertilizers that are used in agricultural fields and the increasing number of hydroponic companies that contain a central system of waste disposal, water pollution is a concerning problem.

3.7 Results

There are measurements for almost all anions (Na^+, K^+, Mg^{2+}, Ca^{2+}) and cations (Cl^-, F^-). The focus of research was for nitrogen (NO_2^-, NO_3^-, NH_4^+, TN) and phosphorus (PO_4^-) compounds, that are connected with the use of fertilizers and it is an indicator about pollution in groundwater and the water of Samos.

3.7.1 Nitrogen compounds

Nitrogen compounds are the mains examination compounds that concern the research that took place in the island. Nitrogen compounds contain nitrate, nitrite and ammonium levels. All measured by IC chromatography and the measurement became in mg/l. To receive total amount of nitrogen everything was converted also in mmol/l. Nitrogen compounds were the indicator to show us the quality of water samples in combination with phosphate levels. The main problems were noticed in areas next to cultivation fields and areas that there is also intrusion of salt water. In Table 3.2 are also given in details nitrogen amounts.

Table 3.2: Details of nitrogen compounds in Samos

mg/l	Samos 1	Samos 2	Samos 3	Samos 4	Samos 5	Samos 6	Samos 7	Samos 8	Samos 9	Samos 10	Samos 11	Samos 12	Samos 13	Samos 14	Samos 15
NO2	0.00	0.02	0.03	0.00	0.32	0.01	0.00	0.00	0.00	1.80	1.97	3.20	0.05	1.74	0.90
NO3	1.09	1.53	1.31	3.99	3.53	27.51	3.33	31.39	83.05	46.62	24.03	106.87	43.93	3.05	52.25
NH4	2.77	2.93	3.10	2.11	10.92	3.20	3.11	2.93	191.83	3.61	5.65	294.50	5.71	5.55	4.83

mmol/l	Samos 1	Samos 2	Samos 3	Samos 4	Samos 5	Samos 6	Samos 7	Samos 8	Samos 9	Samos 10	Samos 11	Samos 12	Samos 13	Samos 14	Samos 15
NO2	0.0000	0.0004	0.0007	0.0000	0.0069	0.0002	0.0000	0.0000	0.0000	0.0391	0.0428	0.0695	0.0011	0.0378	0.0195
NO3	0.0176	0.0247	0.0211	0.0644	0.0570	0.4439	0.0537	0.5065	1.3400	0.7522	0.3877	1.7244	0.7088	0.0492	0.8431
NH4	0.1535	0.1624	0.1718	0.1170	0.6053	0.1774	0.1724	0.1624	10.6332	0.2001	0.3132	16.3242	0.3165	0.3076	0.2677
TN	0.1711	0.1875	0.1936	0.1813	0.6692	0.6215	0.2261	0.6689	11.9732	0.9914	0.7437	18.1180	1.0264	0.3946	1.1303

3.7.1.1 Nitrite levels

The nitrite levels are classified into three main categories. Firstly are the locations near to the main mountain of island, here nitrite levels were low. Secondly, locations next to cultivated areas those levels were higher than expected. A last category that is noticeable was the salt marsh that the water quality there was totally different than everywhere else due to the intrusion with sea water.

3.7.1.2 Nitrate levels

The extensive use of fertilizers and contamination from other sources (e.g. domestic wastes) it is noticeable in high nitrates levels. Nitrate contamination is an important issue on Samos. Nitrates affect groundwater quality and should be attributed exclusively to anthropogenic contamination, such as the extensive agricultural activities.

The research provides a very concerning result. The island again can be classified into 3 categories. The first category is the category that nitrate levels are lower than the limit that EU will obtain the following years and it is also the limit in USA (<10 mg/l). These samples received from springs that are mainly located in mountainous range of island and two more springs that are located in lower height in south central part of the island and the last one in the northeast part of island. The quality of water in these points is very good and these are the sources of drinking water.

The second category is the category that nitrate levels are higher than 25 mg/l till the limit of EU/WHO (50 mg/l). There are 6 samples in that range. The areas that these samples were obtained were next to areas with big population and in agricultural areas that are used the water for agricultural purposes. The areas next to populated places achieved the lower results between

24 and 32 mg/l NO_3^--N. The results show that there is a contamination of the system in those areas that are in south of island. There is connection between population, tourists and the water demand that is huge especially summer months. The places next to agricultural areas are cultivated and all hydroponics factories are there. Nitrate levels are higher and close to high limit (50 mg/l). It is visible that fertilizers that are used affect the hydrological basin of the area. That affects the water quality, but continuously vegetable and fruit production and finally in that chain people that live there. It is also visible that the samples that obtained from river basin next to hydroponic factories achieved peak levels a little bit higher than limit (52.3 mg/l). It is really concerning that there is not waste managing system in these factories and through away waste water directly to torrents next to their location.

The last category is the category that nitrate levels are much higher than limit (>> 50mg/l). These areas are at south central and southeast part of Samos. The area that is expected to achieve those results was salt marsh with the highest nitrate levels. The second area, is also next to the sea and it is concerning because is next to populated areas and close to airport. It is also a protection area for migratory birds that are coming from Asia to Europe and stop there.

3.7.1.3 Ammonium levels

Ammonium levels are classified into 3 categories again. Low levels of ammonium samples (< 4mg/l). The samples that had low ammonium levels, obtained from central part of island in mountain springs and field areas. The second category contains samples between 4-11 mg/l. In this category there is a spring from central mountainous place that the water is moving to North West part of island. The other samples received from east part and focused on cultivated field and hydroponics companies. Finally the last category is the category of nature protection zones. Ammonium levels are really high more than 150 mg/l.

3.7.2 Phosphate levels

Phosphate levels were noticeable at all sample points. This is an indicator along with nitrate levels for the agricultural pollution of groundwater. It is concerning that even from the springs samples from mountainous area phosphate levels were high. The island was separated in 4 categories depending on the amount of phosphate level. In Table 3.3 is visible in details phosphate levels.

Table 3.3: Phosphate levels in Samos

PO4	Samos 1	Samos 2	Samos 3	Samos 4	Samos 5	Samos 6	Samos 7	Samos 8	Samos 9	Samos 10	Samos 11	Samos 12	Samos 13	Samos 14	Samos 15
mg/l	17.20	25.67	16.51	12.73	16.73	17.44	26.23	24.67	163.92	42.06	43.10	206.25	17.07	26.08	28.81

The first category contains the points that phosphate levels were lower than 20 mg/l. These water samples are from springs on mountainous areas of central Samos and from a sample in north east area next to Vathy.

The second category is samples with phosphate concentration between 20-30 mg/l. These areas are cultivated with olive trees and vegetables. Phosphate levels are increasing in river basins and in springs and that areas are next to populated areas. Phosphate levels increased where the cultivations are transformed from olive trees to vegetables and fruits.

The next category is the category between 30-50 mg/l. In that category there are two samples received from water basins next to agriculture fields and next to area with hydroponic companies. This is very concerning and need high attention to that area because it not only the water that used in that fields but also it is concerning because that effects finish though the food chain in human. A great attention to those areas and especially to hydroponics factories must be received. The problem as it is noticed in nitrate levels is the recirculation of liquid solutions in those factories.

In last category are places next to sea and they have a protection role in the area for birds. In those areas phosphate levels are really high (>150 mg/l). These results expected but it is also a notification for areas next to them. Next to that areas also there are cultivated areas so protection and a normalized use of fertilizers must be done. The high phosphate levels result in the eutrophication of the area and the correct environmental management is proposed.

3.7.3 Anions and cations

Except from nitrogen species and phosphate levels, with IC chromatography measured more anions and cations that are also connected with water quality. The detailed results are given in Table 3.4. There are measurements of chloride, sodium, calcium, magnesium, fluoride and potassium levels.

Table 3.4: Anions and cations in Samos

mg/l	Cl^-	Na^+	Ca^{2+}	Mg^{2+}	F^-	K^+
SAM 1	68.49	6.63	78.06	8.15	1.03	0.48
SAM 2	73.95	7.60	62.23	19.69	0.86	0.65
SAM 3	85.86	8.38	95.65	28.06	0.59	0.43
SAM 4	82.83	3.29	120.47	10.85	0.41	0.20
SAM 5	91.62	13.64	101.04	57.88	3.61	1.32
SAM 6	101.58	9.04	74.21	31.09	3.12	1.06
SAM 7	107.16	8.60	80.42	32.79	4.09	1.50
SAM 8	157.09	8.04	104.60	35.38	1.55	0.62
SAM 9	606.45	370.17	461.56	41.89	3.75	38.93
SAM 10	142.01	30.04	121.44	20.41	2.63	1.65
SAM 11	216.56	34.62	70.66	15.95	2.39	2.34
SAM 12	669.96	614.51	601.10	78.49	8.49	31.79
SAM 13	77.01	10.04	103.56	49.55	1.50	1.80
SAM 14	98.84	33.90	63.60	29.67	5.16	1.89
SAM 15	114.29	29.47	129.94	20.22	2.62	3.13

All measurements became simultaneously with nitrogen compounds and phosphate levels measurements.

In detailed, chloride levels are an indicator for the quality of water. The limits for drinking water are 250 mg/l (WHO). The samples from mountainous areas of and from springs received the lowest chloride levels and the highest more than acceptable limits received in protection zone samples. This was also the result of intrusion of sea water in water basins of those areas.

Sodium levels are also important for water quality and pollution of the area. The highest acceptable limits for drinking water are 200 mg/l (WHO). Sodium levels in the majority of samples were less than 10 mg/l. At the protection zones, sodium levels were much higher, (> 200 mg/l).

Magnesium is another indicator. There is no limit for drinking water but as an indicator for health it should be lower than 125 mg/l (WHO). Half of the samples have magnesium levels less than 25 mg/l. These areas are mainly areas with cultivations and springs. Samples with the highest levels but again in acceptable limits were protection zones samples.

Fluoride measurements were separated in 2 categories. The first one with lower than 1.5 mg/l fluoride (EU limit). In this category only 6 samples were lower than this limit. The second category was samples higher than 1.5 mg/l and the peak level is in salt marsh with 8.49 mg/l.

Potassium levels were really low, in 13 samples lower than 3.2 mg/l and in protected areas that levels increased to 31-39 mg/l. There is no higher limit for potassium levels (WHO).

Finally calcium levels are the last measurement that received. The acceptable levels for drinking purposes were 200 mg/l. All samples received lower levels except from protective zone samples. The lowest levels received in spring samples.

3.8 Discussion

Along the east coast line areas are plain and there are many vegetables and fruits fields that are used to deliver the main production for the whole island. The last years, there is a turn of the farmers to compact agricultural production of vegetables with hydroponics factories that discard their wastes (liquids/fertilizers/feed solution) to local river basins without any treatment. This increases the pollution levels in water quality and causes eutrophication. Fertilizers are used that contain N and P compounds and extend the existing problem. The problem of eutrophication if protection measurement will not receive will be visible in the near future. The downgraded of water quality is expected in combination with the stress of water sources.

3.8.1 Samos problem with Total Nitrogen

Results of the research that took place in summer of 2013 and the water analysis of the groundwater of the area show there are some opportunities to explore solutions to increase the water quality. According to the analysis that took place in Archipelagos Institute and in Strathclyde University there are five areas where total nitrogen levels are really high and actions are implemented to eliminate the problem. The highest levels were in areas that are cultivated and the amount of pesticides and fertilizers that used is really high. In those areas also there are heavy industries and hydroponic factories. According to Samos municipality (2011), the total population is 33,000 people for winter months and during the touristic period this population can be increased to 100,000 people. The increase in demand stresses the natural sources especially during the summer months. The areas at high risk of nitrogen pollution are in south east part of Samos and more specifically in Pythagoreio. That is the most touristic area with 8,000 people. The most pressing issues are located next to airport where is a plain area full of cultivation of olive trees, lemon and orange trees and vegetables that is the source area for fresh products.

The water impact of island is enough to use it for drinking purposes in present but is definitely in the near future, the lack of water will become more evident. The pollution affects the water quality and it is important to focus on the problem that the water resources face and provide a suitable solution in for the area based on the substrate materials that the same area produces.

CHAPTER 4

METHODS

4.1 Introduction

This chapter describes the methods used to analyse all samples: porosity; HRT; pH; redox potential, dissolved oxygen (DO), microplate-spectrophotometric methods for NO_2^-, PO_4^-, NH_4^+, NO_3^-, total organic carbon (TOC), ion chromatography (IC), and quantitative PCR (q-PCR). The analytical procedures for various water and wastewater parameters are according to the Standard Methods (1998).

pH, conductivity and oxidation reduction potential were measured using a multi meter (Mettler Toledo-Seven Multi meter). Dissolved oxygen (DO) was determined by a DO probe with a DO meter (Hanna HI9145), following Section 4500-O G: Membrane Electrode Method in the Standard Methods (1998). Anions and cations measured with ion chromatography and with spectrophotometer. There is also the description of calibration (Appendix I) of all these methods and the uncertainty of analysis and of every calibration. Finally, according to the calibration of the methods there is a question about the accuracy of methods and how the confidence in results.

4.2 Porosity and Hydraulic Retention Time (HRT)

The preparation for measurement of porosity was following the procedure:

1. Set up a cylindrical column, securing it to the stand with a clamp.
2. Add the substrate material to the column, making sure the stopcock is closed before the process.
3. Determine V_T. This is the total volume of the cylindrical column. The total volume is the area that contains a cycle multiply by height.
4. Fill the graduated cylinder with water. Weigh the water-filled container on the scale and mark down this mass.
5. Slowly add water into the column containing the substrate materials. Continue to pour water into the column until the substrate materials are filled with water.
6. Use the scale to weigh the water container again with the remaining amount of water and record this mass.

7. Determine V_v. This is the volume of water added to the sediment. To calculate this value, subtract the final mass of the water container from the beginning mass.

Divide the value that is calculated for V_v by the value that is calculated for V_T. Multiply this resulting number by 100 and express the porosity value as a percentage. (Porosity= Volume of void space/ Total volume of the solid).

The Hydraulic retention time (HRT) is a measure of the average length of time that a soluble compound remains in a constructed bioreactor. Hydraulic retention time is the volume of the storage unit divided by the influent flowrate. HRT for each column was obtained by dividing the pore volume with the flow rate at the time of measurement. Hydraulic Retention Time (HRT) is the amount of time in time units (hours/days) for solution to pass through a storage unit.

4.3 pH

pH is an important variable in the assessment of water quality as it affects many biological and chemical processes that occur in water and its treatment. pH is a first indicator of the environmental conditions (Rivett et al., 2008). Environmental conditions of experiment are not stable, and immediate changes in water upon contact with substrates are visible in direct and immediate measurement of pH (Gray, 2009). The reactions are affected with substrate materials and microbial activity. pH is a measure of relative amount of free hydrogen and hydroxyl ions in the water.

When water dissociates it yields a hydrogen ion and a hydroxide.

$$H_2O \overset{\leftrightarrow}{Kw} H^+ + OH^- \qquad \text{(Equation 4.1)}$$

Water that has more free hydrogen ions is acidic, whereas water that has more free hydroxyl ions is basic.

To be precise in equilibrium calculations instead of using concentrations ions activities are used. Activities are not a theoretical construct and can be measured for every solution. In pH, there is a measurement of the activity H^+ ions and not the concentration $[H^+]$.

Activities for hydrogen-ion in a given solution can be determined through simple pH measurements, and the activity coefficient (γ) can be evaluated using the relationship where C is concentration.

$a = \gamma C.$ (Equation 4.2)

Activities can be defined in terms of molar concentrations (M) or molal concentrations (m)

When calculating pH, where [ ] refers to molarity, M.

$K_w = [H^+][OH^-] = 1 * 10^{-14}$ at 25°C (Equation 4.3)

Due to this property pH can be defined using the following equation,

$pH = -log_{10}[H^+]$ (Equation 4.4)

The range of pH is from 0-14, T=25 ºC, with 7 being neutral (pure water $[H^+] = [OH^-] = 1x10^{-7}$). pH of less than 7 indicate acid solution (Acidic Solution: $[H^+] > 1x10^{-7}$), whereas a pH greater than 7 indicates a basic solution (Basic Solution: $[H^+] < 1x10^{-7}$).

pH measurements are a routine test carried out on environmental water samples along with temperature, conductivity and major anion and cation monitoring. This allows a full chemical picture to be complied of the water source. (Appelo and Postman, 2005)

pH measurements are obtained using a pH probe (Intab Redox-Pro pH) attached to a meter such as a Mettler Toledo pH and conductivity meter Figure 4.1.

Figure 4.1: Mettler Toledo pH, conductivity and redox potential meter (www.globalspec.com)

To obtain a pH value, the probe is dipped in a buffer solution (pH=4), a buffer solution (pH=7) and buffer solution (pH=10) to ensure that the probe is correctly calibrated. Once probe is

calibrated, it is dipped into the aqueous sample, allowed to equilibrate, and the pH value obtained is recorded. The probe should be rinsed with nano-pure water provided by Barnstead™ Nanopure™ by Thermo Scientific between each sample recording to avoid measurement contamination from samples. The nano-pure water has 18.2 megohm ionic purity with no bacteria.

Nano-pure water is deionized water that is cleared with different way. The ideal system for critical applications requiring 18.2 megohm water with less than 2 ppb Total Organic Carbon. The water produced is perfect for applications requiring low organics including High Performance Liquid Chromatography (HPLC), Ion Chromatography (IC) and TOC Determinations. A dual wavelength quartz UV lamp (185 and 254mm) oxidizes organics down to virtually undetectable levels and also maintains minimal bacterial levels.

All samples were run in triplicate (n=3). All regression analysis became with linear regression. At all measurements error bars (10%) used and analysed only the price that where on the limits. The LOD in pH calibration was LOD=0.0044 and LOQ=0.0135.The linear regression approach used also in pH analysis. All the details for calibration are given in Appendix I.

4.4 Oxidation Reduction Potential (ORP-Redox)

Oxidation-Reduction or Redox potential measurement is another indicator of water quality. It measures the tendency of a chemical species to acquire electrons. The redox potential is a measure of the affinity of a substance for electrons compared with hydrogen The processes of oxidation and reduction involve transfer of electrons. Specifically, oxidation involves the loss of electrons, while reduction takes in electrons. These two processes performed simultaneously as coupled half reactions. The redox reactions are primary feature of the electron transfer between the reactants, and of determines the mobility and reactivity behaviour of redox-reactive chemical components in water.

ORP levels are expected to change in experiments due to the different environmental conditions used in columns and different substrate materials. Due to different influent solutions tap water and groundwater used in experiments are expected to change redox overtime. According to other research (Saeed and Sun, 2011) the redox potential is expected to reduce due to the microbial activity that created.

ORP is measured in millivolts (mV). It is not a measurement of concentration directly, but a measurement of activity levels.

Redox potential is a good indicator that shows the oxidising conditions that exists in an aquifer (Walton, 1981). Redox reaction in groundwater are driven by the oxygen content of recharge water, the distribution and reactivity of organic matter, the redox buffers in an aquifer and the recirculation of groundwater (Drever, 1997). Redox potential can give an indication of possible chemical reactions. Table 4.1 presents various redox reactions in water.

Table 4.1: Approximate Eh Values, ORP reactions in water (pH=7, T=25 ^{o}C; Drever, 1997)

Redox Reaction	Eh (mV)
$O_2 \rightarrow H_2O$	+800
$NO_3^- \rightarrow N_2$	+650
$NO_3^- \rightarrow NH_4^+$	+350
$SO_4^{-2} \rightarrow H_2S$	-200
$CO_2 \rightarrow CH_4$	-250

Oxidation-Reduction Potential measures an aqueous systems capacity to either release or accept electrons from chemical reactions. The measurement of ORP became with Mettler Toledo-Seven Multi-meter. To obtain an ORP value, the probe is dipped in a buffer solution of ORP 240 mV and a buffer solution ORP 470 mV to ensure the probe is correctly calibrated. ORP standard solutions allow testing the precision of ORP electrodes. Once the probe is calibrated, it is dipped into the aqueous sample, allowed to equilibrate and the ORP value obtained is recorded. The probe should be rinsed with nano-pure water between each sample recording to ensure that the value recorded is correct. It should be noted there were on-going problems with this equipment throughout the experimental period. The problems were transcended with the replacement of the probe with one that was similar with the same characteristics and the results were analysed and triplicated before further analysis. In the redox measurement the problem with the probe was faced by replacing the probe that already exist with another one with similar characteristics. The problems also encountered with calibrations any time that there was a problem to ensure that the measurement were accurate at all time. The calibration became with ANOVA statistical analysis package. All the results were accurate and the problems did not affect the examination of the denitrification process.

4.5 Dissolved Oxygen (DO)

DO is another indicator measured. The levels of oxygen are crucial for experiments, microorganisms and denitrification process. The dissolved oxygen in natural waters varies in relation to temperature, salinity, turbidity, photosynthetic activity of algae and plants and air pressure. The solubility of oxygen is reduced when temperature and salinity increases. DO values can characterize the redox system that exists, the possibility of contamination in aquifer and also provide information if there is any recent recharge in the area (Freeze and Cherry, 1979). DO levels can be different with wide range. In a well-aerated stream depending on temperature and salinity the range can be between 8-10 mg/l. In contrast, anaerobic groundwater samples these DO levels are zero (Hounslow, 1995).

There is a connection/reduction between ORP potential with DO and denitrification rates due to the energy requirements within columns. DO levels affect almost all chemical and biological processes within the water.

The concentration of DO is usually expressed in milligrams of oxygen per litre of water (mg O_2/L) or parts per million (ppm). Some meters compare calculated oxygen content with observed concentration and report percentage saturation (% sat) (USGS). It should be noted there were on-going problems with this equipment throughout the experimental period. The problem were periodically, with the calibration of the DO instrument, but all the time were outrun with the correct calibration solution, with triplicate repetition of the samples to minimize the reliability problems. In DO measurement, the problems that exist was again with the calibration of the probe, it was something that at all the measurements was noticed but it was faced with correct calibration and analysis of all the samples. The calibration was not only used before the analysis of water samples but also during the measurements when it was noticed a result that was not expected. All the measurement became in triplicate and statistical analysis became with ANOVA statistic package.

All the measurement for DO became with DO meter Hanna HI 9142 (Figure 4.2).The calibration of DO meter became with zero oxygen calibration solution (Sodium sulphite).The DO is connected with temperature and the salinity of the solution.

Figure 4.2: DO meter (http://hannainst.com)

4.6 Electrical Conductivity (EC)

Electrical Conductivity is a measure of the ability of water to pass an electrical current. Conductivity measurements are directly affected by temperature. While the electrical conductivity is a good indicator of the total salinity, it still does not provide any information about ion composition in water. Significant changes in conductivity could be an indicator that a discharge or source of pollution has entered a stream. The conductivity is increasing when the water temperature is increasing. For this reason, conductivity is reported as conductivity at 25 degrees Celsius (25 ^{o}C). The electrical conductivity of water increases by 2-3% for an increase of 1 degree Celsius of water temperature. The SI unit of conductivity is S/m and more often μS/cm and it is reported to 25 °C.

It is sensitive to variations of soluble solids, primarily minerals. The extent to these ions are separated, the electrical charge on each ion, the mobility of the ion and solution temperature all have influence on the conductivity. Conductivity is a measurement of water quality that reflects the total dissolved solids (TDS) in a water sample. Conductivity is useful in groundwater because shows the levels of mineralization that groundwater has undergone and can become an indicator of residence time. Typical electrical conductivity values for different natural water are visible in Table 4.2.

Table 4.2: Typical conductivity ranges for different waters, T=25 ºC (Kiely, 1997)

Water	Conductivity range (μS/cm)	Water	Conductivity range (μS/cm)
Distilled	0.1-4	River Water	100-1,000
Rainwater	20-100	Groundwater	200-1,500
Soft Water	40-150	Estuarine	200-2,000
Hard Water	200-500	Seawater	<40,000

The conductivity of most fresh water ranges from 10-1000 μS/cm, but can be exceed 1000 μS/cm, especially in impacted waters.

Conductivity is measured with a probe and a meter (Hanna, HI 9142). Voltage is applied between two electrodes in a probe immersed in the sample water. The drop in voltage caused by the resistance of water is used to calculate conductivity per centimeter. The meter converts the probe measurement to micromhos per centimetre and displays the result for the user (APHA, 1992). All regression analysis became with linear regression. At all measurements error bars (10%) used and analysed only the price that where on the limits. The LOD in conductivity calibration was LOD=42.082 and LOQ=127.523.The linear regression approach used also in conductivity analysis. All the details for calibration are given in Appendix I.

4.7 Nitrogen compounds

Nitrogen is an important pollution issue in groundwater. There are many water quality issues for nitrate and phosphate compounds, which are the main nutrients that are connected with eutrophication. In uncontaminated groundwater nitrate concentration is typically less than 5 mg/l. Nitrate (NO_3-N) can become an indicator of contamination by fertilizers and waste organic matter (Tchombanoglou and Schroeder, 1985, Kiely, 1997).

During nitrate reduction, denitrification bacteria use nitrate ions as elector acceptor and oxidise organic carbon to CO_2 (Houslow, 1995). If large amounts of NO_2^- and NH_4^+ are present nitrate levels may be reduced. Also the high ammonia levels are possible to have agricultural sources like animal excrement, sewage or ammoniacal fertilizers that are commonly used (Rivett et al., 2008).

$$5C_{organic} + 4NO_3^- + 4H^+ = 2N_2 + 5CO_2 + 2H_2O \qquad \text{(Equation 4.5)}$$

Inorganic nitrogen in different forms was measured regularly. The analytical procedures of NH_4^+-N, NO_2^--N, and NO_3^--N measurement followed Section 4500-NH_3 F: Ammonia phenate

method, Section 4500-NO_2-B: Colorimetric Method, and Section 4500-NO_3-B: Ultraviolet Spectrophotometric Screening Method in Standard Methods (APHA, 1999), respectively.

The concentrations of both NO_2^--N and NO_3^--N were determined by the Thermo Scientific UV-Vis Helios Zeta Spectrophotometer (Figure 4.3).

The UV-Vis spectrum shows the absorbance of one or more sample component in the cuvette when it is scan through various wavelengths in the UV/Vis region of the electromagnetic spectrum.

In UV-Vis, a beam with a wavelength varying between 180 and 1100 nm passes through a solution in a cuvette. The sample in the cuvette absorbs this UV or visible radiation. The amount of light that is absorbed by the solution depends on the concentration, the path length of the light through the cuvette and how well the analyte the light absorbs at a certain wavelength. The transmittance I/I0 is an indication of the concentration of the analyte in the sample. I/I0 is defined as the transmittance (or transmission) T. If there is no absorption of the light passing through the solution, the transmittance is 100%.The amount of absorbed light is the absorbance, defined as:

$A = - \log_{10} T = -\log_{10} (I/I0)$ (Equation 4.6)

$1/T = 10(A)$ (Equation 4.7)

The relation of absorbance to concentration is given by Lambert-Beer's law (Beer's law):

$A = \varepsilon l c$ (Equation 4.8)

(where A is absorbance (unitless), ε is molar absorption coefficient (or molar absorption constant) of the analyte for a certain wavelength ($l \cdot mol^{-1} \cdot cm^{-1}$), l is path length (cm) through your cuvette and c is the concentration of the analyte ($mol \cdot l^{-1}$)).

By measuring and comparing a series of standard solutions of the analyte, the concentration of the analyte in the sample can be determined. The most important condition for an accurate measurement is: the concentration of analyte in the sample has to be in between the highest and lowest concentration of a series of standard solutions.

Figure 4.3: Thermo Scientific UV-Vis Helios Zeta Spectrophotometer (http://www.thomassci.com)

4.7.1 Total nitrogen (TN)

The method for TN involved the alkaline-persulfate oxidation of inorganic and organic nitrogenous compounds to nitrate (APHA, 1999). Concentrations of nitrate were screened spectrophotometrically with two wavelengths. The 220-nm run wavelength measured nitrate concentrations, while the 275-nm wavelength corrected for any organic carbon interference

4.7.2 Summary

The measurement by UV/Vis spectrophotometer described in details in Appendix I. In the Table 4.3 there are the limits of detection (LOD) of all UV/Vis compounds that analysed by this method. All measurement replicated 3 times and all regression analysis became with linear regression. At all measurements error bars (10%) used and analysed only the price that where on the limits.

Table 4.3: LOD and LOQ for UV/Vis in spectrophotometer

	Limits of detection (LOD)	**Limit of quantification (LOQ)**
NO_2^--N	0.0625	0.1894
NO_3^--N	0.1126	0.3415
NH_4^+-N	0.9506	2.8807
PO_4-P	1.0155	3.0773

4.8 Phosphate compounds

Phosphorus is an essential nutrient for living organisms and it is often the limiting factor for growth of algae and primary productivity in surface water (APHA, 1999).

In natural waters and wastes, phosphorus exists mainly as dissolved orthophosphates and polyphosphates and organic phosphates bound salts. It can be observed continuously changing forms due to decomposition and composition. Phosphorus is rarely found in high concentrations in freshwater as it is absorbed by plants. As a result, it is possible to observe significant seasonal variations of concentrations in surface waters.

It is important to measure phosphate levels along with nitrate levels because there are associated with eutrophication. It is expected to receive wide range of phosphate results, especially in substrate materials that contain high organic levels like waste tea materials and hazelnut husk wastes. It is important to minimize not only nitrogen compounds but also phosphate compounds.

The phosphorus concentration (PO_4-P) in water was analysed by (APHA), Standard Method section 4500 PE–Ascorbic Acid Method, (Standard Methods, 1998).

4.8.1 Total phosphorus

The method involves the acidic persulfate oxidation of particulate and dissolved organophosphate to inorganic orthophosphate. The phosphate then reacts with molybdate and potassium antimonyl tartrate, producing a phosphomolybdic acid. The ascorbic acid reduces the heteropoly acid to form a blue colour which is proportional to phosphate concentrations (APHA, 1999).

4.9 Total Organic Carbon (TOC)

Total Organic Carbon (TOC) is an indirect measure of total organic molecules present in water and is typically measured in parts per million or milligram per litre (ppm or mg/L) as carbon.

The measurement of TOC involves subtracting the measured inorganic carbon (InC) from the measured total carbon (TC), which is the sum of organic carbon and inorganic carbon: TOC = TC – InC.

Every sample contains dissolved carbon species that are measured with TOC. TOC levels can be increased from both anthropogenic sources like landfill leachate and agricultural runoff and natural sources that is the decomposition of organic matter that produce fulvic acid. This is an important quantity to monitor (Kebbekus and Mintra, 1998).

4.9.1 TOC analysis

TOC was measured by a TOC analyser (Teledyne Tekmar Dohrmann Series Apollo 9000) using the high-temperature combustion method (Figure 3.3).The basic instrumentation of a TOC is provided in Figure 4.4.

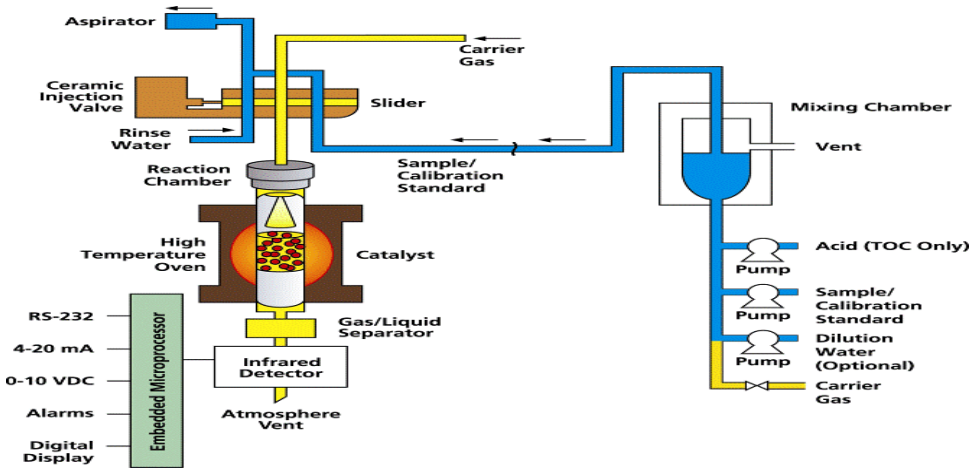


Figure 4.4: TOC basic components (www.instrument.org)

Figure 4.4 shows the basic set up of TOC analyser. The instrumental procedure is the following. The sample firstly treated by phosphoric acid to remove any carbon dioxide (CO_2) gas that may be present in the sample vial from atmosphere. Sample is oxidizing through combustion. Finally, CO_2 detected and measured using a non-dispersive Infra-Red (IR) detector. Organic molecules can be oxidized using heat, oxygen, ultraviolet irradiation, chemical oxidants, or combinations of these (Kebbekus and Mintra 1998).

The supplied stock TOC solution (SIGMA-ALDRICH) of 2500mg/l was diluted in nano pure water to create working solutions with concentrations 1 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 50mg/L, 100 mg/L, 200mg/L and 400mg/L.

The working solutions were determined by TOC analysis. Each working solution was injected in triplicate (n=3) and the peaks areas were recorded. The average areas were plotted against the

working solution concentration to form a calibration graph. The stock solutions were stored at the fridge 4^{o}C in the absence of light.

Figure 4.5: TOC analyser (Teledyne Tekmar Dohrmann Series Apollo 9000)

Samples were analysed using a liquid TOC analyser, Teledyne Tekmar Dohrmann Series Apollo 9000 (Figure 4.5). 20 ml of sample was pipetted into every TOC vial. The vials were then capped.

The samples then were run in the following sequence, two blanks (nano pure water) followed by standard solutions vials (1-400 mg/L), to check the calibration was still valid. After that followed by two blanks, followed by five groundwater samples, followed by a blank and five groundwater samples sequence.

All samples were run in triplicate (n=3) and the average peak area recorded and used to calculated the concentration of TOC present in the sample. The concentration of carbon in the samples was calculated using the calibration graphs that it was created. All measurement replicated 3 times and all regression analysis became with linear regression. At all measurements error bars (10%) used and analysed only the price that where on the limits. The LOD in TOC calibration was LOD=42.441 and LOQ=128.6114.The linear regression approach used also in TOC calibration and TOC analysis. All the details for calibration are given in Appendix I.

4.10 Ion Chromatography

4.10.1 Introduction

The method was chosen due to amount of details that received from analysis. The amount of results that can be measured in the same time for anions and cations was also an advantage to choose that method. Additionally it was the cheapest and fastest method for all that results.

4.10.2 Theory of ion chromatography

An aqueous sample is introduced into a stream of an ionic eluent (the mobile phase), which is pumped through a separation column packed with an ion exchange resin, (the stationary phase).

The stationary phase must have an affinity for the ions being resolved. The stationary phases usually consist of small beads which provide a large surface area for ion exchange and are very robust, stable over a large pH range, and efficient in order to yield the best results (Smith,1988, Jakson et al., 2002).

At the mobile phase the eluents that are used for anion exchange chromatography are aqueous solutions which contain anions most commonly a carbonate or bicarbonate solution. For cation resolution the eluents used are usually acidic and commonly are a 5mM solution of hydrochloric acid (HCl) or 2.5mM nitric acid (HNO_3) plus 2HCl plus 2.5mM *m*-phenylenediamine solution (Smith, 1988, Lopez Ruiz, 2000).

The process that takes place from stationary phase until reachs equilibrium is the following:

$$Resin - N^+R_3HCO_3^- + Anion^- \rightarrow Resin - N^+R_3Anion^- + HCO_3^- \qquad \text{(Equation 4.9)}$$

The anions in the sample will compete with the anions in the mobile phase for the active sites on the resin. There is between the sample and the stationary phase for a specific period of time. Continuously the ions transferred at the end of the column by the eluent ions before interacting with the column again at a different active site on the resin. This is a continuous process during the ions move at the stationary phase (Colenutt and Trenchard, 1985, Seki, 1980).

The rate of migration down the stationary phase varies for different anions depending on their affinity to the stationary phase. The selectivity coefficient is the measure of how efficient an ion is as an eluent according to its affinity to the stationary phase. The selectivity coefficient can be defined as:

$$K^{Cl-HCO_3^-} = \frac{[Resin-N^+R_3HCO_3^-][Cl^-]}{[Resin-N^+R_3HCO_3^-][HCO_3^-]}$$ (Equation 4.10)

Anions with high selectivity coefficients have strong interactions with the stationary phase and are held for a longer period of time before being eluted from the column in relation to the chloride anion. Generally polyvalent anions have a weaker interaction with the stationary phase than monovalent species and as they are larger ions, they move through the resin slower and so are eluted last. For cation analysis a cation exchange resin is used. In this process the cations in the sample compete with cations in the eluent for the active sites on the resin as shown in the equation below

$$Resin - SO_3 - H^+ + Cation^+ \rightarrow Resin - SO_3 - Cation^+ + H^+$$ (Equation 4.11)

The retention time of each individual ionic species, is used for identification purposes. The weaker the interaction between the analyte species and the stationary phase the shorter the retention time will be and the earlier the ion will be eluted (Colenutt and Trenchard, 1985).

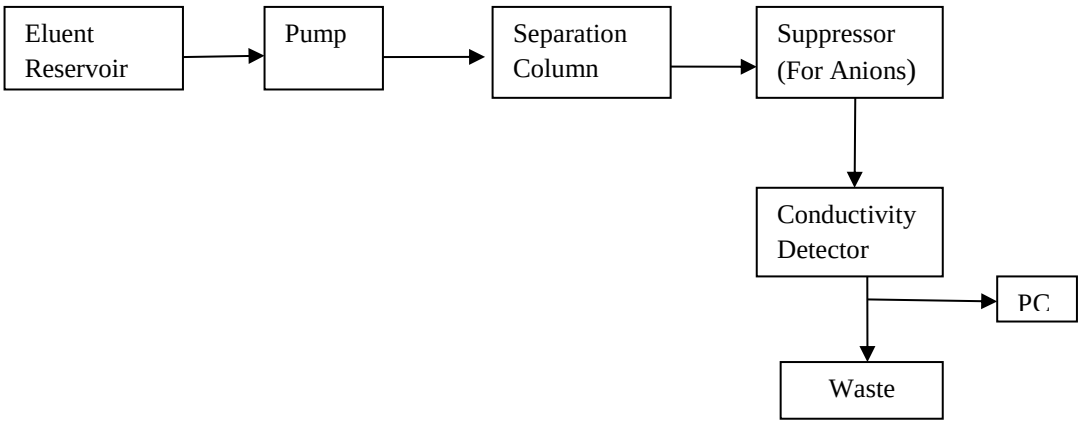


Figure 4.6: Schematic diagram of Ion Chromatograph components

Figure 4.6 shows the basic components of an Ion Chromatograph (IC). The basic components of an IC are the following: a solvent delivery system, sample injection valve, ion exchange chromatography columns, a conductivity detector and a computer for recording results. There are different detectors that are used for different separations.

A suppressed conductivity detector is used for anion detection and an unsuppressed conductivity detector is used for cation detection (Radojevic and Bashkin, 1999).

A conductivity detector is generally used for ion analysis because ions are excellent conductors. There are two methodologies of electrical conductivity detection. Suppressed conductivity method removes counter ions of the eluent after separation, thus reduces background noise. The other methodologies, non-suppressed conductivity detection, uses a low-conductivity eluent instead.

In High Performance Liquid Chromatography (HPLC) equipment which IC is part of them, all the tubing inside an IC are constructed from metal free inert plastic (PEEK) tubing instead of stainless steel. The reason is because stainless steel would corrode due to the corrosive nature of the eluents that are frequently used. Systems can be used which resolve either anions or cations separately or resolve both anions and cations in one sample run using dual analysis systems. (Jackson and Chassaniol, 2002).

Figure 4.7: Metrohm 850 Professional IC

Figure 4.7 shows the IC that is used for the analysis of the water samples. The IC is coupled with an autosampler which allows up to 150 samples to be analysed in sequence. As a result, the IC can be run continuously which reduces analysis times. It should be noted there were on-going problems with this equipment during the experimental period. The problems all the time faced with professional way, and the only issue in some samples was that they were not analysed immediately but remained in the freezer (-80 ^{o}C) till the analysis time. That happened in experiment 1. All the analysis became in triplicate to minimize the quality assurance errors and did not affect the final results that received from IC. In Chapter 5 in the first experiment the water samples were frozen (-80 ^{o}C) and defrosted before the analysis of them in IC. All samples even the initial influent solution frosted and to ensure that there was not affected the concertation of nitrogen compounds was measured before and after that process. The concertation remained the same at all cases and according to that hypothesis.

4.10.3 Stock solutions

Stock solutions for anions and cations prepared. Anion stock solution with fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphate are prepared to concentration of 100mg/L in nano pure water. This was prepared from purchased 1000 mg/L single ion IC standards. A cation stock solution was also prepared with the same concentration containing lithium, sodium, ammonium, potassium, calcium and magnesium in nano pure water. This was prepared from purchased 1000 mg/L single ion IC standards. The stock solutions were kept at temperature close in a fridge with temperature 4^{o}C and in the light absence.

4.10.4 IC calibration

4.10.4.1 Anions calibration

The stock anion solution was diluted in nano pure water to prepare working solutions with concentrations, 0.5 mg/L, 1 mg/L, 5mg/L, 10 mg/L, 20 mg/L and 40 mg/L.

Dilution is the process of making a concentrated solution less concentrated. The formal formula for calculating a dilution is $C_1V_1 = C_2V_2$, where C_1 and C_2 represent the concentrations of the initial and final solutions, respectively, and V_1 and V_2 represent their volumes.

The working solutions were determined by IC analysis and a calibration graph was produced. For anion calibration 20µL of the working solution was introduced to a Metrohm 850 Professional IC via the accompanying 858 Professional Sample Processer autosampler. The chromatographic column was a MetroSep7, 250mm x 4mm i.d with a MetroSep A Supp 4/5 Guard guard column. The eluent was a 3.6mM sodium carbonate solution in nano pure water at a flow rate of 0.7ml/min. The column temperature was kept constant at 50^{o}C. Each anion was identified by its retention time using the suppressed anion detector. Injection of each working solution was triplicate (n=3) and the peaks areas were recorded. The average areas were plotted against the working solution concentration to form a calibration graph. This was repeated for every new eluent solution.

4.10.4.2 Cations calibration

The stock cation solution was diluted in nano pure water to form working solutions of concentrations 0.5 mg/L, 1 mg/L, 5mg/L, 10 mg/L, 20 mg/L and 40 mg/L. The working solutions were determined by IC analysis to produce a calibration graph. For cation calibration 20µL of the working solution was introduced to a Metrohm 850 Professional IC via the accompanying 858 Sample Processer autosampler. The chromatographic column used was a MetroSep C4–150 mmx4mm i.d with a MetroSep C4 Guard guard column. The eluent, or mobile phase, was a 0.7mM dipicolinic acid solution / 1.7mM nitric acid in nano pure water at a flow rate of 0.9ml/min. The column temperature was kept constant at 50^{o}C. Each cation was identified by its retention time using the non suppressed cation detector. Injection of each working solution was triplicate (n=3) and the peaks areas were recorded. The average areas were plotted against the working solution concentration to form a calibration graph. This was repeated for every new eluent solution.

4.10.5 IC analysis of groundwater samples

Groundwater samples were analysed using a Metrohm 850 Professional IC with accompanying 858 Sample Processer autosampler. The IC was run in dual analysis mode which allowed for simultaneous detection of both anions and cations in a single sample run. For anion detection the chromatographic column used was a MetroSep7, 250mm x 4mm i.d with a MetroSep A Supp 4/5 Guard guard column. The eluent was a 3.6mM sodium carbonate solution in nano pure water at a flow rate of 0.7ml/min. The column temperature was kept constant at 50^{o}C. Each anion was identified by its retention time using the suppressed anion detector. For cation detection the chromatographic column used was a MetroSep C4–150mm x 4mm i.d with a MetroSep C4 Guard guard column. The eluent was a 0.7mM dipicolinic acid solution / 1.7mM nitric acid in nano pure water at a flow rate of 0.9ml/min. The column temperature was kept constant at 50^{o}C. Each cation was identified by its retention time using the non-suppressed cation detector.

Groundwater samples of 10 ml were injected in each vial. The samples ran in the following sequence, matrix blank (nano pure water) followed by standards, to check the calibration was still valid, followed by two matrix blanks, followed by five groundwater samples, followed by blank vial, and five groundwater sequence.

A triplicate of every sample analysed and the average peak area recorded and used to calculate the concentration of ions present in sample. The concentrations were calculated by using the calibration graphs that produced. All regression analysis became with linear regression. At all measurements error bars (10%) used and analysed only the prices that were on the limits. The LOD in IC calibration for nitrogen compounds and phosphate is given in Table 4.4.The linear regression approach used also in IC calibration and IC analysis. All the details for calibration are given in Appendix I.

Table 4.4: LOD and LOQ for Ion chromatography

	Limits of detection (LOD)	**Limit of quantification (LOQ)**
NO_2^--N	0.4269	1.2938
NO_3^--N	0.2896	0.8776
NH_4^+-N	0.3270	0.9911
PO_4-P	0.3444	1.0438

4.11 Quantitative real-time PCR

Samples from columns were analysed by others for this project to confirm the presence of denitrifying bacteria. Though microbiology research was not part of this thesis, a description of the methods used by others to verify that denitrifying were present are given here. Quantitative real-time PCR (qPCR) was used for gene detection because it is rapid, detects genes in both culturable and non-culturable bacteria, and it is quantitative, which allows statistical analysis between gene levels and experimental treatments. Specifically genes were quantified, which were associated with the non-haeme containing (*nirS*) and copper-containing (*nirK*) nitrite-reductases that encode the key enzyme classes responsible for the conversion of nitrite (NO_2^-) to nitric oxide (NO) within the denitrification pathway (Philippot, 2002). Targeting such genes has been used to determine denitrifier community composition (Braker et al., 1998; Philippot and Hallin, 2005; Henry et al. 2004; Graham et al., 2010) in environmental samples. The ultimate goal here was to quantify gene abundances of the two key nir genes as a measure of denitrifier population numbers, and explain treatment differences in nitrate reduction.

DNA samples were extracted using MoBio PowerSoil DNA kit (Carlsbad, CA, USA) according to manufacturer's instructions. Previously developed primers for *nirS* (Throbäck et al. 2004) and nirK (Henry et al., 2004) were used in qPCR reactions involving BioRad ssoAdvanced Green PCR reagent (BioRad, Hercules, CA, USA) and BioRAd iCycler. Reaction conditions involved initial DNA denaturation at 98 ^oC for 3 minutes; 40 cycles of denaturation at 95 ^oC (10

seconds), annealing at 55 ºC (10 seconds) and elongation and fluorescence detection at 60 ºC (10 seconds). Cloned *nirS* and *nirK* gene fragments (Graham et al., 2010) were used to prepare DNA standards with known quantities of target DNA (102–107 copies/mL). Quality control included post-analytical melt curves for detection of possible PCR artefacts and spiked DNA (10^6 copies/mL) into UV-irradiated DNA extracts.

4.12 Conclusion

More details about methodology and calibration of each instrument can be found in Appendix I. The results of calibration and replication indicate the results determined in each experiment are significant and are therefore acceptable.

CHAPTER 5

COLUMNS EXPERIMENT WITH SAND AND WHEAT STRAW AS SUBSTRATE MATERIALS

5.1 Introduction

The initial experimental part of the research program started with simple column experiments designed to evaluate materials studied in existing publications (Gibert et al., 2008). Two substrates were chosen for study and the experiment varied the percentages of them in each column. As an initial experiment the main purpose was to check how denitrification was reacting under different environmental conditions.

The main hypothesis under investigation is whether column experiments can simulate a natural denitrification system and whether the selected substrate materials were capable to treat water. The main aim of the experiment was to examine materials that are low value and are available as waste in substantial amounts. A denitrification process with low organic carbon levels and in some condition with not at all carbon, was expected and the performance of denitrifier bacteria was measured as reduction of all nitrogen compounds. The process can be characterized as heterotrophic and due to the lack of nutrient in the environment and more specifically in columns, can be characterized as oligotrophic environment.

It was expected that the system could establish suitable conditions to receive the best treatment. The substrate materials used in that experiment were: sand and wheat straw. The sand was used as an inert material that represented aquifer materials and the main reactive material was wheat straw.

Wheat straw is an abundant agricultural residue with low commercial value. Wheat (*Triticum aestivum L.*) is the world's most widely grown crop, cultivated in over 115 nations under a wide range of environmental conditions (Talebnia et al., 2010). Wheat straw, like any other biomass of lignocellulosic composition, is a complex mixture of cellulose, hemicellulose, lignin, and a small amount of soluble substrates, which are known as extractives and ash. The overall chemical composition of wheat straw could differ slightly depending on wheat species of each area, the soil, and the climate conditions of the area. Cellulose, hemicellulose and lignin content of wheat straw generally are in the range of 33–40, 20–25, and 15–20 (%w/w), respectively

(Prasad et al., 2007). Lignocellulosic waste materials obtained from energy crops, wood and agricultural residues represent the most abundant global source of renewable biomass (Lin and Tanaka, 2006). Wheat straw is an important substrate material that contains high levels of carbon and can force the system to denitrification process.

Table 5.1: Column experiments with substrate materials

		Substrate materials	HRT	pH	T (ºC)	Porosity	N Removal	Half Life
Calderer et al., 2014	Columns	Woodchips	0.67-0.75 days	6.9-7.9		38-72%		
Tanaka et al., 2007	Columns	Agro industrial wastes	2.9 hours	7.5	20		70%	
Robinson Lora et al., 2009	Columns	Crab shell chitin		6.5			40-60%	
Saeed and Sun 2011	Columns	Gravel, zeolite, woodchips	0.63-0.92 days	5.3-6.5			72-87%	
Rudolf von Rohr et al., 2014	Columns	Biod. dissolved organic mater.	4-10 hours				30%	
Alcala Jr et a., 2009	Columns	Woodchip, grass		6.5-8.1			90%	
Bratieres et al., 2008	Columns	Sand, gravel, soil					70% + PO4 rem. > 85%	
Lee et al., 2004	Columns	Activate sludge	1.8-3.8 hours	6.5	30		83-95%	
Gibert et al., 2008	Columns	Woodchips, soft, hard wood			22		98%	
Aslan and Turkman, 2005	Columns	Wheat straw			30		60-90%	30-126 days
Robertson et al., 2010	Columns	Woodchips	0.96-1.3 days		0-36	60-70%	78%	0.29-27 days
Jing et al., 2010	Columns	Walnut shell	24 hours	6-7.5	12.5		80%	14.8 hours

There are several studies with wheat straw as substrate material (Aslan and Turkman, 2005), including SBR (Trois et al., 2010), column studies (Table 5.1), reed bed systems (Zhao et al., 2009) and PRB systems (Gibert et al., 2008). The main purpose of those studies was to investigate the reduction of nitrate and nitrogen compounds. The denitrification process in column studies showed that a 60% reduction of nitrate could be achieved, and under optimal conditions levels more than 95% (Aslan and Turkman, 2005). The suitable conditions that are important to the best optimize in the denitrification process include temperature (Ovez, 2006), carbon source (Soares and Abeliovich, 1998) that can ensure the continuous carbon source availability, velocity of the spiked solution in the experiment (Su and Puls, 2007, Xu et al., 2013) that ensures the stable flow rate. This experiment was designed to compare new work with existing published information.

5.2 Description of experiment

The experimental setup was separated into two components. The first component was use of a pre-treatment tank (reed bed) to provide a sustained microbial population capable of

denitrification, and the second was the experimental columns that represent a lab scale reactive barrier. In the first experiment, the aim was to focus only on nitrogen compounds that exist in groundwater. Groundwater from the NITRABAR site in N. Ireland was passed from an initial pre-treatment, which contribute a reed bed tank with *Phragmites Australis* plants. The pre-treatment tank helped the groundwater to homogenise chemistry, and to react with other chemicals, pesticides or pollutants that may exist from any agricultural activity in the area that collected. It also helped to create the suitable conditions for sustain activity in microorganisms. After pre-treatment, specific amount of KNO_3 was spiked to an initial concentration. The only chemical that added in groundwater was KNO_3, and it was important to focus only in nitrogen compounds and minimize any other contamination in water. The next part of setup was the experimental columns. Columns contained different percentages of substrate material to investigate the denitrification process under possible conditions in nature. The conditions of experiment kept stable at room temperature (20±5 ^{o}C).

The NITRABAR system is a trench containing a mixture of natural materials, which removes nitrate from shallow groundwater before it enters rivers or lakes. Both soil and groundwater contain bacteria which naturally degrade nitrate into nitrogen gas. The NITRABAR trench creates the conditions for these bacteria to flourish. NITRABAR is intended for placement between a field and a surface watercourse and may be used strategically to deal with major fluxes in a catchment or reduce flux to sensitive receptors. The NITRABAR Project aims to demonstrate a field-scale permeable reactive barrier for removing nitrate from shallow groundwater at an agricultural site and assist others in the replication of the technology across Europe.

The columns that used were perplex columns with internal diameter 5 cm and length 55 cm. The detailed approach of columns is provided in the next paragraphs. The initial situation for the experiment was to homogenise the substrate materials that are used in this chapter. Sand of local farmers received, sieved to remain particles > 0.66 cm and then cleaned with water and burned in an oven for 10 hours in 250 ^{o}C. The wheat straw received also from local farmers, chopped in particles < 2 cm and according to experiment mixed with sand and insert in columns with specific in each column. Until the start of the experiment there was a stabilization period of 10 days. In that period there was the same flowrate of tap water in the columns as it would use in experiment. The steady flowrate ensured by using peristaltic pump. The flowrate in the columns was upside movement as it is mentioned in other researches (Gibert et al; Jang et al., 2010). During that period there was a preparation period to face any possible problem with trapped bubbles along the columns. To avoid those problems was important to ensure that the

experiment will take place under specific conditions. Problems with trapped bubbles faced using shakers to remove all trapped oxygen. To avoid the high levels of trapped O_2 the flowrate was upside opposite to gravity and with initial flowrate very low till the time that all the columns were saturated, full of water. Then the velocity of water increased till the proposed flowrate along the experiment.

5.2.1 Groundwater

Groundwater was collected from Northern Ireland, Ballymena, where NITRABAR project took place (Gibert et al., 2008). Groundwater stored in 4ºC before used in the experiment. After the pre-treatment of groundwater, tanks kept stored at 4 ºC. NITRABAR project was a European project designed to remove nitrate levels of agricultural areas using cheap materials and the process was based on microbial activity (denitrifying bacteria).

Figure 5.1: Ballymena groundwater collection point (www.google.co.uk/maps/)

The pre-treated groundwater was spiked with potassium nitrate (KNO_3) to achieve an initial concentration of 0.406 mmol/l (25 mg/l) NO_3-N. The area where groundwater was collected was next to the river where agricultural activities occurred. Furthermore, there was also animals (sheep) activity in the area that may have affected the water quality. The water collected in the end of October 2011, during a rainy period and the flow in the river was high.

The collection of NITRABAR groundwater was chosen for several reasons. The NITRABAR project took place in 2008 and it is a good indicator to find out if the system in the area after 3 years is still working efficient. Moreover, with the treatment that NITRABAR provide to the area the amount of phosphate levels remain stable and there is a sustainable microbial activity in the system. Finally, due to denitrification activity that the project designed, denitrifier bacteria exist in the system that can provide and forward the denitrification process along column experiments.

5.2.2 Pre-treatment tank

An artificial wetland tank (Figure 5.2) was used to pre-treat groundwater. The dimensions of wetland were 150cm x 10 cm x 60 cm. The media of the tank was sand and six *Phragmites Australis* to create a reed bed. The level of sand in the tank was at 45cm, and at the top level groundwater moved along the tank with surface and underground flow rate. In the one side of tank there was an input valve to add groundwater and at end of the tank there was also an output valve to discharge the pre-treated groundwater.

The pre-treatment tank was installed to simulate areas next to the river slopes where reed beds are growing. The sand sieved to have diameter less than 0.66cm, and small amount of soil from the roots of reed beds plants also existed. The system in reed bed was acclimated one month before the experiment to increase the root density in sand and to stabilize the system for the investigation period. The water used in that period was tap water without any addition of chemicals.

Figure 5.2: Pre-treatment tank /Reed bed

Groundwater velocity passing through the tank and water volume was controlled by a peristaltic pump. Tubes with diameter 0.25 cm were used to connect the tanks with groundwater to the artificial reed bed. The amount of groundwater that was pre-treated in reed bed was 8.3 litres/ day. The groundwater stored in 25 l tanks until it was used in experimental columns.

The reed bed was used as pre-treatment for the microbial activity. It is used to balance the microbial activity that already exists in groundwater and to simulate the laboratory environmental conditions. The next point for the reed bed was to absorb any chemicals that probably exist in the solution due to the environmental conditions and finally to absorb any pesticides that exist due to the cultivations that exist in the collection area.

Pre-treatment tank used to stabilise the microbial activity that exist in groundwater from NITRABAR. The reed bed can absorb high levels of pesticides that possible exist in GW. Except from that, the groundwater was received exactly after the barrier system in NI and the microbial activity of the barrier exist in the groundwater. With the artificial wetland the main focus on the research was the denitrification bacteria. The activity of them remained alive and active. From that process the groundwater even spiked immediately in columns part or remained in fridge (4 ^{o}C) till the application time on columns. The characteristics of influent groundwater analysed but pesticide analysis was not taken place and it was speculative approach to the possibility of existence. The water after the pre-treatment tank spiked with KNO_3 and then feed into columns with specific flowrate.

5.2.3 Columns design

The columns consisted of Perspex plastic. In this experiment six Perspex Cylinder columns were used. The length of columns was 55 cm and the diameter 5 cm. Along the columns there were five sample points. The connection to the initial tank was with tube with diameter 0.32mm Tyger Tygon and the stable spiked water quantity in the columns was moved by peristaltic pump (Ismatec 8 channels).

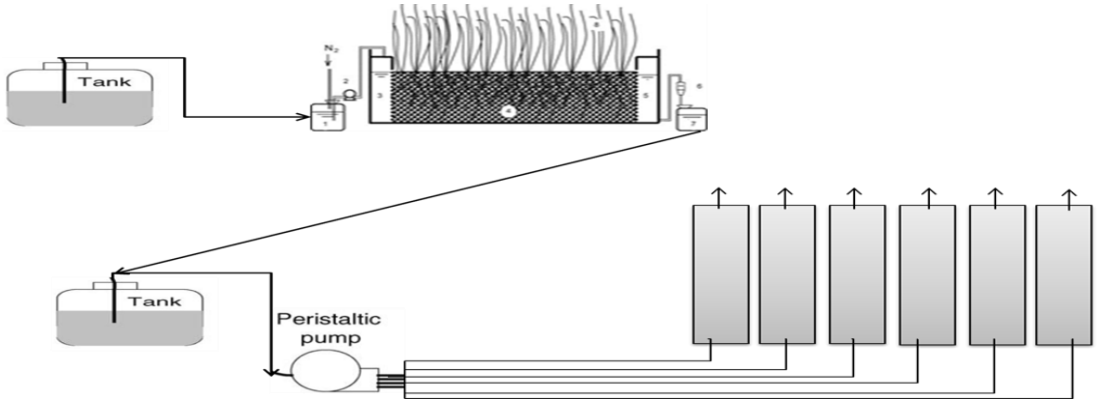


Figure 5.3: Design of experiment

Columns were chosen because it was an easy way to simulate the ground conditions in laboratory. Additionally, environmental parameters could be controlled such as oxygen levels, light, temperature, addition of chemicals, and to use the substrate materials that selected. With all these parameters under control, the interaction of selected substrate materials with groundwater and the differences treatments could be observed. The initial question for this experiment was how much the nitrate levels can be reduced in that system. According to another study the reduction levels should be > 60% (Aslan and Turkman, 2005).

5.2.4 Experiment details

In the first two experiments six columns were used. The substrate materials that used were sand and straw in different percentages. The design of the columns is shown in Table 5.2 and Figure 5.4.

Table 5.2: Design of columns, experiment with sand and wheat straw

Column 1	100% (v/v) sand
Column 2	20% (v/v) straw, 80% (v/v) sand
Column 3	40% (v/v) straw, 60% (v/v) sand
Column 4	60% (v/v) straw, 40% (v/v) sand
Column 5	80% (v/v) straw, 20% (v/v) sand
Column 6	100% (v/v) straw

The sand (Figure 5.5) was sieved to use particles < 0.66mm. The wheat straw (Figure 4.5) was taken from local farmers, chopped to pieces < 2 cm, and packed at the columns uniformly with the sand.

Figure 5.4: Columns design

Water samples collected in 50 ml plastic sterilized bottles from the output of each column and analysed for pH and conductivity immediately after the sampling time and remained in the freezer until the analysis for NO_3, NO_2, NH_4, PO_4 and TN with spectrophotometer.

Figure 5.5: Sand and wheat straw substrate materials (www.designpanoply.com, www.mushroomsource.ca)

The substrate material homogenised using cone and quarter method. This is the method that is used in the majority of laboratories processes. The process is the following:

1. Decontaminate all laboratory equipment according to appropriate Laboratory SOPs.
2. Don appropriate PPE and gloves. Clean gloves must be worn for each sample composited.
3. With the top 1/3 and bottom 1/3 of sample material in the homogenization tray/bowl/pan, chop-up the sample into small chunks using a clean, stainless steel wallboard knife or other suitable implement.
4. Remove non-soil debris, including sticks and vegetation, as much as possible.
5. Scooping from the edge, form a mound in the centre of the tray/bowl/pan.
6. Divide the mound into two equal piles and form each pile into a mound.
7. Divide each into two piles.

8. Mix the piles together that are opposite from each other into a single mound.
9. Repeat steps 7, 8 and 9 until the sample is thoroughly homogenized (a minimum of 3 times).
10. Transfer the thoroughly mixed sample to appropriately labelled sample containers for the required analyses.

The sterilization of water sample bottles became using boiling water. In a big bucket all the water bottles insert there and remain in boiling water for more than 90 minutes and afterwards the bottles placed in steam sterilizers for more than 30 minutes.

Water samples collected from the output tube of the columns. The analysis of the results became as fast as possible depending on the availability of the equipment and instruments for the analysis. The water samples that did not analysed in the collection time remained frozen (-80 ^{o}C) till the analysis time to ensure that all the nutrients remain there.

5.3 Results experiment 1

Samples collected for 18 days in experiment 1 of experiment. During the first 3 days, the sampling was more intensive and 2 samples per day were collected. The measurements were separated to direct measurements (pH, conductivity), and the non-direct measurement (NO_3, NO_2, NH_4, PO_4 and TN).

The hydraulic retention time (HRT) in part 1 of experiment was 7 hours and measured according to the flow rate and the porosity of substrate materials that used. The porosity measured according to the definition $n = \frac{V_V}{V_T}$ where V_V is the volume of void space and V_T the total volume of materials (solid and void components). The average porosity along the columns was 0.45 (SD=0.05).

The HRT was selected according the literature that exist and the half-lives that other researcher mention as the optimal period for denitrification process (Table 5.1, Chapter 2). According to this, a comparison between the initial solution and output solution and the degradation activity in the end of the process can be made. Finally, with the selected HRT and the flow rate set up, it was expected to provide at about 80% removal of total nitrogen.

The temperature of the experiment was room temperature (20±5 ^{o}C), similar to other studies (Karanasios et al., 2010). Measurements were taken at specified times, and the samples were

collected by sterilized syringes to 50 ml plastic sterilized bottles. Water samples were kept frozen (-80^{o}C) until analysis.

Water solutions (groundwater) without any additional chemical ran through the columns for seven days to create the suitable conditions and environment before the experiment.

The analysis of the results will follow the same way in all the chapters. There is a separation between lag and stable phase. The lag phase is the initial phase where there is a detailed start of the growth of microbial activity in denitrification process. Adaptation lag phase is the initial lag phase that the microbial activity is low and combined with the growth phase. The initial lag phase is the phase that the microbial activity is delayed till the time that start the growth phase. In the growth phase the microbial activity is increasing. In the adaptation lag phase there is also the exponential phase where the microbial activity achieves the highest levels in denitrification process. The number of new bacteria appearing per unit time is proportional to the initial population. If growth is not limited by the carbon source, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each specific time period. In that time period the microbial activity achieves the highest levels in denitrification process. For Thesis the adaptation lag phase is used and contains the lag phase and the exponential phase. The process is clear for the degradation rates where it is separated in two parts. The adaption lag that has already described and the stable phase that is clear enough that starts when stationary phase starts in the bacterial growth curves and in the kinetics growth curves.

There is a separation between the graphs that created to analyse the microbial activity of Nitrogen compounds and all other measurements. The N compounds graphs at all the chapters are presented in concertation VS pore volume. Additionally, at the tables with the results exist also the correspond in days. The degradation rates described in normalized concertation VS pore volume. That approach adopted to be more visible and clearer the results of the kinetics that follows Monod Kinetics Laws. The approach was detailed to fit the kinetics with actual microbial activity. There is a description of instantaneous rate constants along the experiments. These instantaneous rate constants were analysed over specific time periods (PV). The Pore Volume (PV) is the total volume of the volume multiplied by the porosity of it and it is the total volume of water in the column at any time. The flowrate (volume per time) is the amount of solutions that come into and out of the column. The approach is an instantaneous approach that used to describe in detail the conditions and the kinetics in specific time period. For each specific day there is the specific HRT that is stable in each experiment and the K for the specific

day. Finally, there is also a separation of all other measurements that are designed in the graphs concertation VS time, but as it is mentioned there is also the conversion with pore volume in the table of each chapter. This approach in kinetics is adopted in all the chapters and experiments.

Table 5.3: Results of experiment 1 (all results received after (N=3 replicates) triplicate analysis)

		Influent	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
pH	mean	7.90	7.81	7.45	7.35	7.19	6.91	6.86
	SD	0.12	0.22	0.32	0.40	0.45	0.63	0.88
	% change		-1.12	-5.61	-6.93	-8.90	-12.42	-13.11
Conductivity μS/cm	mean	382.27	501.77	479.64	583.41	545.91	481.32	486.68
	SD	73.15	219.62	218.41	300.33	278.02	348.75	249.83
	% change		31.26	25.47	52.62	42.81	25.91	27.31
NO_2-N μmol/l	mean	2.31	3.06	37.21	23.38	24.33	7.92	12.90
	SD	0.04	0.05	1.72	1.67	1.74	0.62	1.32
	% change		32.48	1509.40	911.11	952.14	242.74	458.12
NO_3-N μmol/l	mean	406.18	84.39	83.18	118.82	102.11	138.27	171.01
	SD	0.09	4.97	5.89	5.85	5.30	7.98	9.10
	% change		-79.22	-79.52	-70.75	-74.86	-65.96	-57.90
NH_4-N μmol/l	mean	20.31	28.45	17.26	17.01	19.76	20.26	19.76
	SD	0.28	0.54	0.15	0.18	0.29	0.33	0.24
	% change		40.07	-15.01	-16.25	-2.73	-0.25	-2.73
TN μmol/l	mean	428.80	115.91	137.65	159.21	146.19	166.45	203.67
	SD	0.30	5.22	5.87	5.85	5.52	8.05	9.69
	% change		-72.97	-67.90	-62.87	-65.91	-61.18	-52.50

In Table 5.3 there are average results from direct and indirect measurements along the experiment 1 of experiment. Except from the average results there are also the differences that exist from the initial conditions. More detailed approach for all measurements exists in Appendix II.

5.3.1 pH levels

The pH along the first experiment in the influent solution is stable with an average value 7.9 (SD=0.12). At all columns along the experiment, pH levels remained neutral with a reduction from the initial solution 1% till 13% in columns but always neutral which are the most suitable for denitrification process.

5.3.2 Conductivity levels

Conductivity was measured in μS/cm. It is also an indicator for nutrient uptake because it is connected with total dissolved solids (TDS). TDS describes all solids (usually mineral salts) that are dissolved in water. The TDS and the electrical conductivity are in a close connection. The

more salts are dissolved in the water; the higher is the value of the electric conductivity. At influent groundwater solution the conductivity was 382.27 μS/cm. The conductivity levels increased at all the columns along the experiment with the highest levels in column 3 (583.41 μS/cm).

5.3.3 Nitrogen species

The Figure 5.6 shows in detail all the results of nitrogen species from experiment 1. As it is noticed at all the columns there is an initial phase that the reduction of nitrogen levels achieved (NO_3^--N, TN). This is phase has duration between 4-6 days and was expected as the microbial populations adapted within the columns. Discussion of these results will follow. The analysis of results became in C VS Pore Volume. The Pore Volume used to describe and to compare more easily the different HRT that are used in the experiments. The Pore Volume is connected with the turnover period in the columns and the HRT.

The reduction of nitrogen compounds achieved as it is mentioned in 4-6 days, which is 8.5 PV periods. That phase is the initial phase and as it is described is the microbial kinetics is the adaptation lag phase, where the microbial activity is increasing achieving the best rates.

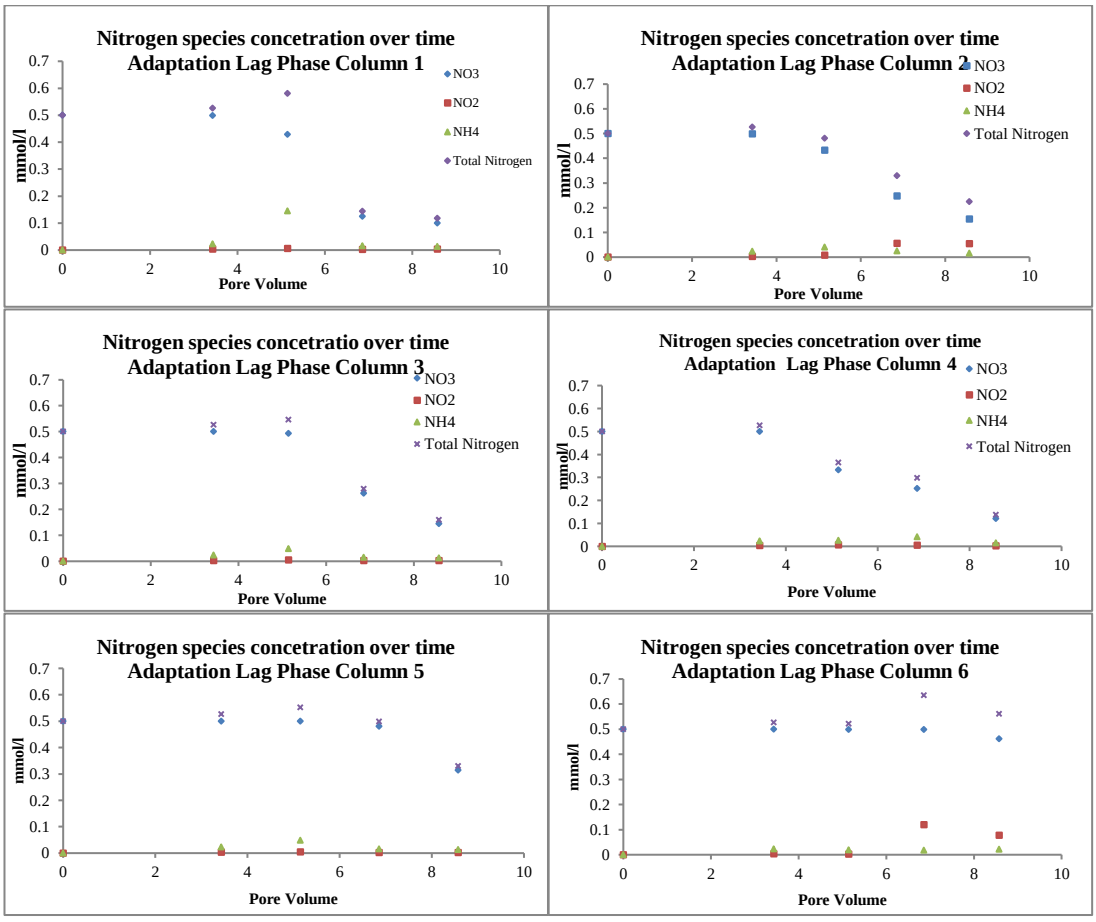


Figure 5.6: Nitrogen species concertation on adaptation lag phase experiment 1

In the initial adaptation lag phase the reduction on nitrogen species is more than 50%. At all the columns the amount of NO_2-N and NH_4-N remain in very low levels in contrast to NO_3-N and TN (Figure 5.6).

In column 1, NO_3-N is reducing along the adaptation lag phase and is the nitrogen compound that provide the higher amount of TN. Total Nitrogen (TN) follows nitrate levels except in PV=5 where there is an increase due to the increase of NH_4-N.

In column 2, NO_3-N reduced more than 70% in the adaptation lag phase. TN is also reduced in the same levels along that period. From the PV=5 till PV=8 there is an increase in NO_2-N but this increase in minor in contrast to the NO_3-N.

In column 3, NO_3-N reduced more than 70% in the adaptation lag phase. TN follows also the reduction of NO_3-N. In that column NO_2-N, NH_4-N remain at all the duration in very low levels (<0.05 mmol/l). In contrast to column 1 and 2 there is an initial stable period till PV=5 in NO_3-N and TN and then there is the reduction of them. This occurs due to the time that influent

groundwater demand to assimilate the conditions of the column and the increase of microbial activity in the column.

In column 4, NO_3-N and TN reduced more than 70%. In that column the initial stable phase in less than PV=4 and the reduction after that continue till achieving the best results. In that column also the NO_2-N and NH_4-N remain in very low levels. As it is noticed in all first 4 columns the amount of organic carbon is high and the microbial activity from the influent groundwater can provide the results in that time period.

In column 5, the amount of wheat straw is increasing and the amount of organic carbon that can be provided is higher. The time that the influent groundwater demands to assimilate the conditions and to provide the expected reduction is higher. The amount of NO_2-N and NH_4-N is very small and the amount of NO_3-N initially and TN remain very high and stable till PV=7 and the reduction is less than 40% in the adaptation lag phase.

In column 6, the substrate material that exists in column is only wheat straw and the trend that is followed is same like column 5. The amount of NH_4-N is very small and remains in low levels at all the duration of adaptation lag phase. There is an increase of NO_2-N after PV=7 that affect the TN levels. The amount of NO_3-N remains almost stable at all the duration of adaptation lag phase with minor reduction (10%). TN levels increased in the adaptation lag phase showing that only the influent groundwater and the HRT in the columns is not enough to provide the reduction that was visible in the columns 1,2,3,4.

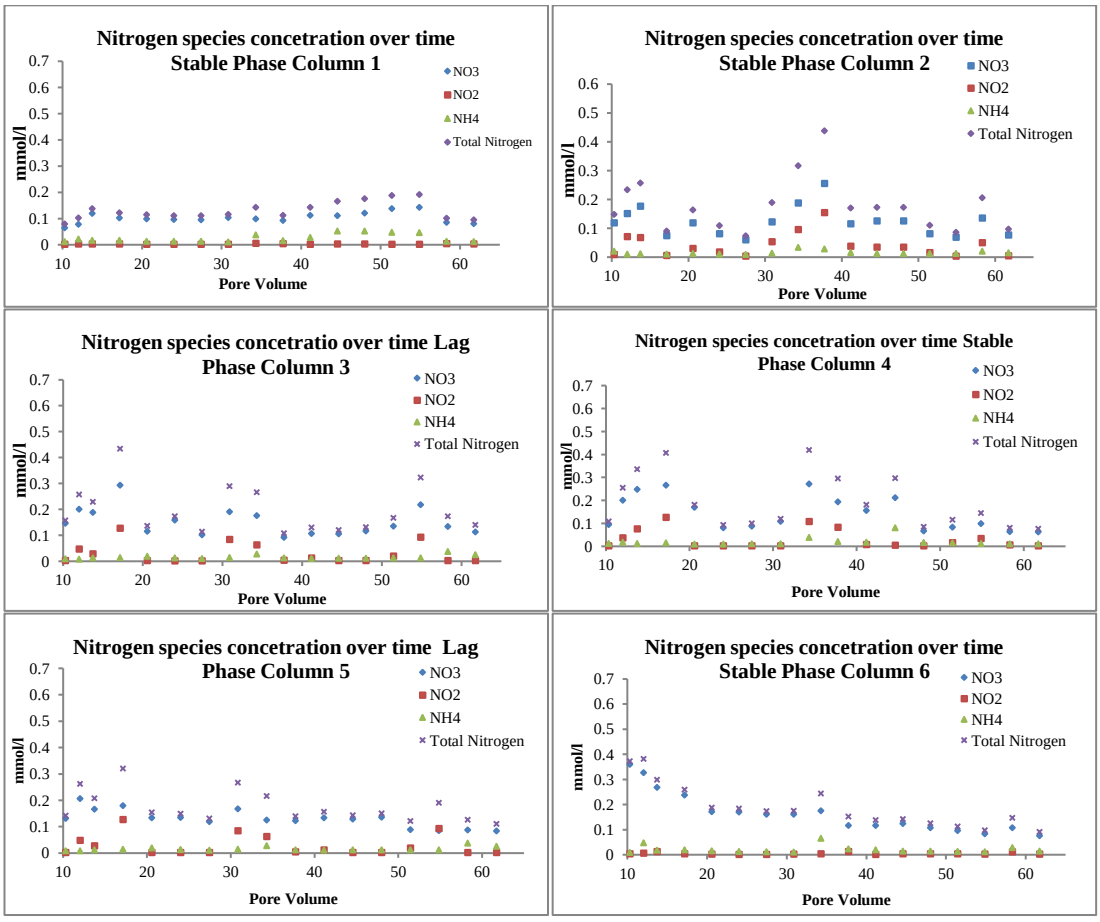


Figure 5.7: Nitrogen species concertation on Stable Phase experiment 1

After the adaptation lag phase as it is also described in the microbial kinetics there is the Stable phase of the experiment (Figure 5.7). The Stable phase starts from Day 6 till the end of the experiment (Day 19).

In column 1, the Stable phase is more visible and stable than any other columns. The reduction from the start of Stable phase is more than 70%. The Stable phase can be separated in smaller periods, the first one between PV10-15 where there is an increase in NO_3-N and TN, afterwards there is a stable period till PV=30. Another minor increasing stable period is till PV=55 and finally there is the final stable period till the end of experiment PV=65. The increases and decreases in the amount of nitrogen compounds is a result of different influent groundwater that is spiked into the column. Additionally, the environment in the column can faster assimilate the groundwater and provide the suitable conditions for denitrification process. The interesting result in column 1 is the total amount of reduction that is achieved. The organic carbon that exists in column is provided only from groundwater and that shows that the activity from the existing in water microbes can enhance the whole process.

In column 2, the Stable phase is not as stable as in column 1. There are 3 increasing periods between PV10-15, 28-38, 50-58. The interested point is that not only NO_3-N and TN were increasing but also the amount of NO_2-N increased. The total reduction in the end of the experiment achieved levels more than 70%. The changes in the trend are again characteristic and can be connected with the changes in the influent spiked water. The amount of NO_2-N is also connected with the denitrification process and the amount that exists improves that the HRT is not enough to finish the process.

In column 3, the reduction levels achieved 75%, but again there are smaller stable periods in the Stable phase. The stable periods are between PV 20-28, 30-34, 36-52, 58-65. Again there are increasing periods before the stable periods and the amount of NO_2-N is characteristic. There is the same trend as in column 2 with demand of higher HRT in the columns to finish denitrification process. Additionally, the change of influent water affects the results in the same time period as in column 2.

In column 4, the Stable phase is the phase with the less stable periods. There are only two stable phase between PV 20-32, 48-65. The reduction in those stable periods is more than 75% showing that there is a good balance between sand and wheat straw and can provide the organic carbon for denitrification process. Between PV10-18 there is an increasing period and between PV 34-48 a decreasing period, these is also in agreement with the other columns and the time period that the water changed. The amount of NO_2-N is countable and can assume even in that column that HRT is not enough for the microbial activity to assimilate the amount of nitrogen compounds that can.

In column 5, the amount of wheat straw is increasing providing in the system higher amount of organic carbon. That is visible from the reduction of all nitrogen compounds along the stable period. In that column the stable periods last longer and the spread in results is visible only in the time that the water changes and these are the same days as in the previous columns. Those time periods the amount of NO_2-N is increasing and is also a conclusion that higher HRT in the columns is important. The reduction in TN and NO_3-N achieved levels more that 75%.

In column 6, there is only wheat straw on the column. The amount of organic carbon that is provided to the system is the highest but it is not achieving the best reduction rate (70%). The reduction on nitrogen compounds is more stable with reduction period at all the duration of the experiment. There are also some peaks as it is visible at the other columns in the time period when the influent groundwater was changed. In contrast to other columns there are higher levels of NH_4-N than NO_2-N and NO_3-N and TN are very close at all the duration of the experiment.

5.3.3.1 Nitrite levels

Nitrite levels along the experiment were significant because in all columns there were peak periods that concentration was noticeable. Except column 1 where nitrite levels along the experiment were lower than 6.52 μmol/l; at all other columns there were noticeable amounts of nitrite levels. In column 2, nitrite level peaked in the 11th day of experiment with 0.154 mmol/l and along the experiment with high amounts. In column 3 the peak level of nitrite was noticed in 5th day with 0.127 mmol/l and again high levels along the experiment. The same was noticed in column 4 with the peak day the 5th day of experiments and 0.126 mmol/l NO_2-N. In column 5 the levels of nitrite remained lower than previous columns along the experiment with the highest levels in 9th day and 0.056 mmol/l. Finally, in column 6, there was an initial increase of nitrite levels and then the levels kept really low. The peak day in column 6 was the 2nd day with 0.119 mmol/l NO_2-N.

5.3.3.2 Nitrate levels

Nitrate levels as it is noticed in the Table 5.4 and Figure 5.7 showed similar patterns of reduction. On the first 3-6 days the reduction of nitrate levels was noticed at all columns and it was the reduction period following a stable period of denitrification.

Table 5.4: Nitrate levels experiment 1

μmol/l	Pore Volume	Influent	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	3.42	407.09	404.99	406.93	408.38	405.15	405.64	406.44
	5.14	408.70	220.24	327.54	375.95	256.06	533.75	613.29
Day 2	6.85	405.47	89.71	133.92	209.59	171.84	384.50	387.56
	8.57	408.06	70.67	64.38	110.69	87.61	143.60	304.47
Day 3	10.28	406.76	39.05	70.67	110.69	64.38	81.16	289.62
	12.00	408.38	41.14	45.50	112.78	114.88	132.31	220.24
Day 4	13.71	405.31	81.16	68.57	116.98	121.17	127.47	194.91
Day 5	17.14	406.28	66.48	47.60	106.17	83.26	138.12	177.65
Day 6	20.57	404.83	66.48	55.99	74.87	129.73	98.10	125.37
Day 7	24.00	403.54	64.38	39.05	116.98	55.99	100.20	127.47
Day 8	27.42	405.80	64.70	37.11	72.61	61.31	88.74	121.01
Day 9	30.85	407.73	74.87	36.95	62.28	79.38	72.77	122.63
Day 10	34.28	405.47	45.50	41.14	67.77	98.42	62.28	89.71
Day 11	37.71	407.57	70.67	66.15	72.61	85.68	98.42	76.96
Day 12	41.14	405.15	64.38	43.24	66.48	104.88	91.81	76.96
Day 13	44.57	406.12	43.24	64.38	79.06	101.81	98.42	91.97
Day 14	48.00	405.31	51.79	64.38	88.74	38.72	104.88	76.96
Day 15	51.42	406.93	68.57	41.14	83.90	40.34	62.28	66.15
Day 16	54.85	407.09	72.61	41.63	77.45	37.27	58.09	56.47
Day 17	58.28	403.54	53.89	43.24	72.61	35.82	55.99	50.02
Day 18	61.71	404.83	51.95	44.37	66.15	38.72	52.44	46.79
Day 19	65.14	405.96	50.18	45.98	61.31	34.04	50.99	39.53

In column 1 (100% sand), the reduction of nitrate levels started from the first day achieving reduction more than 50%. Along the experiment the lowest levels noticed in the 3rd day (39.05

μmol/l), after that an upside down period till the end of the experiment. The average levels were 84.39 μmol/l (SD=80.27). It is interesting to note that denitrification occurs in the control, likely due to the dissolved organic matter and microorganisms from the pre-treatment reed bed tank.

In column 2, the reduction of nitrate levels achieved in 3 days with a more stable rate. After that initial period there was a reduction period till day 9 with the lowest levels (36.95 μmol/l) and till the end of experiment the average nitrate levels were 83.18 μmol/l (SD=95.07).

In column 3, the initial reduction of nitrate levels is slower and the reduction that was expected achieved in 6th day of experiment with 74.87 μmol/l. The lowest amount noticed the last day of experiment and the average levels along the experiment was 118.82 μmol/l NO_3-N (SD=94.34)

In column 4, the reduction of nitrate levels followed a faster route with reduction more than 75% achieved in the 3rd day (64.38 μmol/l). After that, it followed an increasing period till 12th day of experiment and then a reduction till the last day where the lowest levels was noticed. The average levels along the experiment was 102.11 μmol/l NO_3-N (SD=85.46).

In column 5, the first day was an in initial peak of the concentration of nitrates levels achieving 533.75 μmol/l and then started the reduction till the 10th day of experiments with nitrate levels 62.28 μmol/l. Then there was an increase and decrease of nitrates till the last day when the lowest amount was noticed. The average levels in that column along the experiment was 138.27 μmol/l NO_3-N (SD=128.72).

In column 6, as it is noticed also in column 5, in the first day there was an initial increase of nitrates till 613.29 μmol/l. After, there was a reduction period till the end of the experiment but with lower rates. The lowest amount noticed in the last day of experiments and the average levels along the experiment were 171.01*10^{-3} mmol/l NO_3-N (SD=146.80).

5.3.3.3 Ammonium levels

Ammonium levels remain low at all the duration of experiment. The highest levels are noticed in column 1 at first day of experiment. At all columns there is a reduction period till the 10th day that the concentration is < 27.71 μmol/l. After the 10th day there is an increase till 83.15 μmol/l with a reducing rate till the end of experiment.

5.3.3.4 Total Nitrogen (TN) levels

Total Nitrogen levels (Table 5.5 and Figure 5.7) are the total amount of nitrate, nitrite and ammonium levels. As it is noticed TN levels follow the same reduction rate as nitrate.

Table 5.5: Total Nitrogen levels experiment 1

μmol/l	Pore Volume	Influent	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	3.42	415.32	417.41	415.48	417.41	410.80	412.09	414.83
	5.14	415.32	267.03	345.29	393.37	268.33	544.88	620.88
Day 2	6.85	416.28	96.49	182.65	215.89	187.49	399.34	480.99
	8.57	416.28	77.13	109.56	116.50	93.74	199.75	368.04
Day 3	10.28	411.44	44.37	83.26	115.20	69.22	90.84	294.46
	12.00	411.44	49.70	101.65	150.70	146.99	149.09	238.96
Day 4	13.71	412.41	87.94	122.79	141.67	180.55	132.15	208.30
Day 5	17.14	412.90	73.58	54.54	204.75	181.20	143.44	185.71
Day 6	20.57	411.44	72.12	82.45	82.13	134.08	102.78	131.34
Day 7	24.00	411.77	69.70	55.67	122.14	60.18	104.39	132.31
Day 8	27.42	411.12	69.86	42.60	76.96	65.67	92.78	125.69
Day 9	30.85	412.09	79.38	80.51	129.24	83.42	118.11	127.79
Day 10	34.28	422.58	60.67	121.82	122.46	190.23	78.74	110.85
Day 11	37.71	431.45	78.25	188.78	79.55	152.64	106.81	93.10
Day 12	41.14	412.09	73.74	76.00	78.90	115.53	100.52	83.74
Day 13	44.57	418.54	60.35	93.26	84.39	128.11	103.91	98.91
Day 14	48.00	418.54	68.90	93.26	94.07	45.50	110.36	83.90
Day 15	51.42	414.51	84.06	56.96	101.97	56.80	67.28	72.28
Day 16	54.85	412.41	87.61	48.24	150.38	65.83	62.93	61.31
Day 17	58.28	412.41	60.51	86.48	85.19	43.56	62.12	66.80
Day 18	61.71	411.44	57.76	52.44	75.19	43.73	58.41	52.28
Day 19	65.14	410.48	55.50	53.89	66.96	41.14	56.63	43.73

The highest average levels were noticed in column 5 and column 6 with 149.88 μmol/l (SD=129.85) and 186.19 μmol/l (SD=156.32) respectively. The lowest levels were noticed in column 1 (95.09, SD=84.27), in the column 2 in the 8th day 42.60 μmol/l (115.80, SD=94.63).

5.3.4 Degradation rates

There is an initial time period that is more than 1 week till 10 days to stabilize the system in the columns with the several substrate materials. That time period is not analysed in the chapters and it is the stabilization period that is mentioned in the start of the chapters. The solution that is used is tap water and groundwater depending on the experiment. That initial period is critical to stabilize the conditions in the columns and to create the microbial environment for the growth of the bacteria that are responsible for nitrogen removal. The period that is described in the adaptation lag phase is the periods that the spiked solution (water / groundwater with KNO_3) is pumped in the columns, and there is a period that the denitrification bacteria demand to start the process. That period is the time that the system in the columns demands to stabilize the Nitrogen compounds and to remove the biggest amount of NO_3-N levels. During the adaptation lag phase

the description of kinetics is not very clear due to the fast reactions that take place in the column between solution and denitrifier bacteria colonies on the substrate materials.

Degradation rates in Figure 5.8 are following the reduction of TN levels at paragraph 5.3.6. At all columns the initial adaptation lag phase has duration 4-6 days depending on column and the substrate materials. The initial period of adaptation lag phase is a period that cannot be described and the main issues are focused on the Stable phase from Day 6 till the end of the experiment. The degradation rates measured according to TN levels that are very close to NO_3-N. The NO_2-N and NH_4-N as it was described in the previous paragraphs were noticed only in the time that the influent groundwater was changed. For that reason, the most responsible approach on degradation rates became with TN levels. The degradation rates are following the Monod Kinetics.

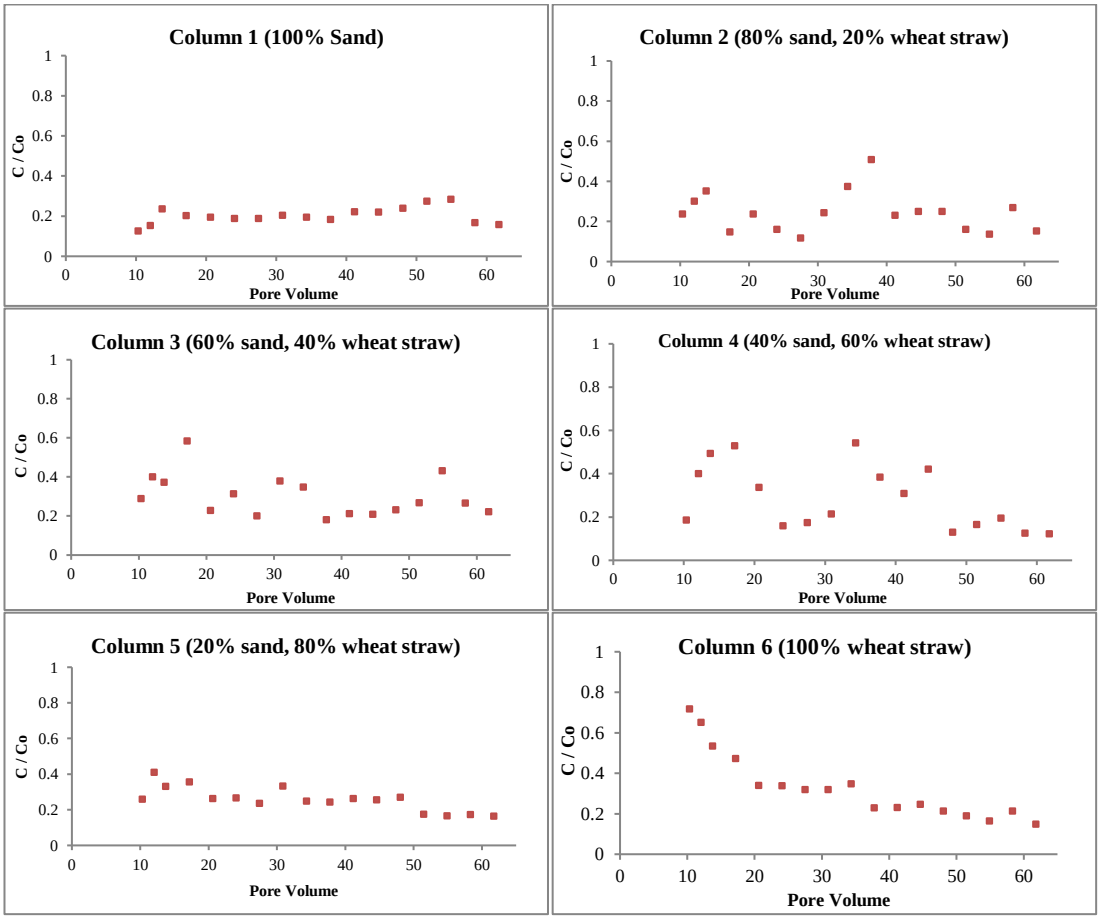


Figure 5.8: Degradation rates experiment 1

In column 1, the research can be separated in 3 periods. The first one from PV10-15 where there is an increasing rate in the column. That increase is connected with the change of influent water and the HRT that is not enough to assimilate the microbial activity of the solution. The second

period is a stable period from PV 15-55 where the microbial activity is stable and has already reached the maximum level on denitrification process. The last period is again a stable period from PV 55-65. The level is lower than the previous one and it is again stable. The change is connected with the influent groundwater. The stable flow rates in column 1 are following zero order kinetics and the increasing period is following first order kinetics.

In column 2, the degradation rates have not specific trend. There is stable phase (PV 40-55), increasing phases (PV 10-15, 15-20, 28-40) and decreasing phases (PV 20-28, 55-65). The changes in the phases as it is mention in the previous paragraph are connected with the change of influent groundwater and the carbon source levels that are provided by the internal ecosystem in the column. The stable phase follows the zero order kinetics and all the other phases the first order kinetics.

In column 3, the experimental period can be separated in 4 groups. There are two increasing groups in PV 10-20, 20-35, a stable group in PV 35-55 and a decreasing group in PV 55-65. The spread of results is wide and the trend follows the column 2. The changes in the trends are also connected with the change in the influent solution and the HRT that is not enough to assimilate the microbial activity of the solution and the column. The organic carbon that is provided by the system is higher due to the higher levels of wheat straw.

In column 4, the spread of results is following columns 2 and 3. There is one increasing period (PV 10-18), two decreasing periods (PV18-22, 35-48) and two stable periods (PV 22-32, 48-65). The trends are again the same as the previous columns and the changes are connected with the changed of influent groundwater. The stable phases are following the zero order kinetics and all the other the first order kinetics.

In column 5, the spread of results in not visible and there are only one increasing phase (PV 10-15) and two stable phase (PV15-48, 48-65). The change in the phases is due to the change of spiked solution. In that column is also important that the amount of organic carbon that is provided by the system is higher and the ecosystem in the column can more easily assimilate the amount of organic carbon and the microbial activity is working with perfect conditions. In contrast to the other columns, here is visible that even the HRT that was chosen is working providing results that expected.

In column 6, is the column that only substrate material is wheat straw. Depending on other researches (Aslan, 2005, Aslan and Turkman, 2005) and the columns before, it was expected that with the higher amount of organic carbon that a system can provide, the denitrification

process can achieve the best results. In column 6, that is not confirmed. The reduction of nitrogen levels need more time to be achieved and the degradation rates are following different approach than all other columns. There is an initial decreasing period (PV 10-20), followed by two stable periods (PV 20-38, 38-65). The changes in the results are connected with the influent solution. The decreasing period is connected and followed by first order kinetics and the stable phases are following zero order kinetics.

In Table 5.6 there are the characteristics of Monod kinetics ($C=C_oe^{-\lambda t}$) that describe in more detailed the denitrification process in the columns. Additionally, there is the half-life to find out the time that demands to reduce the TN levels. In the adaptation lag phase is the phase that the reduction achieves levels more than 50%. In that phase there is the fast reduction due to the carbon that exist in waste materials that are used and the description of the adaptation lag phase fits better in 1^{st} order kinetics but it is not so clear due to the fast reaction as it is mentioned before.

In the stable phase, in the experiment that takes places is not easy to define only with one kinetic the trend in the experiment and it should be separated in smaller phases that described by zero and first order kinetics. The detailed description about the kinetics was given in previous paragraphs.

The description of the kinetics became with normalized concertation VS pore volume. That approach used to organize and to present the results from the microbial activity in the columns with better way. This approach used to describe clearer the kinetics that exist in the process. The approach is an instantaneous approach that used to describe in detail the conditions and the kinetics in specific time period. For each specific day there is the specific HRT that is stable in each experiment and the K for the specific day. For the specific day, it is measured the instant K. From that instant K analysed the instant zero order reaction. That was measured for each day. With each HRT as time it is received a unique K for the day that is measured and there is not a measure of K day to day. Curves or lines or other relationships day to day cannot be used to infer changes in mechanism for K.

Table 5.6: λ values and $T_{1/2}$ values on adaptation lag phase and stable phase

Adaptation Lag phase		Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Days	λ_{min}	0.006	0.194	0.001	0.005	0.001	0.270
	λ_{max}	7.053	4.915	4.235	5746	4.604	2.138
Hours	$T_{1/2\ min}$	2.350	3.380	3.914	2.905	3.626	7.780
	$T_{1/2\ max}$	31.430	33.770	382.570	11.910	123.080	61.970

Stable phase		Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
$Days^{-1}$	λ	5.045	5.331	4.481	5.040	4.990	4.810
	SD	0.611	1.360	1.151	1.840	0.880	1.250
Hours	$T_{1/2}$	3.050	3.696	4.344	4.584	3.901	4.968
	SD	0.016	0.057	0.068	0.105	0.073	0.138

5.3.5 Discussion of experiment 1

According to Table 5.3 and Figures 5.7 and 5.8 can be seen that the denitrification process is working in all columns. The removal rates from column 1 till column 5 have almost the same degradation rates and interestingly the lowest removal rate received in column 6 only with straw. The questions that rise from these experiments are the following.

1) Why the good removal rate in column 1 which contains only sand.
2) Why the poorest removal rate in column 6 which contains only straw.

The good removal activity in column 1 is an indicator that groundwater from Nitrabar project even after the pre-treatment in reed bed contains levels of carbon that can promote the denitrification process for the time period of experiment. The carbon is not a limiting factor for the duration of experiment for column 1 (100% sand). The columns 2 to 5 combine inert material with substrate material in different percentages and the removal rates range between 61-68%.

However, in column 6 (100% straw) the removal was the lowest than any other column with only 52%. The removal rate is an issue for that column. Due to the highest amount of carbon due to substrate material it was expected to achieve the same or even better removal than other columns. With that result is visible that only the carbon source material is not receiving the best results and the need on inert material is necessary.

All the results support the denitrification hypothesis for nitrate removal levels. The only result that changes from the initial hypothesis was in column 6. It is shown that the denitrification process is not strongly linked to the percentage of straw.

Perhaps there is a need for an inorganic substrate for the microorganisms to thrive. Study of microbiology is not part of this thesis, and as the focus is on systems within the ground, future column experiments will always include inorganic substrates.

According to these initial results, the next step of hypothesis was to extend the time period that solution remains in columns. The increase of HRT was the next hypothesis.

5.4 Results Experiment 2

According to the results of experiment 1 the main issues of the experiment was to provide more acceptable reduction in nitrogen compounds. The main issue from the results was that the time period when the influent water was changed the amount of NO_2-N was increasing showing that the denitrification process was not completed. For that reason, the main change in the experiment 2 was the HRT. The HRT changed to 50 hours. To estimate the time of HRT as it was happened in experiment 1 the measurements became according to the turnover times based on peristaltic volumetric flow in the influent and output quantity and the void volume in the columns that comes from the porosity. The columns remain the same in both experiment and the only change that happened was the HRT. According to the methodology that is provided in chapter 4 the estimated porosity for sand was 0.45 and for wheat straw 0.65. To determine that all the columns can provide the same HRT measurements became separately for each column to calibrate the influent solution with larger/smaller input tube. For experiment 2 of experiment, the HRT and the time period between samples was changed. Samples received every 4 days and the duration of experiment was 26 days. The HRT was 50 hours. With the change of HRT the dynamics in the columns were affected and expected in combination with the microbial activity and the organic carbon that wheat straw can provide to receive the expected results. In that experiment there is the introduction of TOC measurements to provide more details in the results and there are also measurements of PO_4-P.

Table 5.7: Results of experiment 2

		Influent	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
pH	mean	8.01	8.14	7.95	7.55	7.51	7.44	7.88
	SD	0.16	0.11	0.29	0.43	0.44	0.44	0.54
	% change		1.61	-0.75	-5.67	-6.19	-7.12	-1.66
Conductivity µS/cm	mean	434.71	359.29	342.86	327.86	328.14	332.86	343.57
	SD	25.28	43.25	45.87	37.02	50.07	5119.00	65.71
	% change		-17.35	-21.13	-24.58	-24.52	-23.43	-20.97
NO_2-N µmol/l	mean	2.58	3.32	27.33	4.01	5.09	4.72	9.75
	SD	1.02	0.46	21.31	4.05	3.92	3.80	8.63
	% change		28.92	960.24	55.42	97.59	83.13	278.31
NO_3-N µmol/l	mean	409.88	246.24	58.25	49.17	55.07	52.12	61.24
	SD	1.87	19.16	78.92	77.66	71.04	78.93	82.26
	% change		-39.92	-85.79	-88.00	-86.57	-87.28	-85.06
NH_4-N µmol/l	mean	4.31	4.33	4.09	3.98	4.30	4.02	4.05
	SD	0.97	0.86	0.76	0.70	0.38	0.75	0.81
	% change		0.55	-4.96	-7.54	-0.18	-6.80	-5.88
TN µmol/l	mean	416.76	253.90	89.67	57.16	64.46	60.85	75.05
	SD	3.13	18.87	75.93	77.04	70.24	78.04	82.56
	% change		-39.08	-78.48	-86.29	-84.53	-85.40	-81.99
PO_4 mg/l	mean	0.15	0.13	0.09	0.09	0.08	0.09	0.08
	SD	0.08	0.02	0.01	0.01	0.00	0.01	0.03
	% change		-17.13	-43.67	-43.58	-44.79	-43.30	-45.90

In Table 5.7 are the average results of experiment 2 for all the direct and indirect measurements for experiment and the change from the initial conditions

5.4.1 pH levels

In experiment 2, pH levels are higher than experiment 1. In column 1 the lowest value noticed in the first day of experiment and the last day with the highest value. In column 2 the lowest pH was in the first day and the highest again the last day. At all the columns the lowest pH was noticed in the 1st day and the highest in column 3 and 6 in the 9th day and for the column 5 in the 5th day and finally at the column 4th in the 17th day. The average levels were higher and there is increase at column 1 and at all the other columns a decrease 0.75-7.12%. The pH levels remained in the ideal range for denitrification process.

5.4.2 Conductivity levels

The conductivity levels in contrast to experiment 1 were higher in the initial solution and at all columns there was reduction between 17-25%. It had a wide spread (250-460 μS/cm). The highest (359.29 μS/cm) noticed in column 1 and the lowest in column 3 (327.86 μS/cm).

5.4.3 Nitrogen species

The detailed results of experiment 2 are shown in Figure 5.9 and 5.10. There are two groups of results as it was happened in experiment 1. There is initially the adaptation lag phase that has duration between 3-5 days and that is the time period where the microbial activity is rising accorded to Monod kinetics and that is the period where the main reduction of nitrogen compounds received. The second group of results is the Stable phase where the reduction has been achieved and there is the periods that the kinetics can be investigated in long term period and to provide the results that expected.

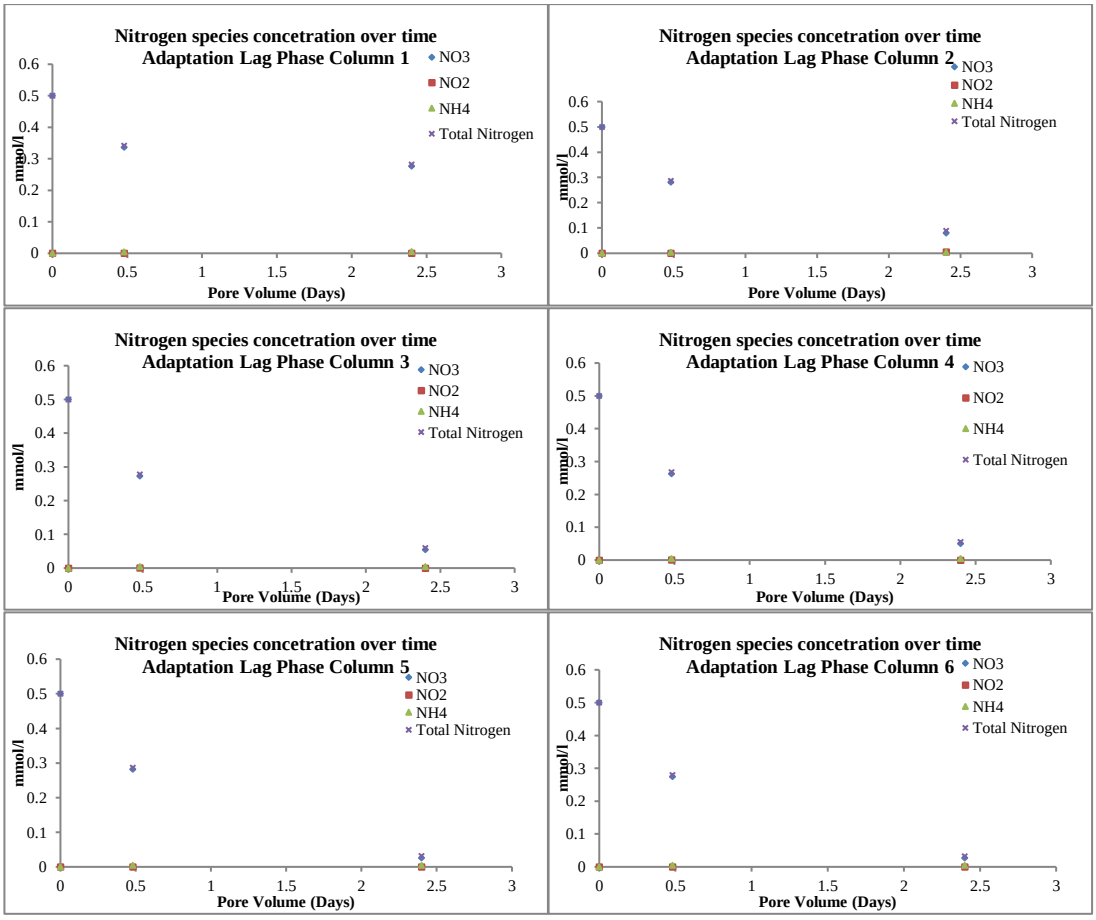


Figure 5.9: Nitrogen species concertation adaptation lag phase experiment 2

In experiment 2 the approach of the reduction of nitrogen compounds is more specific in the adaptation lag phase. The column 1, where the only substrate material was sand, now cannot provide the same reduction as it was noticed in experiment 1. The reduction levels are in 45% only. In contrast the column 2, 3, 4 can provide better results and reduction that achieved levels 80%. Finally, in the last columns 5 and 6 where the amount of wheat straw is higher and the provided organic carbon is increasing the reduction that is noticed at all nitrogen compounds is more that 85%. Here there is also not visible the change of influent water and the amount of NO_2-N and NH_4-N was not changed in that period.

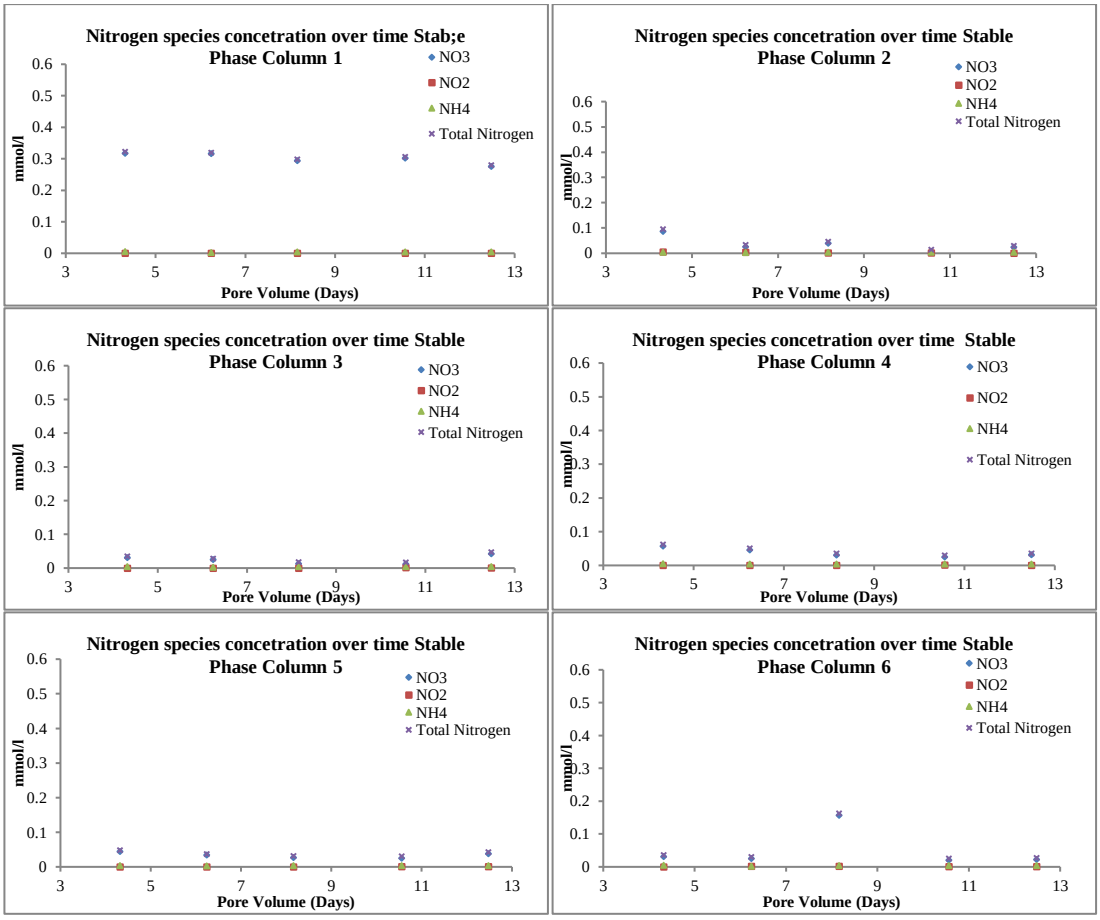


Figure 5.10: Nitrogen species concertation Stable Phase experiment 2

In column 1, the stable phase is separated in two parts. The NO_3-N and TN are following the same trend and the same is happening with NO_2-N and NH_4-N. The reduction in NO_3-N was only 45% and remains stable at all the duration of the experiment, showing that the system cannot provide the organic carbon to achieved higher reduction levels. Moreover, the NO_2-N and NH_4-N remain stable at all the duration of the experiment and supported the hypothesis that HRT is enough to provide a complete denitrification process in the columns. In contrast to experiment 1 here is not visible the change in the results due the changes in influent solution.

In column 2, the reduction levels of NO_3-N and TN are increasing and achieving levels higher than 85%. The NO_2-N and NH_4-N remain in very low levels without affecting the system. The changes in the influent solution are detected by the increase and decrease of NO_3-N and TN in the column.

Columns 3, 4 and 5 have the same trend as column 2. It is important because all of them have both of substrate materials and can provide to the system the demanding organic carbon to finish with success the denitrification process. As it is noticed in the previous columns the

amount of NO_2-N and NH_4-N remain close to zero at all the columns and it is not affected by the influent solution. The change of solution is detected in NO_3-N and TN levels which are increasing and decreasing along the experiment. At all columns the reduction is more than 85%.

Finally, column 6 is the column that only wheat straw is substrate material. The NO_2-N and NH_4-N remain in very low levels and NO_3-N and TN levels are reducing but not with the same way as in the previous columns. Here the change of the water is visible because the system needs more time to assimilate the influent groundwater and to provide the reduction in the same levels as before. Even here the reduction in NO_3-N and TN achieves levels more than 85%.

5.4.3.1 Nitrite levels

Nitrite levels except from column 2 and column 6 at all days of experiment were lower than 13.01 μmol/l for the duration of experiment. At column 2 from start till the 17th day of experiments nitrite levels were higher than 21.14 μmol/l with the highest in 5th day with 51.24 μmol/l. At column 6 between 13th and 17th day nitrite levels were at 21.15 μmol/l.

5.4.3.2 Nitrate levels

The nitrate levels (Table 5.8) were following a different approach than experiment 1. The initial concentration was the same 0.409 mmol/l NO_3^--N (SD=1.87).

Table 5.8: Nitrate levels experiment 2 **(**μmol/l)

μmol/l	**Pore Volume**	**Initial**	**Column 1 (100% sand)**	**Column 2 (80% sand, 20% straw)**	**Column 3 (60% sand, 40% straw)**	**Column 4 (40% sand, 60% straw)**	**Column 5 (20% sand, 80% straw)**	**Column 6 (100% straw)**
Day 1	**0.48**	411.60	276.23	229.76	222.99	214.60	230.57	224.44
Day 5	**2.40**	409.83	224.44	56.47	40.34	37.11	16.62	17.75
Day 9	**4.32**	411.60	258.32	63.09	21.30	42.92	32.43	21.14
Day 13	**6.24**	406.60	256.39	14.52	18.07	33.56	25.33	16.46
Day 17	**8.16**	408.54	237.99	26.46	6.62	20.17	17.59	122.95
Day 22	**10.56**	411.44	246.70	1.94	4.68	15.81	15.81	12.10
Day 26	**12.48**	409.51	223.63	15.49	30.17	21.30	26.46	13.88

In column 1, nitrate levels were higher than expected. The average along the experiment was 0.246 mmol/l, (SD=19.16) and the highest was noticed in 1st day with 0.276 mmol/l and the lowest in the last day with 0.223 mmol/l NO_3^--N. The reduction along the experiment achieved levels close to 40%.

In column 2, the reduction was visible and achieving levels more than 85%. The highest received in 1st day with 0.229 mmol/l and the lowest in 22nd day with 1.94 μmol/l NO_3^--N. The average along the experiment was 58.25 μmol/l NO_3^--N (SD=78.92).

In column 3, there was reduction more than 88%. The highest noticed in 1st day with 0.222 mmol/l and the lowest in 22nd day with 4.68 μmol/l NO_3^--N. The average along the experiment was 49.17 μmol/l NO_3^--N (SD=77.66).

In column 4, nitrate levels were slightly higher than column 2 and 3 with average 55.07 μmol/l (SD= 71.04). The highest noticed in 1st day with 0.214 mmol/l and the lowest in 22nd day with 15.81 μmol/l NO_3^--N.

In column 5, nitrate levels were following column 4. The highest noticed in 1st day with 0.230 mmol/l and the lowest in 22nd day with 15.81 μmol/l NO_3^--N. The average along the experiment was 52.12 μmol/l NO_3^-N (SD=65.85).

In column 6, nitrate levels reduced more than 85%. There is a peak at 17th day with 0.122 mmol/l except from the highest levels at 1st day 0.224 mmol/l. The lowest noticed in 22nd day with 12.10 μmol/l and the average along the experiment was 61.24 μmol/l NO_3^--N (SD=82.26).

5.4.3.3 Ammonium levels

Ammonium levels at all columns and along the experiment were lower than 5.54 μmol/l NH_4^+-N. The low ammonium levels and the low nitrite levels in combination are showing that HRT was long enough to complete the denitrification process in contrast to part 1 of experiment.

5.4.3.4 Total Nitrogen levels

The TN levels (Table 5.9) follow nitrate levels. The levels in column 1 were higher than the other columns with average 0.254 mmol/l TN.

Table 5.9: Total Nitrogen levels experiment 2 (μmol/l)

μmol/l	Pore Volume	Initial	Column 1 (100% sand)	Column 2 (80% sand, 20% straw)	Column 3 (60% sand, 40% straw)	Column 4 (40% sand, 60% straw)	Column 5 (20% sand, 80% straw)	Column 6 (100% straw)
Day 1	**0.48**	419.26	283.93	238.01	229.81	223.00	237.40	232.30
Day 5	**2.40**	417.76	233.78	112.48	46.52	43.89	23.51	24.03
Day 9	**4.32**	417.83	266.90	117.84	27.14	49.37	38.71	28.13
Day 13	**6.24**	410.67	262.20	62.66	22.30	39.51	30.00	40.31
Day 17	**8.16**	414.38	245.35	52.52	12.46	31.88	26.96	148.30
Day 22	**10.56**	418.81	254.02	18.16	20.90	31.81	31.87	28.10
Day 26	**12.48**	418.62	231.11	26.01	40.96	31.76	37.52	24.18

In all the other columns the levels of TN are lower than 90 μmol/l and with the lowest noticed in the 22nd day of experiment. The TN levels in combination to all other nitrogen species were the indicator for denitrification process. It is noticeable that TN levels reduced more than 78% at all cases and it is a results that is in agreement with all the other researches (Salining et al., 2007, Talebnia et al., 2010)

5.4.4 Phosphate levels

Phosphate levels (Table 5.10) at all the duration of the experiment were lower than 0.2 mg/l with the highest in the column 1 only with sand. This observation will be studied in more detail in the next chapter. As an initial approach the only output that can be provided by this experiment is that the higher amount of wheat straw can also provide higher reduction of phosphate levels.

Table 5.10: Phosphate levels experiment 2 (mg/l)

mg/l	Initial	Column 1 (100% sand)	Column 2 (80% sand, 20% straw)	Column 3 (60% sand, 40% straw)	Column 4 (40% sand, 60% straw)	Column 5 (20% sand, 80% straw)	Column 6 (100% straw)
Day 1	0.084	0.122	0.080	0.084	0.084	0.099	0.126
Day 5	0.084	0.101	0.076	0.072	0.080	0.076	0.070
Day 9	0.171	0.123	0.072	0.078	0.088	0.088	0.103
Day 13	0.095	0.104	0.080	0.087	0.082	0.085	0.093
Day 17	0.114	0.144	0.105	0.104	0.083	0.078	0.063
Day 22	0.253	0.153	0.094	0.090	0.086	0.092	0.069
Day 26	0.273	0.143	0.098	0.091	0.090	0.091	0.057

5.4.5 Degradation rates

Degradation rates in Figure 5.11 followed the observed reduction of TN levels in the first experiment (paragraph 5.4.3.4). At all columns there was a visible initial adaptation lag phase. That phase had duration 4-6 days (PV 0-4) depending on substrate materials of each column.

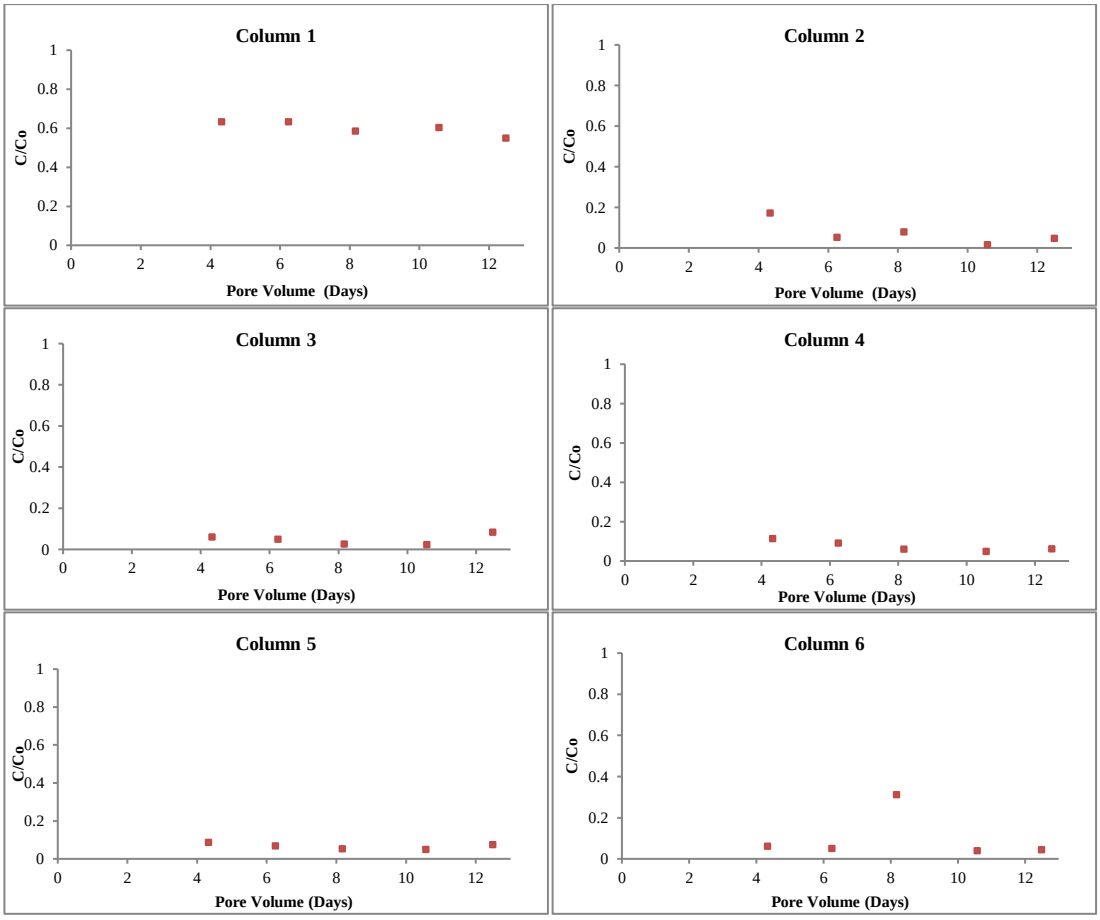


Figure 5.11: Degradation rates experiment 2

The initial adaptation lag phase cannot be described in detail by the noticed period of that experiment and cannot provide results in the way that the microbial activity reacts in the column in the first 3-5 days. The degradation rates can be described in the stable phase. All the degradation rates as it is mentioned in experiment 1 are following Monod Kinetics due to microbial activity that exists. Additionally, from the reduction that is noticed in the previous paragraph, the separation of phases can be easily become. As it is mentioned there is a description of instantaneous rate constants with specific time between each measurement. That time was the PV time.

In column 1, the degradation rate at all the duration of the experiment can be described by the zero order kinetics without separated the period in smaller periods.

In column 2, the change of influent water affects the results and separated the experimental period in 3 smaller periods. The first one is from PV 4-6 where there is a reduction period and the kinetics are following the first order kinetics and two stable periods PV6-8, 10-13 which are following the zero order kinetics.

In column 3, the influent water affects the microbial kinetics and the separates the period in two smaller periods. The first one is from PV4-10 which is a stable period and follows the zero order kinetics. The last period PV10-13 is an increasing period that follows the first order kinetics.

In column 4, the results of groundwater solution are visible and affect the kinetics. There is a decreasing period from PV 4-10 which is following the first order kinetics and the last period PV 10-13 is a stable period which is described by zero order kinetics.

The column 5 follows the same trend like column 4 and the changes in the kinetics are exactly the same.

In column 6, there is a change in kinetics. There are two stable periods which are described by zero order kinetics (PV 4-6, 10-13) and there is an increasing period (PV6-8) and decreasing period (PV8-10) that are described by first order kinetics.

According to Table 5.11 degradation rates are characteristics to describe the kinetics in the columns and the microbial activity that affect the denitrification process. In the adaptation lag phase is the phase that the reduction achieves levels more than 50%. The highest reduction in nitrogen compounds noticed in the Columns 2,3,4,5 that there is a combination of substrate materials. In that phase there is the fast reduction due to the carbon that exist in waste materials that are used and the description of the adaptation lag phase fits in 1st order kinetics. In the stable phase, in the experiment that takes places is not easy to define only with one kinetic the trend in the experiment and it should be separated in smaller phases along the experiment and that periods can be described by zero and first order kinetics depending on the trends. The detailed approach of the kinetics is described in the previous paragraphs.

Table 5.11: λ Values and Half Life in adaptation lag phase and Stable Phase Experiment 2.

Adaptation Lag phase		Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Days	λ_{min}	0.189	0.275	0.290	0.308	0.275	0.287
	λ_{max}	0.284	0.881	1.065	1.098	1.416	1.400
Hours	$T_{1/2\ min}$	5.86	18.88	15.61	15.14	11.74	11.88
	$T_{1/2\ max}$	88.00	60.39	57.28	57.28	60.56	57.99

Stable phase		Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
$Days^{-1}$	λ	0.244	1.372	1.499	1.259	1.306	1.267
	SD	0.028	0.402	0.261	0.163	0.112	0.464
Hours	$T_{1/2}$	67.104	14.040	12.070	13.700	12.640	14.660
	SD	0.333	0.184	0.105	0.070	0.040	0.311

In column 1 the adaptation lag phase was 5 days and after there was a stable phase. In column 1 due to sand as substrate material the reduction was not the expected. The λ value along the experiment is 0.242 (SD=0.036). The half-life in column was 67.104 hours (SD=0.333). Here there is a considerable difference in the half-life that is likely due to full use of dissolved organic matter, and therefore the control is different than the other columns for the longer HRT.

In column 2, the adaptation lag phase had duration 5 days. The stable phase was very consistent. The λ value along the experiment was 1.145 (SD=0.537). The half-life in column was 14.04 hours (SD=0.184).

In column 3, the initial phase had duration 5 days. The stable phase was consistent as column 2. The λ value along the experiment was 1.264 (SD=0.506). The half-life in column was 12.07 hours (SD=0.105).

In column 4, the adaptation lag phase had duration 5 days as all the columns. The λ value along the experiment was 1.100 (SD=0.379). The half-life in column was 13.70 hours (SD=0.07).

In column 5, the λ value along the experiment was 1.174 (SD=0.409). The half-life in column was 12.64 hours (SD=0.04).

In column 6, the λ value along the experiment was 1.146 (SD=0.505). The half-life in column was 14.66 hours (SD=0.311).

5.4.6 Discussion experiment 2

The longer HRT in columns was studied to see if HRT is important as mentioned in the hypothesis, if there is connection between the removal rate and the retention time. The initial solution was the same in both experiments and the microbial activity remained the same. It was expected due to the higher HRT that the removal rates would be more effective. In all columns the reduction of nitrogen compounds increased and the λ value decreased except from column 1. In column 1 (control) the higher HRT created problems with carbon availability during the experiment. For that reason, the removal rates reduced only 40%. In all the other columns due to the available carbon substrate material, the removal rates increased and showed the best results. The removal rate reached levels 90% at all columns even in column 6 (100% straw). That was another interest point for column 6 showing that the flow rate is very important factor for denitrification process.

5.5 Discussion

The columns separate into four categories, the first was the control column with 100% sand as substrate medium. The second category was the columns that the majority of substrate medium was sand than wheat straw (column 2, 3). The third category was the category where wheat straw proportion was higher than sand (column 4, 5). The last category was column 6 with 100% straw.

Control column was the column that the only substrate material was sand. It is used as control column.

The pH levels in experiment 1 were the highest than any other columns. In experiment 2, pH levels were high but not as high as in experiment 1.

Conductivity levels in experiment 1 had wide range and achieving the highest levels that noticed in experiment at all columns. At experiment 2 the values were balanced without wide range.

Nitrite levels at both parts of experiments were really low. In experiment 1, at all the duration of experiment less than 6.09 μmol/l NO_2-N achieved. In experiment 2, less than 4.34 μmol/l NO_2-N achieved.

Nitrate compounds were the most important part of the experiments. It was noticed in experiment 1; reduction more than 75% with average levels about 84.39 μmol/l. In contrast at experiment 2, the reduction was only 45% with average levels more than 0.246 mmol/l at all the duration of experiment.

Ammonium levels were really low in both parts of experiment. The only difference was that in experiment 1, the levels were 10 times higher than the second experiment with average levels 0.5 μmol/l. In experiment 2, the average levels were 5.54 μmol/l.

NO_3-N levels followed TN levels and at experiment 1, of experiment lower than experiment 2 with average levels 95.09 μmol/l and 253.90 μmol/l respectively.

Finally, phosphate levels were low at both experiments with levels < 0.15 mg/l at in columns.

The second group of columns are columns with higher percentage in sand than substrate material. These columns are column 2 and column 3. The pH in part 1 was much lower at the

start of experiments due to the connection of groundwater with substrate materials. The lower levels were increasing during the experimental period and they ended up with almost the same average levels along the experiment with slightly higher levels in column 3 where the amount of wheat straw is higher. In experiment 2, pH levels were much higher than experiment 1, and it was noticed that higher levels exist in column 2 with higher amount of sand.

Conductivity levels at experiment 1 were following almost the same observation. In column 2, there was smaller range of results and the conductivity tends to decrease along the experiment. In column 3 the range of results was wider and there was no reduction as noticed in column 2. After the 13th day there was again an increase gap (600 μS/cm) that was not noticed previous in columns. In experiment 2, the attitude of the columns was the same. The conductivity levels were much lower than part 1, with average levels in column 2 (342 μS/cm) and in column 3 (327 μS/cm), without wide range of results. The difference that noticed was that in column 2 there was an increasing rate along the experiment and in column 3 there was a decreasing route during the experiment.

Nitrite levels in experiment 1 received noticeable amounts in both columns. The levels were higher in column 2 with average levels along the experiment 37.16 μmol/l NO_2-N and in column 3, 23.25 μmol/l NO_2-N. The range of results in that part was wide. In experiment 2, nitrite levels were lower but again in column 2 noticeable (27.38 μmol/l NO_2-N). In column 3 the levels were really low with average amount (3.91 μmol/l NO_2-N).

The results from the columns in experiment 1 were the same for nitrate levels. There was a decrease in both columns and it was noticeable that the reduction was faster in column 2 with lower amount of wheat straw. The reduction in column 2 was 75% with average levels 83.18 μmol/l NO_3-N along the experiment. In column 3 the reduction was about 70% with average levels 0.118 mmol/l NO_3-N. In experiment 2, there was reduction more than 85% at both columns with better result in column 3. The average levels in that part was for column 2 58.25 μmol/l NO_3-N and for column 3 49.17 μmol/l NO_3-N.

Ammonium levels experiment 1, were really low and all cases less than 27.71 μmol/l NH_4-N at both of the columns. In experiment 2, ammonium levels were really low, less than 5.54 μmol/l NH_4-N at all cases.

Total Nitrogen levels are the sum of all nitrogen species. In experiment 1, higher amount of TN noticed and it was noticeable that the average levels were higher in column 3 (0.141mmol/l N)

than in column 2 (0.116 mmol/l N). In experiment 2, TN levels were much lower and here the lowest levels noticed in column 3 (average 57.16 μmol/l N) than in column 2 (89.67 μmol/l N).

Finally, phosphate levels at both experiments were really low and < 0.1 mg/l. Phosphate will be studied in future experiments.

The third group were columns with higher percentage of wheat straw than substrate material. These were column 4 and column 5. The pH levels were lower than other columns and it was observed that the more wheat straw the lower the pH is (perhaps due to organic acids, but as the pH was near neutral this was not considered a controlling factor). There was an increasing trend in both columns but pH levels were lower in column 5 (average pH=6.91) than column 4 (average pH=7.19). The levels were much higher and more neutral in the second experiment when compared with the first with average levels in column 4 pH=7.51 and in column 5 pH=7.43.

Conductivity levels were similar to experiment 1. There was wide range of results in column 4 with many increases and decreases during experimental period and very high average levels (545.9 μS/cm). Column 5 follows a decreasing trend along the experiment without fluctuations. The average levels were 481.3 μS/cm. In experiment 2, conductivity levels were much lower than experiment 1. Both of columns follow the same trend from the start till one point were increasing and after that there was decrease. The average levels were almost the same with column 4 (328 μS/cm) slightly lower than column 5 (332 μS/cm).

Nitrite levels were low in both experiments. In experiment 1, there was a higher amount of nitrite in column 4 with average levels 24.12 μmol/l NO_2-N and in column 5 that levels were much lower (7.82 μmol/l NO_2-N). In experiment 2, nitrite levels were lower than experiment 1, and in both columns the average levels were the same (4.78 μmol/l NO_2-N).

Nitrate levels were reducing at both of the columns in experiment 1. The reduction that achieved in column 4 was 74% with average levels along the experiment (0.102 mmol/l NO_3-N) and in column 5 was 72% with average levels along the experiment (0.138 mmol/l NO_3-N). In experiment 2, the reduction levels were more than 85% in both columns with the best result in column 5. The average levels in column 4 were 55.07 μmol/l NO_3-N and in column 5 52.12 μmol/l NO_3-N.

Ammonium levels were low in both columns in experiment 1. The levels were the same in both columns with average levels along the experiment19.95 μmol/l NH_4-N. In experiment 2,

ammonium levels were again much lower than 5.54 μmol/l NH_4-N at all the duration of experiment.

Total Nitrogen levels in experiment 1, were noticeable and higher than expected. It was noticed that in column 5 with higher amount of wheat straw the TN levels were higher (average levels 0.149 mmol/l N) than column 4 (average levels 0.125 mmol/l N). In experiment 2, TN levels were much lower and in contrast to experiment 1 in column 5 there were lower levels than in column 4 with 0.064 mmol/l and 0.060 mmol/l N, respectively.

Finally, phosphate levels at both experiments were really low and < 0.1 mg/l at all time.

The last category was the column with wheat straw only. It is used as an additional control column. This column investigated the non-preferable (micro-aerophilic) for the system conditions that exist only wheat straw as substrate material and there is no mixture with any other material. In Column with the wheat straw the lowest levels of pH received in experiment 1 of the study with the lowest average levels (pH=6.86). In experiment 2, pH levels were not lower than other columns in contrast, this column achieved third highest average levels with pH=7.87 at all duration of experiment.

Conductivity levels in experiment 1 as it is noticed at all columns had wide range with average levels 486.7 μS/cm. It was the lowest levels of conductivity in experiment 1. In experiment 2, conductivity levels were much lower than experiment 1. The average levels were 343 μS/cm, which was the highest conductivity level in the columns in experiment 2 of experiment.

Nitrite levels were really low at both of the parts of experiment. In experiment 1, average levels were 12.82 μmol/l NO_2-N and in experiment 2 9.78 μmol/l NO_2-N.

Nitrate levels in experiment 1 remained in high levels with reduction only in 57% with average levels along first experiment 0.171 mmol/l NO_3-N. In experiment 2, the reduction levels were much higher more than 85% and average levels 0.061 mmol/l NO_3-N.

Ammonium levels were low in experiment 1 with average levels 19.95 μmol/l NH_4-N. In experiment 2, ammonium levels are much lower less than 5.54 μmol/l NH_4-N.

TN levels in experiment 1 were higher than all the other columns with average levels 0.186 mmol/l N. In experiment 2, TN levels were much lower with average levels along the experiment 0.075 mmol/l N.

Finally, phosphate levels at both experiments were < 0.13 mg/l.

According to the results in the first experiment, it is visible that wheat straw is working as substrate material. The conclusion from that experiment is that there is not a strong correlation of denitrification potential with correct mixture of the materials. However, the choice of correct flow rate is important to receive the best reduction. In connection with flow rate, HRT is important part and the more the ware solution remains in column the better reduction rates achieved. The research is in agreement with other researchers that focus on wheat straw as substrate material (Soares et al., 1991; Aslan and Turkman, 2005).

With these results it is clear that the system is working and perhaps change of the substrate may have vantage to further research. The main point for this thesis is to investigate new materials that work in denitrification process. Therefore, further study will focus on low cost materials available as agricultural waste.

The results from both experiments are showing the denitrification process takes place. It is noticeable that the faster HRT is an important factor and that a system with lower retention time is more preferable. That is described in the results of chapter 4 showing that in part 1 and first experiment the HRT was not enough to assimilate the influent water solution. That was also visible with the detection levels of NO_2-N and NH_4-N that were higher in the time when the influent water changed. In contrast in part 2 where the HRT increased detection of NO_2-N and NH_4-N were low and did not affect the system. Additionally, the NO_3-N and TN reduction was much higher in part 2 (all columns except from column 1 < 85%) than part1. This is also in agreement with degradation rates that exist. The system in part 1 cannot be stabilised so easily and the changes of influent water affect the system. The amount of organic carbon is in connection with the amount of denitrification microbial activity that exist in the solution and that is why in part 1 there is reduction in NO_3-N and TN which is not visible in part 2.

The environment for microbial denitrification in both cases is the ideal with pH and conductivity levels to spread in the ideal range. Additionally, the degradation rates were following the same trend, with an initial adaptation lag phase between 3-6 days and then a stable phase. The HRT and the half-lives (between 4-70 hours) that achieved in the experiments are in agreement with the other researchers (Patterson et al., 2005, Robertson, 2010). Finally, the retention time in both experiments is much faster than the half life time showing that along the experiment the microbial activity is active and the denitrification process taking place.

5.5.1 Evaluation of experiments

The hypothesis that was under investigation was if the system of columns with sand and straw as substrate material can be used to reduce total nitrogen levels. The chapter 5 was separated in two experiments. In the one, the hypothesis was to find out if the system was working in a sustained way according to other published results and HRT that is in acceptable limits (Lee et al., 2004; Tanaka et al., 2007; Calderer et al., 2014). The groundwater that used received from an area that designed for removal of nitrogen levels through denitrification process. The denitrifier bacteria already exist in solution. The pre-treatment tank used to clear all chemicals and pesticides that exist in the solution and to sustain the microbial activity for groundwater solution. From the first experiment the main outcomes that the research focuses on were the following. The denitrification process takes place in columns. Microaerophilic conditions are met in the chapter 5 where after denitrification process there are some levels of nitrogen compounds along the experimental process. The reduction is achieving levels that are higher than 50% at all nitrogen compounds but still exist a countable amount of nitrogen compounds that is connected with the conditions that exist in columns. The removal rate at all columns received levels between 52-73%. The column that contains only inert substrate material (sand) is also working properly with high removal rate, showing that the carbon is not limited for the duration of the experiment. Additionally, the reduction in the column only with reactive substrate material (straw) and not inert material did not receive the best reduction, showing that a combination of them is the best design for a proper denitrification system. The HRT in combination with flowrate and the microbial activity that established in columns could not provide the expected results. Additionally, in the last column the accumulation period that the system needs is longer than the columns with sand and wheat straw. It is also connected with the porosity of the system and the granular consistency that exists and can create more easily the microbial colonies in the environment that exist at least an inert material.

The system is working and the next step was to change the flow rate and HRT time in columns for the solution to provide the results that expected and the first experiment could not provide. The new hypothesis for the second experiment was to find out if higher HRT in columns is combined with better results and higher levels of nitrogen removal. Parallel phosphate levels were analysed to find out if there is connection of the selected materials with denitrification process and phosphate removal. The process remains the same with the pre-treatment tank. The results that received confirmed the hypothesis. There is a connection between HRT and removal rates. The removal rates achieved higher levels than experiment one between 39-87% and there were some differences. In column with sand (control) the degradation rates were the lowest. The

longer HRT was connected with carbon availability and that was observed to the removal rates that reduced from 73% to 39%. In contrast in column with straw only the removal rates increased from 52% to 82%. Phosphate levels along the experiment reduced at all columns and the worst results received in sand column. Further investigation between phosphate levels and denitrification process must be done in future experiments showing in details the combination of these two processes and if they can work simultaneously.

The denitrification process in columns is connected with microbial activity. In both parts of experiment there was an initial adaptation lag phase. This phase was between 4 and 6 days at both parts of experiment. The biodegradation follows Monod kinetics ($C/Co=e^{-\lambda t}$) as it was described in details in both experiments.

Table 5.12: λ value, % removal, $T_{1/2}$, and Retention Time experiment 1and 2 in stable phase

λ Value	Experiment 1	Experiment 2
Column 1	5.045	0.242
Column 2	4.524	1.145
Column 3	3.843	1.264
Column 4	4.346	1.100
Column 5	3.962	1.174
Column 6	3.418	1.145

% Removal	Experiment 1	Experiment 2
Column 1	72.97	39.08
Column 2	67.90	78.48
Column 3	62.87	86.29
Column 4	65.91	84.53
Column 5	61.18	85.40
Column 6	52.50	81.99

T ½ (hours)	Experiment 1	Experiment 2
Column 1	3.05	67.10
Column 2	3.69	14.04
Column 3	4.34	12.07
Column 4	4.58	13.70
Column 5	3.90	12.64
Column 6	4.96	14.66

RT (hours)	Experiment 1	Experiment 2
Column 1	0.96	9.21
Column 2	0.84	13.68
Column 3	0.86	12.12
Column 4	0.50	13.51
Column 5	3.67	11.68
Column 6	2.16	6.09

Denitrification processes are likely connected with the chemistry of solution that passes through the columns and the time that it remains in columns. There is a combination of porosity and velocity in the process. Focus on the Monod kinetics and the parameters that affect the denitrification process, λ value was connected with the process that happened and *t* with the media that used. The residence time factor *t* is connected with the porosity of the materials in the columns (residence time will decrease with decreasing porosity). It is important to consider then the effects of a change of the porosity which may results if there is biofouling in columns.

Biofouling or biological fouling according to the definition is the accumulation of microorganisms, plants, algae, or animals on wetted surfaces. Such accumulation is referred to as epibiosis when the host surface is another organism and the relationship is not parasitic (Rivett et al., 2008; King et al., 2012). The result of biofouling is to create another volume countable in columns which were not noticed at both experiments.

As an example, to explain differences between substrate columns and the control column the porosity in the control column would need to be reduced by 80%, and according to the dimensions of the columns the new porosity would drop from 0.45 to 0.09. This was impossible and it was not noticed along the experiments. The only factor that affected the process was λ Values (Table 5.12). The quantity of water that gets in columns was the same amount that came out and this remained stable with the use of peristaltic pump. According to Darcy's law $Q = -kA\frac{dh}{dl}$ the quantity of water is a factor of porosity and a factor of velocity (Soares et al., 1991). The porosity as it is mentioned remain the same and velocity is the only factor that was changing along the experiment.

Finally, phosphate levels at all experiment were really low and there is a need to study a system where phosphate is measurable and for which phosphate removal be important. This will be the focus of the next series of experiments.

CHAPTER 6

COLUMNS EXPERIMENT WITH SAND, MULCH AND PERLTE AS SUBSTRATE MATERIALS

6.1 Introduction

In Chapter 5, the experiment with sand and wheat straw shown that a denitrification process took place in the columns and the reduction of nitrogen levels was observed. The reduction was > 70% in columns studies. The new hypothesis in columns studies is to investigate new materials that can be used for denitrification process and which may also be useful in phosphate removal.

A new material considered for investigation was perlite. The properties of perlite absorb heavy metals and phosphate compounds. It is now investigated if these properties can used in combination with organic substrates that reduce nitrogen compounds. The new hypothesis was to investigate the reduction of nitrogen levels in columns studies that was combined with substrate material the perlite. The total nitrogen reduction that expected is significant with levels more than 50% and a reduction of phosphate levels.

6.2 Substrate materials

In this experiment the substrate materials that used were sand, perlite and mulch. Except from the materials that are used there are several other that investigated in similar studies. These kinds of materials were crab shells chitin (Robinson-Lora and Brennan, 2009), newspapers (Volokita et al., 1996), sawdust, cotton (Su and Puls 2006, Della Rocca, 2005, 2006), liquorice (Ovez, 2006), wood chips (Greenan et al., 2006, Saliling et al., 2007, Leverenz et al., 2010), compost (Gibert et al., 2008), softwood (Gibert et al., 2008), hardwood (Gibert et al., 2008), willow (Gibert et al., 2008) , atrazine (Hanter and Shaner, 2010). In these studies there was investigation under horizontal and vertical flow rate (Tunsciper, 2009, Garcia et al., 2004, Narvaez et al., 2011, Schipper et al., 2010a,b). The denitrification process was under investigation and it was separated in autotrophic and heterotrophic denitrification and depended in carbon sources that exist (Della Rocca et al., 2006, 2007). The carbon source was only the

substrate materials and there was no other source to add carbon amount on the experimental design system with columns.

Previous experiments that investigate mulch as substrate material include wetland studies (Moutsopoulos et al., 2011, Saeed and Sun, 2011a, Albuquerce et al., 2009), PBR (Gibert et al., 2008, Guo and Blowes, 2009, Robertson, 2010), SBR (Kulkarni, 2013, Rodriguez et al., 2011, Trois et al., 2010) and column studies (Xu et al., 2013, Saeed and Sun, 2011b, 2012, Essandoh et al, 2013). The process all the time was different depending on the conditions of each research and the composition of every material.

Perlite is a new material that has not been previously studied along with in-situ denitrification and will be used in experiments here. There are several studies about perlite and removal of heavy metal, leaching waste materials from nursery wastes, and phosphorus removal studies, but no one of them focused on the nitrogen compounds removal through denitrification process with effective results in column studies.

6.2.1 Perlite

Perlite is a volcanic glass formed when lava cools very rapidly trapping small quantities (2-5% w/w) of water and it is typically formed by the hydration of obsidian (Jamei et al., 2011). It occurs naturally and has the unusual property of greatly expanding when heated sufficiently (< 500 ^{o}C). Perlite (Figure 6.1) expands to about 13 times its original volume when it is heated to a temperature of approximately 871 ^{o}C.

Figure 6.1: Perlite (www.succseed.com)

During the heating process, the mineral particles of perlite pop like popcorn and form a granular, snow-white material that is very light (80-128 kg /m^3) (Jamei et al., 2011).

It has a highly adsorbent surface and a very low bulk density which makes it an ideal carrier or low cost filler for many compound formulations. In addition, because of the physical shape of each particle of perlite, air passages are formed in the growing media thereby providing excellent aeration. Expanded perlite is physically stable and chemically inert (Gonzaga et al., 2009).

In horticultural perlite is as useful to the home gardener as it is to the commercial grower. It is used with equal success in greenhouse growing, landscaping applications and in the home in house plants (Yamashita et al., 2011). This is the result of the characteristics of perlite that can provide the suitable aeration conditions in greenhouse commercial use, providing also the suitable porosity for the routes of the plants to grow up faster and to absorb the nutrient materials faster. The same results in smaller scale are also noticed in house plants that the use is more about garden architecture and design and not to provide a production.

In the agrichemical industry, perlite is used as a carrier for pesticides and herbicides, fertilizer bulking, and pelletized seeds. Perlite is used in environmental applications to absorb oil, and to control and clean up pollution. Beer, wine, juices, chemicals, pharmaceuticals, oils, acids, sugars, bio diesels, and water (potable, swimming pool, and storm runoff) are all filtered with perlite that has been expanded and crushed to form a maze of microscopic pathways. Due to their unique physical structure, perlite filter aids offer high flow rates with optimum clarity. They are especially applicable to highly viscous liquids such as syrup or gelatinous slurries requiring fast flow rates. Productivity, clarity and flow rates may be increased through the use of perlite filter aids (Gironas et al., 2008).

The use of perlite is very effective to reduce heavy metals like Pb, Cu (Dyer et al., 2004; Silber et al., 2010). There is an investigation with recognised a reduction of nitrates at 83% and phosphates at 91% using perlite to treat leachates (Ozel et al., 2012). In agro-industrial wastewater the reduction due to denitrification process at NO_3 noticed more than 30% and at phosphates more than 70% (Tanaka et al., 2007).

Perlite is selected except from all the previous reasons, for one more. It is the connection point of research with East Mediterranean area and more specifically Greece which is my country. Greece is the country which has the largest major global production of perlite (USGS). The main advantage is the low cost of production of perlite. It can be easily used not only for laboratory use but also for field works with success.

6.2.2 Mulch

The denitrifying material that selected here was mulch. Mulch (Figure 6.2) is a combination of many things that contain higher amounts of carbon. Studies till now shown that mulch is a media that is effective for denitrification. It is used as media in column studies and PRB studies (Gilbert et al., 2008) providing high amount of carbon to the process.

Mulch contains organic residues: grass clippings, leaves, hay, straw, kitchen scraps comfrey, shredded bark, whole bark nuggets, sawdust, shells, woodchips, shredded newspaper, cardboard, wool, animal manure and other materials. Many of these materials also act as a direct composting system, such as the mulched clippings of a mulching lawn mower, or other organics applied as sheet composting. Rock and gravel can also be used as mulch. In cooler climates the heat retained by rocks may extend the growing season (Kasirajan and Ngouajio, 2012).

Figure 6.2: Mulch (mulchandmore.webs.com)

The difference from other studies was the composition of mulch. It is used the Laboratory Soil Homogenization/Compositing (Cone and Quarter Method). Focused on the initial hypothesis that only easily found and cheap materials used in the experiments, the composition was the following: 70% (w/w) compost soil, 15% (w/w) wheat straw and 15% (w/w) sawdust. The mixture was homogenised and remain for 15 days in columns with water recirculation to be ready to use for the experiment.

6.3 Design of experiment

The design of experiment is the same as Chapter 5. There was the same wetland (Figure 6.3) with the same reed bed (*Phragmites Australis*). The groundwater that used selected also from Ballymena area in Northern Ireland the same time period as in Chapter 4. The pre-treatment tank used to clean groundwater from all possible chemical compounds that exist within.

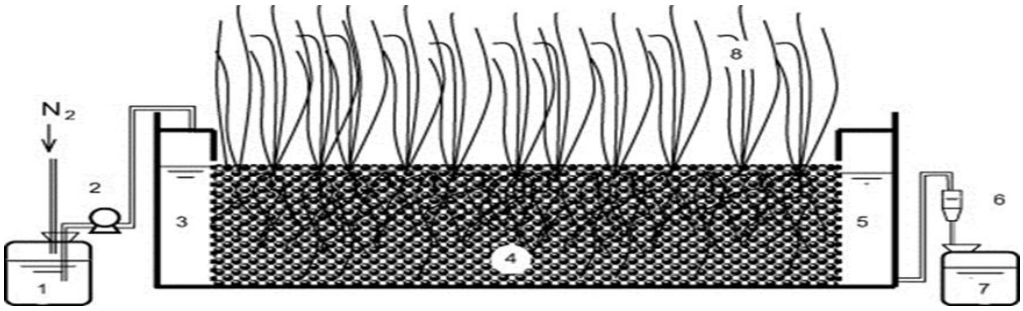


Figure 6.3: Reed bed tank/ pre-treatment tank (www.specifiedby.com)

The retention time through wetland is the same as in chapter 4 (3 days - 8.3 litres per day). The stable flow rate ensured with the use of peristaltic pump with stable rotation speed. The pre-treated groundwater kept in 4 ^{o}C till the time of application in columns. The second component of experiment was the columns experiment. In this experiment seven columns used to investigate all the possible scenarios with different substrate materials.

6.4 Columns design

Seven columns were used (Table 6.1 and Figure 6.4). The dimensions and diameter were the same as Chapter 5 (length 55 cm, diameter 5 cm).

In this experiment the substrate materials used were sand, perlite and mulch. The same particles sizes as in in Chapter 5 were used for the sand with< 0.66 cm diameter, and perlite was also sieved to use < 1 cm particles.

Mulch was a mixture of compost soil 70% (w/w), wheat straw with particles less than 1 cm, 15% (w/w) and sawdust, 15% (w/w). The mixture was selected to create initial carbon levels that are high enough to create suitable conditions for denitrification process. The compost soil primarily, wheat straw and sawdust contain high levels of carbon. According to other studies (Saeed and Sun, 2011a,b, 2012, Su and Puls, 2007), there should be an initial amount of carbon to force denitrification process.

Figure 6.4: Design of columns experiment

Columns were chosen because they can simulate groundwater conditions in the laboratory. Also laboratory conditions control other parameters like oxygen levels, light connection, temperature, and addition of specific chemical in specific amount and to use the substrate materials that were chosen for each experiment. With all these parameters under control the expected outcomes was to see the interaction of selected substrate materials with groundwater and differences in the approach that may exist.

The actual design of the columns in this experiment is given in Table 6.1 in details. The columns design follows a sequential barrier that every levels consist of a specific substrate material and they are not mixt all together. The Layer 1 is the first layer that the treated solution inserts into the column and the Layer 4 is the last layer that the solution is removed out from the column to discard.

Table 6.1: Design of experiment with sand, mulch and perlite

	Column 1	**Column 2**	**Column 3**	**Column 4**	**Column 5**	**Column 6**	**Column 7**
Layer 1	30% perlite	25% perlite	25% perlite	25% sand	20% perlite	20% perlite	25% perlite
Layer 2	20% mulch	25% mulch	25% sand	25% mulch	30% mulch	30% sand	25% perlite
Layer 3	20% sand	25% mulch	25% sand	25% mulch	30% mulch	20% mulch	25% perlite
Layer 4	30% perlite	25% perlite	25% perlite	25% sand	20% sand	30% sand	25% perlite

The porosity measured according to the steps that described in chapter 4 for each column separately. The first step was to measure the weight of void columns without the addition of substrate materials. Then there was measurement of each substrate material for each column. The next step was to measure the columns with the substrate materials and finally to measure the column with substrate materials and spike water into the columns. The water inserted to remove all trapped oxygen. Finally, according to the equation of measurement of porosity

(Porosity = Volume of Void Space / Total Volume of the solid) the porosities of the columns measured. There is a total porosity for each column and not for each substrate material separately. The porosities of each column are given in detail in Table 6.2.

Table 6.2: Porosity of Columns

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Porosity	0.63	0.62	0.61	0.52	0.59	0.54	0.75

The HRT measured with the same method as it was measured in chapter 4 using turnover time and peristaltic pump flow. To achieve the same HRT at each column in the influent tube connection, some smaller and some larger diameters tubes used.
The HRT measured depending on porosity, substrate materials and tubes was calculated. The HRT was 16 hours and the duration of experiment was 60 days. The stable flow rate was maintained by using a peristaltic pump (Ismatec) and the same diameter tubes that were connected the initial tank with columns.

With the design that is selected in layers, it is an initial approach to investigate if in future those results can produce a filter that can be used in a commercial way. The layer design was investigated to find out what was happening in each layer. There was another experiment that took place and not included in that thesis using as collection water sample points the length of each layer. The issues for that experiment were a lot with the main problem the recovery time from the collection time till the next sample period and the flow in the columns. For that reason the layered measurement of N-compounds abandoned. The design of the experiment remained and that was an initial approach to find out what was happening in each layer separately. That approach used to combine some materials for commercial use. The initial design of experiment failed but analysed to find out how these reactive barriers react in denitrification process in a layer experiment. For that reason, there are only water samples from the output point of each column.

6.5 Results

All measurements were according to methods that described in Chapter 4. The only change was that measurements in nitrogen compounds and phosphorus compounds became with microplates and spectrophotometer the same way as Chapter 5. There are also measurements about TOC levels to find out in more details the organic carbon activity of each column. All the average results along the experiment are given in Table 6.3 below.

Table 6.3: Results of experiment with mulch, perlite and sand (all results received after (N=3 replicates) triplicate analysis)

		Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
pH	mean	7.97	7.39	7.37	7.86	7.43	7.41	7.43	7.73
	st dev	0.13	0.31	0.30	0.15	0.31	0.32	0.23	0.19
	%		-7.34	-7.62	-1.38	-6.80	-7.02	-6.78	-3.01
Conductivity µS/cm	mean	413.18	419.91	412.65	405.06	423.29	382.85	394.79	373.91
	st dev	33.62	30.85	43.24	40.88	44.74	43.31	60.25	43.35
	%		1.63	-0.13	-1.96	2.45	-7.34	-4.45	-9.50
NO2 mmol/l *10^-3	mean	2.75	3.94	3.71	3.07	3.06	2.68	3.49	2.72
	st dev	0.97	0.92	1.04	0.60	1.05	0.51	1.06	0.63
	% Removal		43.23	34.97	11.74	11.27	-2.44	26.79	-0.97
NO3 mmol /l *10^-3	mean	414.11	213.91	212.48	299.29	183.09	190.34	184.71	286.02
	st dev	15.12	79.10	83.68	61.20	92.93	84.50	96.97	67.05
	% Removal		-48.34	-48.69	-27.73	-55.79	-54.04	-55.40	-30.93
NH4 mmol/l *10^-3	mean	7.44	11.56	7.94	9.88	6.98	9.89	8.57	7.09
	st dev	2.49	3.51	2.16	2.81	2.40	2.89	3.07	2.04
	% Removal		55.42	6.71	32.83	-6.20	32.99	15.21	-4.74
TN mmol/l *10^-3	mean	424.30	229.42	224.14	312.24	193.13	202.92	196.77	295.83
	st dev	15.66	79.14	85.55	61.46	94.84	84.84	98.18	67.97
	% Removal		-45.93	-47.17	-26.41	-54.48	-52.17	-53.62	-30.28
TOC ppm	mean	11.72	7.85	7.86	6.25	7.30	7.05	6.56	5.00
	st dev	3.53	1.92	2.41	2.08	2.19	2.60	1.95	1.47
	% Removal		-33.02	-32.96	-46.70	-37.75	-39.86	-44.02	-57.34
PO4 mg/l	mean	1.46	0.08	0.08	0.06	0.07	0.06	0.06	0.05
	st dev	0.31	0.22	0.20	0.17	0.21	0.18	0.19	0.20
	% Removal		-94.63	-94.85	-95.78	-95.37	-95.63	-95.74	-96.36

The results were separated in 3 groups. The first group was the Control Columns with only sand and perlite, column 3 (50% sand, 50% perlite) and column 7 (100% perlite). The second group was the group that perlite is the dominant material. These columns were column 1 (60% perlite, 20% mulch, 20% sand) and column 2 (50% perlite, 50% mulch). The last group was the group with main substrate material sand. In that group there were three columns, column 4 (50% sand, 50% mulch), column 5 (20% sand, 60% mulch, 20% perlite) and column 6 (60% sand, 20% mulch, 20% perlite).

6.5.1 First group–Results of Control Columns

In control columns the main purpose was to investigate the reaction of groundwater with those materials. Also it was a comparison with a column with only perlite, to investigate the reaction of the new substrate material separated from all the others materials and to receive the most unfavorable possible situation about that. There is no control column with 100% sand as substrate material but a mixture of sand with perlite 50% of each as control column. That designed adopted because these two materials are inert materials and expected to react with the same way along the experiment.

6.5.1.1 pH and conductivity levels Control Columns

The pH levels (Figure 6.5) in control columns followed almost the same attitude as initial solution. The pH levels in column 3 with perlite and sand had more stable values and the range was not as wide as it was happening in column 7. The differences in pH were visible more for column 3 after the day 20 of experiment. From that time period till the end, pH was always lower than initial solution and follows the same trend. For column 7, the difference in pH was visible earlier from day 10 and afterwards. The values were lower than column 3 and again this column follows the trend of initial solution. The average level in column 3 along the experiment was pH=7.86 and in column 7 as it was mentioned lower (pH=7.73).

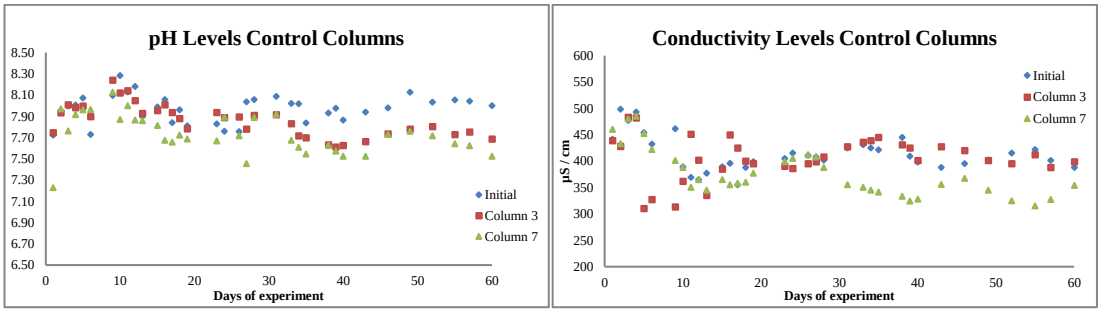


Figure 6.5: pH and conductivity levels Control Columns

Conductivity levels (Figure 6.5) were different in the two columns. In column 3 the range of results was wider in first 20 days. After that conductivity levels followed the initial solution conductivity levels. In column 7 the opposite was happening than in column 3. In first 28 days conductivity was following the initial solution trend. After day 28 till the end there was difference in conductivity levels were much lower than column 3 and the initial solution. The average levels along the experiment also support that. The average levels in column 3 were 406 µS/cm and in column 7 were 376 µS/cm.

The spread of the results that exist in the influent solution and continuously in the columns, mentioned also in chapter 4. It is a combination of the influent groundwater characteristics and the changes along the experiments. Those continuously changes of influent groundwater, changed also the microbiological activity in the columns and in combination with the organic carbon that is provided by the substrate materials of each column are given the spread of results. That noticed at all the columns and all the measurements along the experiment.

6.5.1.2 Nitrogen species Control Columns

Nitrogen species are visible in Figures 6.6, and 6.7 for control columns. The nitrogen levels as it is noticed in Chapter 5 for columns with sand as substrate materials have lower reduction levels than any other columns.

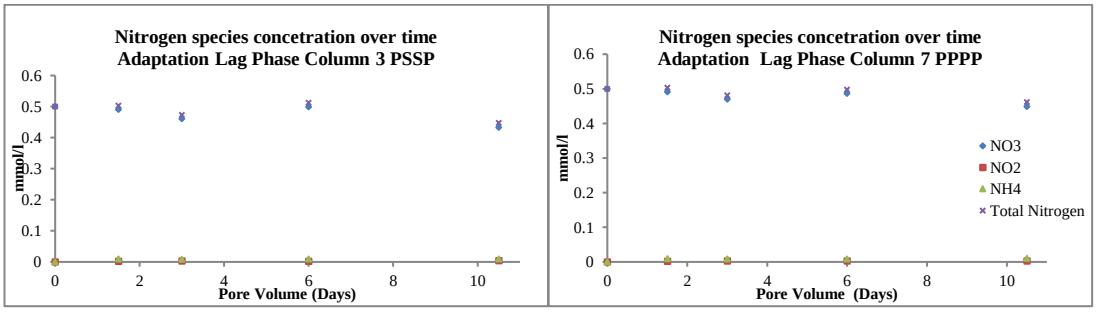


Figure 6.6: Nitrogen species Control Columns adaptation lag phase

The adaptation lag phase in this experiment had duration between 4-7 days. In control columns the same trend was noticed. In both columns the NO_2-N and NH_4-N levels remain stable in very low levels and do not affect the denitrification process. In NO_3-N and TN levels the trend is the same in both columns. There is an initial reduction in both columns but not in the levels that it was expected. The initial reduction in both columns was almost 20%.

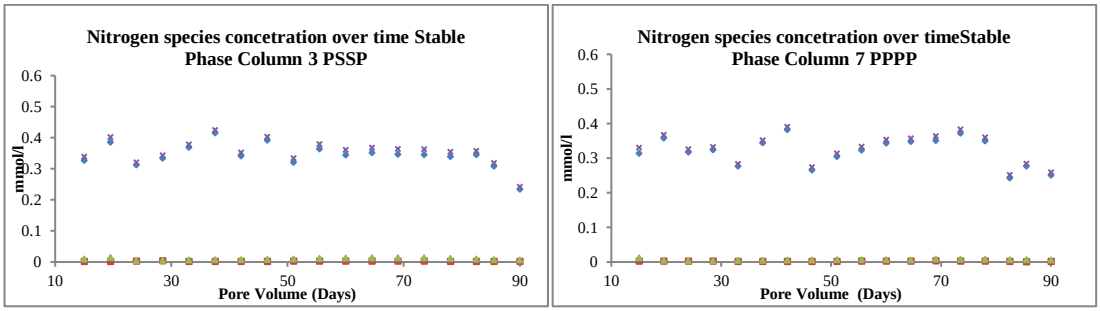


Figure 6.7: Nitrogen species Control Columns Stable Phase

The Stable Phase is the duration of the experiment that the reduction of nitrogen compounds was noticed in more details.

In column 3, there is initial period that are continuously increasing levels of NO_3-N and TN from PV 10-50. After that there is a stable period from PV 50-80 and finally a reduction period from PV 80-90. The reduction levels were between 35-55% with the best results in the end of the experiment. The NO_2-N and NH_4-N remain in low levels without affecting the system. Additionally, there is no significance quantity to ensure that the denitrification process was not complete.

In column 7, there is almost the same trend as it is noticed in column 3. For NO3-N and TN there is an initial uncertain period with increases and decreases from PV10-45. After that there is an increasing but with stable rate period (PV 45-75). Finally, there is a reduction period from PV 75-90. The reduction is in the same levels as in column 3, (35-55%) with the highest reduction in the end of the experiment. The NO_2-N and NH_4-N remain in low levels without affecting denitrification process.

The spread of results in the influent solution and columns are connected with the water that changed along the experiment. This is in connection with the microbiological activity that exists in each column and the time that is needed to assimilate the different groundwater solution.

6.5.1.2.1 Nitrite levels Control Columns

Nitrite levels (Figures 6.6 and 6.7) were low in both columns. The experiment can be separated in two periods. The first one till day 20 where the spread of values was high and the second period till the end of experiment. The change was that in column 3, nitrite levels were higher than initial solution from day 20 and afterward and higher also from column 7. In column 7 nitrite levels were lower from day 20 till day 40. Afterward nitrite levels were higher than initial solution and lower than column 3.

6.5.1.2.2 Nitrate levels Control Columns

Nitrate levels (Table 6.4 and Figures 6.6 and 6.7) in control columns were not reducing as expected. There were the lowest levels of total nitrogen reduction in these two columns. It was expected that result for column with sand but for perlite was the new substrate material that was under investigation. In column 3 the reduction was only 28% with average levels along the experiment 0.299 mmol/l. There was a wide range of results with initial increase in the start and then there was a reduction and the best reduction achieved in the end of experiment. The same was happening in column 7. The reduction rate was slightly higher with reduction 31% and average levels 0.286 mmol/l. Again, the likely dissolved organic carbon residual in the groundwater facilitated some minor denitrification not supported by an organic substrate.

Table 6.4: Nitrate levels Control Columns

mmol/l	Pore Volume	Initial	Column 3 PSSP	Column 7 PPPP
Day 1	**1.5**	0.411	0.403	0.403
Day 2	**3.0**	0.442	0.408	0.417
Day 4	**6.0**	0.454	0.453	0.443
Day 7	**10.5**	0.392	0.339	0.354
Day 10	**15.0**	0.400	0.257	0.244
Day 13	**19.5**	0.401	0.302	0.288
Day 16	**24.0**	0.419	0.26	0.265
Day 19	**28.5**	0.401	0.264	0.258
Day 22	**33.0**	0.433	0.316	0.237
Day 25	**37.5**	0.418	0.344	0.286
Day 28	**42.0**	0.411	0.276	0.313
Day 31	**46.5**	0.422	0.326	0.221
Day 34	**51.0**	0.421	0.264	0.254
Day 37	**55.5**	0.426	0.303	0.273
Day 40	**60.0**	0.409	0.273	0.277
Day 43	**64.5**	0.407	0.277	0.282
Day 46	**69.0**	0.422	0.282	0.291
Day 49	**73.5**	0.412	0.274	0.303
Day 52	**78.0**	0.405	0.266	0.279
Day 55	**82.5**	0.403	0.271	0.19
Day 57	**85.5**	0.402	0.242	0.218
Day 60	**90.0**	0.399	0.181	0.195

6.5.1.2.3 Ammonium levels Control Columns

Ammonium levels (Figures 6.6 and 6.7) were low in both columns. The period was separated in two parts. The first one was till day 12 where the range of results was wide and there was no trend for values. After day 12 there was the same trend at all columns. The difference was in column 3 where ammonium levels were higher than initial solution and column 7. In column 7 the ammonium levels were lower than initial solution till day 45 and then the levels were slightly higher.

6.5.1.2.4 Total Nitrogen levels Control Columns

Total nitrogen levels (Table 6.5 and Figures 6.6 and 6.7) were following nitrate levels. The reduction rates in control column were low. The trend is the same in both columns. TN levels were slightly higher in column 3 than column 7 with average levels in column 3 (0.312 mmol/l) and in column 7 (0.296 mmol/l).

Table 6.5: Total Nitrogen levels Control Columns

mmol / l	Pore Volume	Initial	Column 3 PSSP	Column 7 PPPP
Day 1	**1.5**	0.422	0.415	0.415
Day 2	**3.0**	0.456	0.421	0.428
Day 4	**6.0**	0.467	0.466	0.454
Day 7	**10.5**	0.408	0.353	0.367
Day 10	**15.0**	0.413	0.270	0.260
Day 13	**19.5**	0.415	0.320	0.297
Day 16	**24.0**	0.429	0.269	0.273
Day 19	**28.5**	0.410	0.274	0.266
Day 22	**33.0**	0.441	0.325	0.244
Day 25	**37.5**	0.426	0.354	0.294
Day 28	**42.0**	0.419	0.287	0.321
Day 31	**46.5**	0.432	0.338	0.229
Day 34	**51.0**	0.432	0.277	0.263
Day 37	**55.5**	0.437	0.319	0.283
Day 40	**60.0**	0.418	0.289	0.287
Day 43	**64.5**	0.417	0.294	0.291
Day 46	**69.0**	0.432	0.300	0.304
Day 49	**73.5**	0.421	0.291	0.314
Day 52	**78.0**	0.414	0.281	0.290
Day 55	**82.5**	0.410	0.283	0.199
Day 57	**85.5**	0.409	0.252	0.226
Day 60	**90.0**	0.405	0.189	0.203

6.5.1.3 TOC levels Control Columns

In column 3 (Figure 6.8) TOC levels started with a reduction period until day 16 where the lowest values received at both columns (2.41 mg/l). An increasing period continued till day 49 with the highest TOC levels in column (10.22 mg/l) and finally a reduction period till the end.

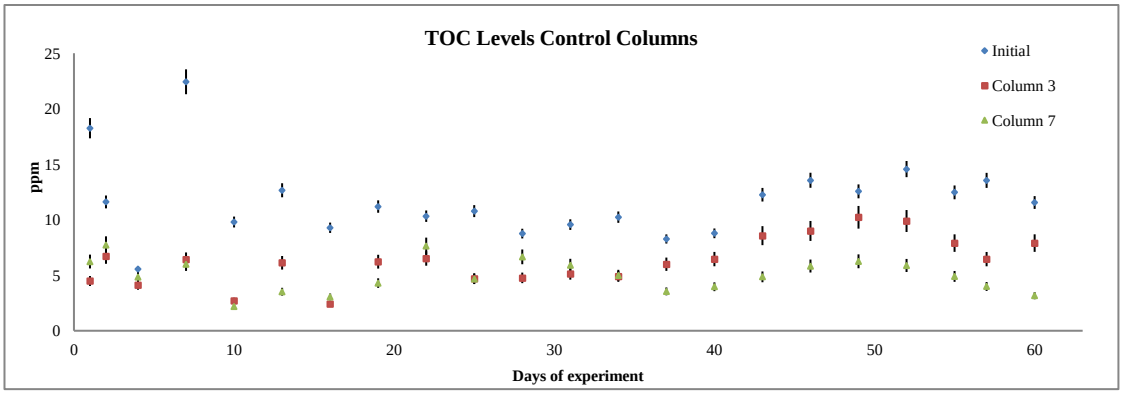


Figure 6.8: TOC levels Control Columns

In column 7 (100% perlite) the lowest levels of TOC were noticed. There were two increasing periods at days 1-2 and days 10-22 and two periods that reduction was noticed at days 2-10 and days 22-60. The highest value received in day 2 (7.73 mg/l) and the lowest in day 10 (2.20 mg/l).

The TOC levels in the influent solution are not stable due to the changes of the water along the experiment. At the initial period of first 8 days the spread of TOC levels is wide. This is

happening due to the pretreatment tank, which provide higher amount of organic carbon. In the duration of the experiment that spread of results is reduced and remained balanced showing that the groundwater that was remain in the fridge till the application time create an internal ecosystem with balance TOC levels. Again there is a spread of results especially when the water was changed but not in the same levels as in the first period. At all the TOC measurements there is an error bar (10%). That is not affecting the measurements of TOC and can describe with insurance the organic carbon activity without quality assurance issues.

6.5.1.4 Phosphate levels Control Columns

Phosphate levels (Figure 6.9) were low in contrast to the influent solution. It is noticeable because the perlite has the characteristic to adsorb phosphate compounds and the reduction was more than 90% at both columns. The results follow nicely the proposed hypothesis that perlite would remove most phosphate from the system.

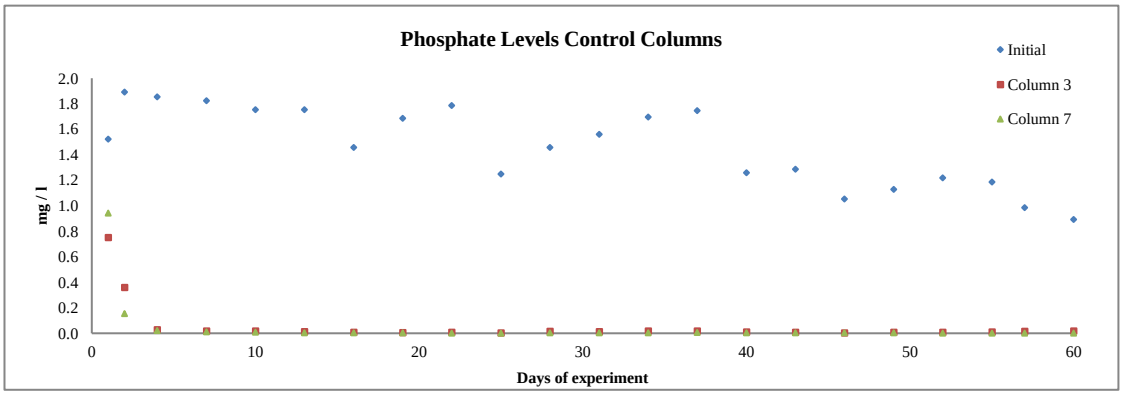


Figure 6.9: Phosphate levels Control Columns

Phosphate levels even in the influent solution even in controls columns remain in very low levels. It is important that in the initial solution that concertation is not stable with increasing and decreasing phases along the experiment. That is happening due to the pretreatment tank. The pretreatment tank can provide groundwater. An amount is spiked immediately in the columns and another tanks remained in the fridge (4 ^{o}C) till the application time. During that time period there is an ecosystem in the tanks that tries to survive, with denitrification bacteria, other microorganisms that exist and there is a balance between them. That reaction of the system is visible in the spread on the initial solution phosphate concentration.

In the columns as it was described in the paragraph 6.2.1 perlite has the ability to adsorb the phosphate levels. That is visible in Figure 6.9. In the first 5 days there is the reduction process

that is noticed in the columns. The reduction is not as fast as the nitrogen removal and demand 5 days to achieve the best removal. After that period the phosphate levels remain stable in low levels.

6.5.2 Second group – Results of Perlite Columns

The second group of columns was columns with perlite substrate materials. The columns 1 with 60% perlite, 20% mulch, 20% sand is the one column in that group and the second column is column 2 with 50% perlite and 50% sand.

6.5.2.1 pH and conductivity levels Perlite Columns

In columns with perlite pH levels (Figure 6.10) started from lower levels in contrast to initial solution due to the substrate materials that were combination of mulch, sand and perlite in column 1 and mulch and perlite in column 2. The two columns followed similar trends along the experiment. There was an increasing period till day 12 for both columns when they arrived at their highest levels. After that there was a decreasing period till day 26. After day 26 there was stable period with decreasing way for both of the columns with lower levels for columns 2. The average levels along the experiment were almost the same with slightly higher pH in column 1 (pH=7.38) than in column 2 (pH=7.36).

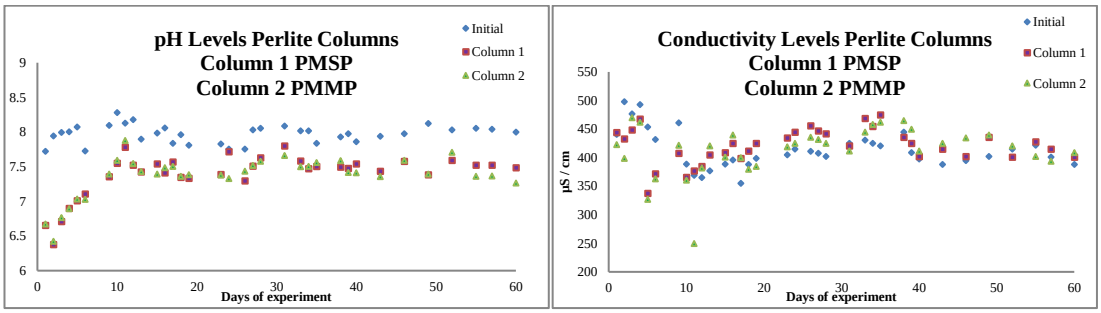


Figure 6.10: pH and conductivity levels Perlite Columns

Conductivity levels (Figure 6.10) follow similar trends as the influent solution. In the start of experiment conductivity levels were lower till day 10. After day 10 the levels were higher than initial solution and till day 36 conductivity levels in columns 1 were higher than in column 2. The period till the end, there was a spread of results between column 1 and column 2 that were very closed at all days. Due to the second period that conductivity levels were higher the

average levels in column 1 were higher along the experiment with 421 μS/cm. The average levels for column 2 were 413 μS/cm.

The initial periods of the experiment till day 10 is the period that the system demands to provide the best environmental conditions to the microbial activity. It is the periods that there is the establishment of new microbial colonies in the substrate materials and the internal calibration of the system. The initial acidic environment becomes more neutral to provide the best conditions for the microbial activity. The best environmental conditions for denitrification process are in neutral environment (Rivett et al., 2008). For that reason, from day 10 till the end of the experiment there is a spread of results but in neutral environment. At all the duration of the experiment the columns pH levels are lower that the influent groundwater solution.

In agreement with pH levels the same is happened to columns with conductivity. There is the initial period of calibration of the system that conductivity levels reduced till day 10 and afterwards there is a balance activity. Of course there is a spread of results due to the change of water along the experiment. In the initial calibration period that microbial activity tries to provide the best results the conductivity levels are higher and are reducing with higher influent solution conductivity levels than columns. After that period the conductivity of influent solution is in the same even lower levels (350-450 μS/cm) than columns showing that the system in the columns is in balance and the conditions are suitable to enhance denitrification.

6.5.2.2 Nitrogen species Perlite Columns

In perlite columns the reduction of nitrogen species is visible in Figure 6.11 and 6.12 with all the details. There is an initial adaptation lag phase of 4-8 days which is the period that the reduction of nitrogen species is stabilized. The second period is the stable period till the end of experiment.

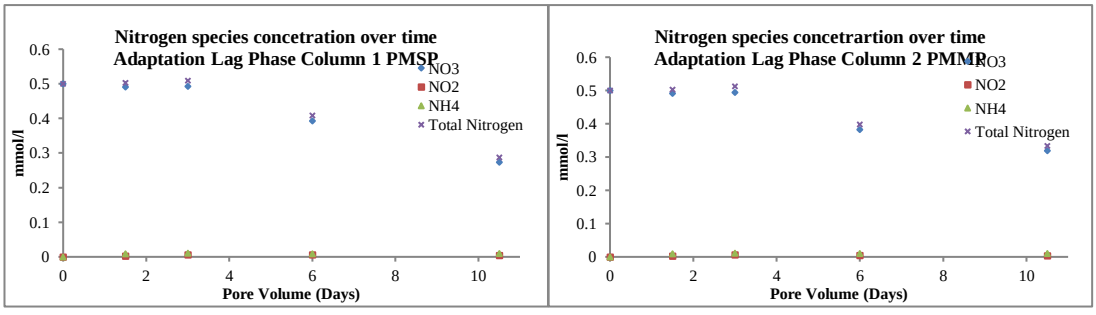


Figure 6.11: Nitrogen species Perlite Columns adaptation lag phase

In the perlite columns the dominant substrate material was perlite. Both columns in the adaptation lag phase are reacting with the same way. In column 1 and 2, NO_3-N and TN levels remain stable the initial periods PV 0-3. After that there is the reduction period PV 3-10. The reduction that is notices in both columns is 45%. NO_2-N and NH_4-N in both columns remain in low levels and do not affect the denitrification process. The HRT is enough to finish the denitrification process.

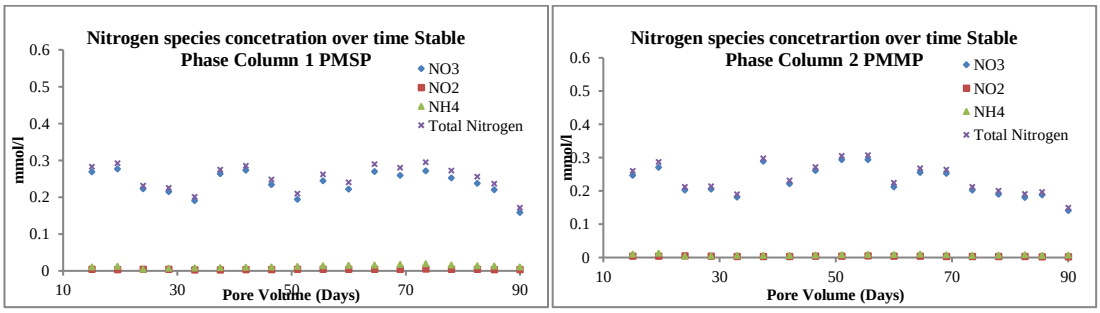


Figure 6.12: Nitrogen species Perlite Columns Stable Phase

In Stable Phase there is the same trend in both columns. There is a reduction till the end of the experiment for NO_3-N and TN. In column 1, there are 4 different reduction periods (PV 10-32, 35-52, 55-60, 65-90). The NO_2-N and NH_4-N remain at all the duration of experiment low without affecting the process. The reduction that was noticed in NO_3-N and TN was between 45-55% with the best reduction in the end of the experiment.

In column 3, the stable period can be described by 3 increasing periods and 2 stable periods. The increasing periods are between PV 10-20, 32-52, 55-70 and the stable periods PV 20-32, 72-90. NO_3-N and TN levels reduced at that experiment again 45-55% with the best reduction in the end of the experiment. NO_2-N and NH_4-N levels remain low at all the duration of the experiment without affecting denitrification process.

The reduction that is noticed in the perlite columns is higher than the control columns. The effect of the perlite on denitrification is discussed later in this chapter.

6.5.2.2.1 Nitrite levels Perlite Columns

Nitrite levels (Figures 6.11 and 6.12) were characterized by 3 time periods. The first period was from the start till day 20. The spread of results was wide without any specific trend. In columns that period was a decreasing period. Nitrite levels at all the duration of experiment were very low showing that denitrification process likely went to nitrogen gas and there was no

transformation of nitrate compounds to nitrite. The second period was the period that nitrite levels were increasing. In column 2 that period follows the initial solution and finished in day 38. In contrast for column 1 that period lasted till day 48. The last period was a decreasing period for all columns with higher level in column 1 than in column 2. The average levels in both columns were very similar with nitrite levels slightly higher in column 1 (4.33 μmol/l) than in column 2 (4.25 μmol/l)

6.5.2.2.2 Nitrate levels Perlite Columns

Nitrate levels (Table 6.6 and Figures 6.11 and 6.12) as it is expected from the hypothesis of experiment were decreasing. The two columns are following the same trend and reduction levels are almost the same. The reduction was slightly higher in column 2 that higher levels of mulch as substrate material exist. More specific in the experiment there were 3 periods. The first period was from the start till day 20. This was a reducing period from initial levels to average levels. After the day 20 till day 40 there was a period that there is no balance in the results with fluctuations. The last period was from day 40 till the end that there was a reduction period at both columns with lower levels in column 2. The reduction levels in column 1 were 48.73% with nitrate levels (0.213 mmol/l) and in column 2 reduction 49.15% (0.212 mmol/l). The reduction was not as high as it was expected in the start but the results in the last days showing that these results can achieve higher reduction levels in longer time period.

Table 6.6: Nitrate levels Perlite Columns

mmol/l	Pore Volume	Initial	Column 1 PMSP	Column 2 PMMP
Day 1	**1.5**	0.411	0.403	0.403
Day 2	**3.0**	0.442	0.433	0.432
Day 4	**6.0**	0.454	0.352	0.342
Day 7	**10.5**	0.392	0.208	0.246
Day 10	**15.0**	0.400	0.208	0.189
Day 13	**19.5**	0.401	0.213	0.208
Day 16	**24.0**	0.419	0.182	0.165
Day 19	**28.5**	0.401	0.165	0.16
Day 22	**33.0**	0.433	0.157	0.151
Day 25	**37.5**	0.418	0.213	0.237
Day 28	**42.0**	0.411	0.216	0.176
Day 31	**46.5**	0.422	0.188	0.215
Day 34	**51.0**	0.421	0.151	0.242
Day 37	**55.5**	0.426	0.195	0.244
Day 40	**60.0**	0.409	0.166	0.164
Day 43	**64.5**	0.407	0.205	0.199
Day 46	**69.0**	0.422	0.202	0.207
Day 49	**73.5**	0.412	0.205	0.161
Day 52	**78.0**	0.405	0.189	0.147
Day 55	**82.5**	0.403	0.177	0.137
Day 57	**85.5**	0.402	0.163	0.145
Day 60	**90.0**	0.399	0.115	0.105

6.5.2.2.3 Ammonium levels Perlite Columns

Ammonium levels (Figures 6.11 and 6.12) were also very low for the duration of experiment. They were higher in column 1 than in column 2. The experiment as it is noticed from the previous results can separated in 3 time periods. The first period was from the start till day 12. In that period there was a wide spread of results with a balance trend. After that there was an increasing period that last more in column 1(day 48) than in column 2 (day 42). After that there was a decreasing period that is more noticeable in column 1 and more balanced reduction in column 2. The average levels along the experiment for column 1 were 12.01 μmol/l and for column 2, 8.12 μmol/l.

6.5.2.2.4 Total Nitrogen levels Perlite Columns

TN levels (Table 6.7 and Figures 6.11 and 6.12) were following nitrate levels. There was a reduction but not as expected. The reduction was slightly higher in column 2 than in column 1 and both columns, following the same trend along the experiment. The average levels in column 1 along the experiment were 0.229 mmol/l (-45.93%) and in column 2 0.224 mmol/l (-47.17%).

Table 6.7: TN levels Perlite Columns

mmol/l	Pore Volume	Initial	Column 1 PMSP	Column 2 PMMP
Day 1	**1.5**	0.422	0.415	0.415
Day 2	**3.0**	0.456	0.449	0.451
Day 4	**6.0**	0.467	0.367	0.357
Day 7	**10.5**	0.408	0.223	0.260
Day 10	**15.0**	0.413	0.222	0.203
Day 13	**19.5**	0.415	0.229	0.224
Day 16	**24.0**	0.429	0.191	0.174
Day 19	**28.5**	0.410	0.176	0.168
Day 22	**33.0**	0.441	0.168	0.160
Day 25	**37.5**	0.426	0.224	0.246
Day 28	**42.0**	0.419	0.229	0.186
Day 31	**46.5**	0.432	0.202	0.225
Day 34	**51.0**	0.432	0.167	0.253
Day 37	**55.5**	0.437	0.213	0.257
Day 40	**60.0**	0.418	0.185	0.177
Day 43	**64.5**	0.417	0.225	0.213
Day 46	**69.0**	0.432	0.223	0.218
Day 49	**73.5**	0.421	0.228	0.171
Day 52	**78.0**	0.414	0.209	0.157
Day 55	**82.5**	0.410	0.195	0.148
Day 57	**85.5**	0.409	0.179	0.154
Day 60	**90.0**	0.405	0.128	0.114

6.5.2.3 TOC levels Perlite Columns

In column 1 there was an increasing period from day 1 till day 3 where the highest value was noticed with 12.34 mg/l. After that a reduction period till day 16 continued with the lowest value (4.47 mg/l). Then followed an increasing period till day 46, another reduction period was in the next 9 days till day 55 and finally an increasing period till the end. The TOC was measured to determine if the addition of perlite had any impact on the available dissolved carbon for denitrification. The results that perlite does not interact with the TOC and therefore should not affect the denitrification reaction with organic substrate.

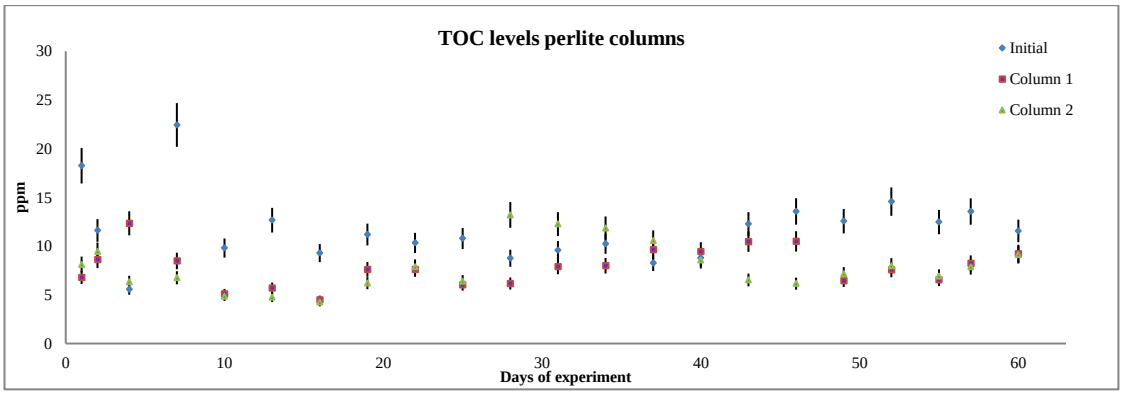


Figure 6.13: TOC levels Perlite Columns

In column 2 experiment started with a reduction period of TOC levels till day 16 of experiment where the lowest levels were noticed (4.24 mg/l). Then an increasing period followed till day 28 where the highest value received (13.19 mg/l). After that a period with reducing level continued till day 46 and finally an increasing period till the end of experiment (Figure 6.13).

As it is mention in control columns, even here there are error bars (10%) for all the measurement that do not affect denitrification process. In this group of columns the initial calibration period is till day 10. After that at all the duration of experiment column 1 has lower levels of organic carbon than influent solution. In contrast in column 2 there is a higher period than influent solution between days 28-36. The spread of results in all the columns is a combination of the influent water change and the microbial activity that exist in the columns.

6.5.2.4 Phosphate levels Perlite Columns

The phosphate levels (Figure 6.14) in both columns were disappeared. It was visible in first two days small amount of phosphate levels and after than the levels remained lower than 0.025 mg/l.

Again, as expected, perlite strongly removes phosphate from the system without detrimental effect on the denitrification process.

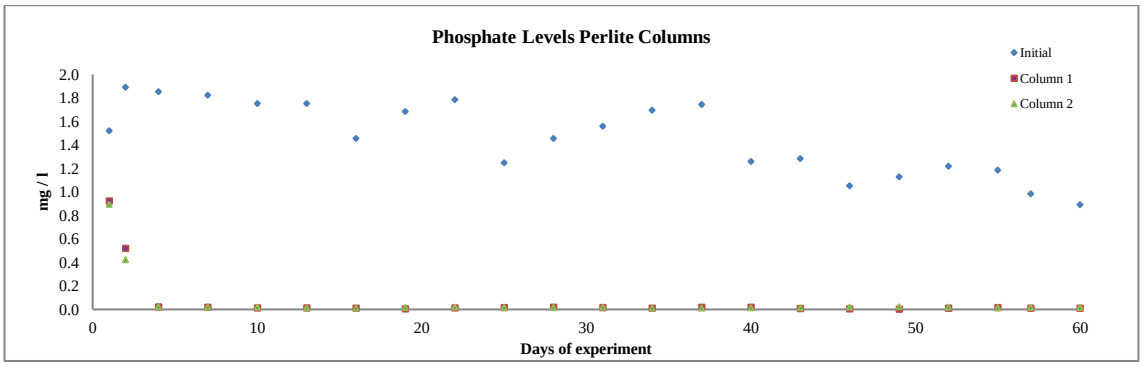


Figure 6.14: Phosphate levels Perlite Columns

The trend of phosphate levels in perlite columns is the same as control columns. There is the same time period of 4 four to achieve the best reduction and after that period the phosphate levels remain in low levels.

6.5.3 Third group – Results of Sand Columns

The last group of columns was the group with the main substrate material sand. These columns were column 4 (50% sand, 50% mulch), column 5 (20% sand, 60% mulch, 20% perlite) and column 6 (60% sand, 20% mulch, 20% perlite).

6.5.3.1 pH and conductivity levels Sand Columns

The pH levels (Figure 6.15) in the group of sand columns are following the same trends. Over the duration of experiment, pH levels were lower than influent solution. The pH levels can be separated in 4 time periods. The first one was from the start till day 12. In that period at all columns, there was an initial reduction and then an increasing period. The reduction was noticed at all columns in first two days and it was more visible with lower pH levels in column 4 and column 5. After that there was a decreasing period from day 12 till day 26. All the columns had similar results. From day 26 till day 32 there was an increase in pH achieving the highest levels. The last period was from day 32 till the end of experiment that there was a reduction balanced period. The average levels for three columns were similar with slightly higher for column 4 and 6 (pH=7.43) than column 5 (pH=7.41).

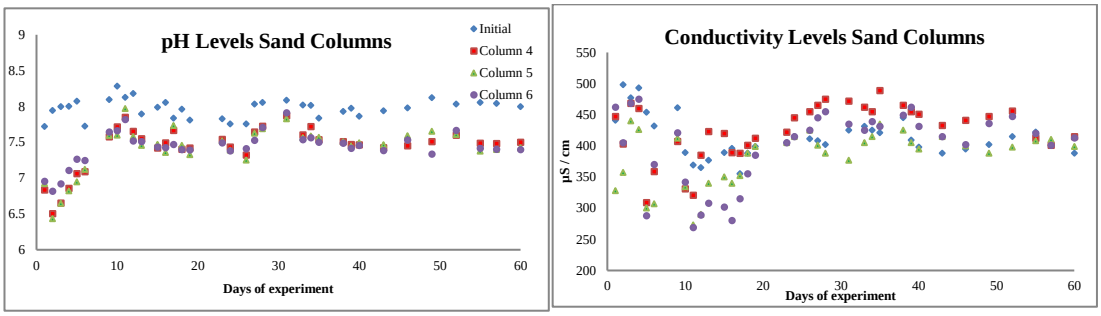


Figure 6.15: pH and conductivity levels Sand Columns

Conductivity trends (Figure 6.15) can separate in two periods. The first one was till day 20. The spread of results was wide and without any specific trend. The second period was from 20 till the end. The spread of the results was not so wide and in general all columns follow the initial solution trend. It was noticed that higher levels received in column 4 and column 6 and lower levels in column 5 at that period. Along the experiment the average levels for column 4 were higher than every other column (424 µS/cm). Column 6 had also high average conductivity levels with 397 µS/cm and lower levels were in column 5 (381 µS/cm).

In these three columns the initial calibration period for pH is till day 10. All columns start from an initial acidic environment and till day the pH increase in neutral levels. After day 10 and till the end of the experiment the trends on pH are following the trend of influent column and all time period in neutral levels that are the best conditions for denitrification process.

In conductivity the initial calibration period last till day 20. The spread of results is wide in that period and the use of perlite create more uncertain initial period especially for column 5 and 6. After day 20 the trend at all columns are almost the same with higher levels than influent solution for column 4 and 6 and lower levels for column 5.

6.5.3.2 Nitrogen species Sand Columns

Nitrogen species are described in detailed in the next paragraphs and seen in Figures 6.16 and 6.17. In contrast to other columns, in these columns the two phases are more obvious. It is shown that the reduction at all columns is higher than all other columns and that is the result of mulch in all columns as substrate material that provide higher levels of organic carbon.

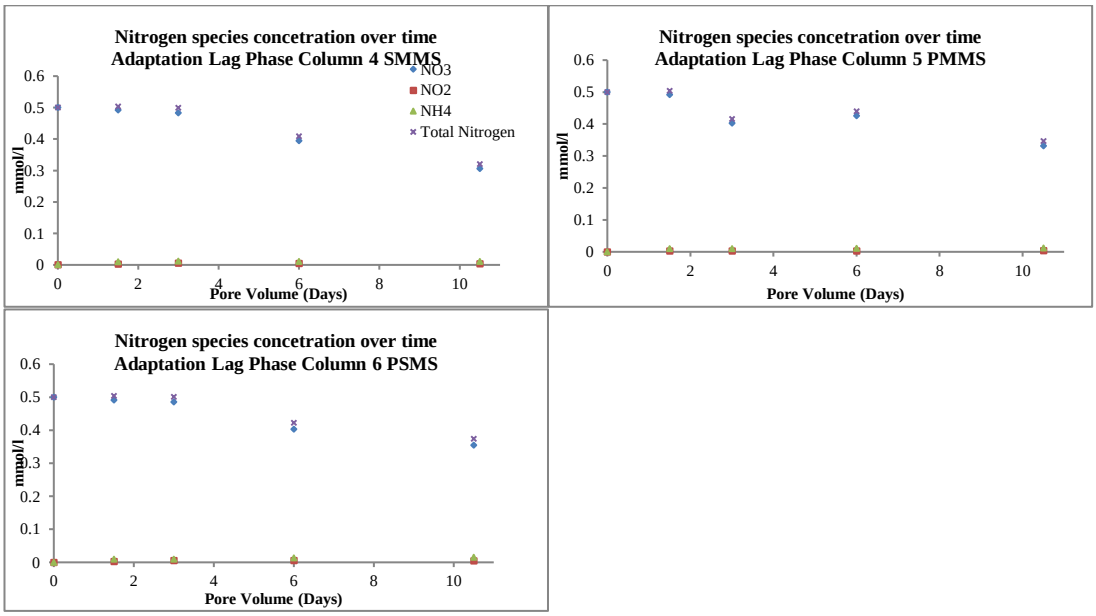


Figure 6.16: Nitrogen species Sand Columns adaptation lag phase

The initial adaptation lag phase has duration till day 8 (PV=11). At all columns 4, 5 and 6 the concentration of NO_2-N and NH_4-N remain in low levels at all the duration of the experiment. The main nitrogen species that are under investigation are NO_3-N and TN compounds. In columns 4 and 6 there is an initial stable period till PV=3 and afterwards there is the reduction period. In column 5 the stable period is till PV=2 and the reduction period starts earlier in the adaptation lag phase. At all columns the reduction is 40% in adaptation lag phase.

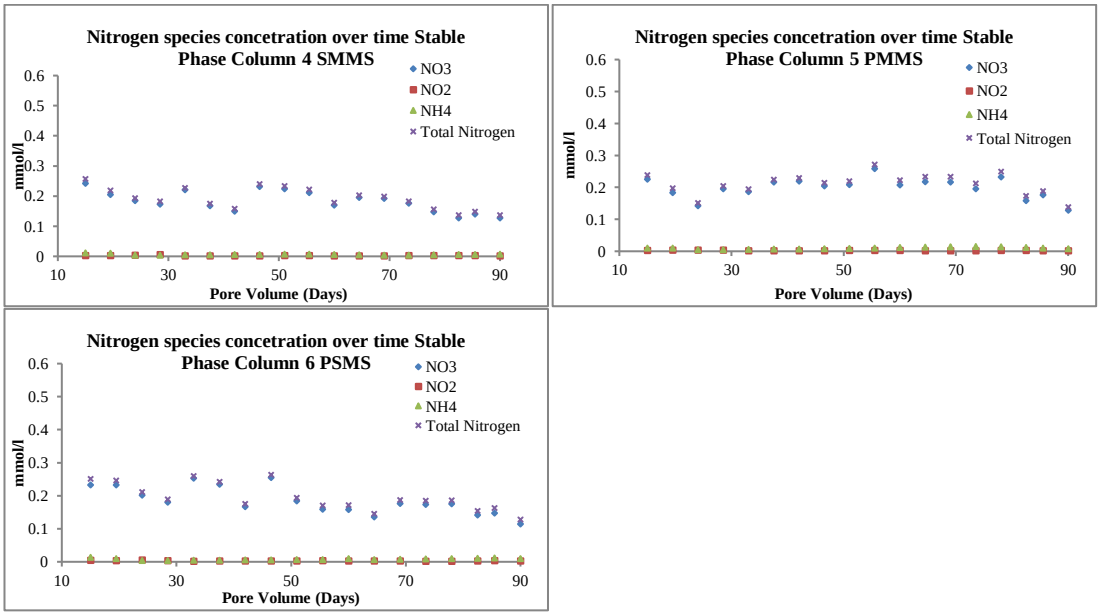


Figure 6.17: Nitrogen species Sand Columns Stable Phase

The Stable Phase is the phase of experiment that the microbial activity reaches the highest efficiency. The NO_2-N and NH_4-N remain in low levels without affecting the denitrification process. Moreover, the HRT that was chosen was enough to assimilate the changes in influent water along the experiment. The NO_3-N and TN affect the system and in these compound is noticed the change of influent water.

In column 4, there are 4 reduction periods (PV 10-30, 35-45, 45-60, 60-80) and one stable period (PV 80-90). The reduction reaches the highest levels in the end of the experiment (70%).

In column 5, there are two reduction periods (PV 10-25, 80-90) and one stable period (PV 25-80). The reduction in that column is 70%.

In column 6, there are 3 reduction periods (PV 10-30, 30-45, 45-65) and a stable period (PV 65-90). The reduction in that column reaches levels of 75% at the end of the experiment. It is very interested because in column 6 there is only one reactive zone in contrast to column 4-5 that there are 2 reactive layers and can provide the same, even better results. That shows that the correct combination of substrate materials is important to provide the best results.

6.5.3.2.1 Nitrite levels Sand Columns

Nitrite levels (Figures 6.16 and 6.17) were low along the experiment. This shows that the denitrification process had gone to completion and there was no transformation of nitrate to nitrite compounds. The experimental period can be separated in 4 sub periods. The first one was from the start till day 20. The spread of results was wide without any general trend. The second and the third time period follow the initial solution trend. The second time period was from day 20 till day 36 which was an increasing period and the third time period was from day 36 till day 50 which was a decreasing period for nitrite levels. In these two periods the column 6 had higher nitrite levels than initial solution and columns 4 and 5 lower levels. The last period was from day 50 till the end where the spread of results was increasing again. The average levels along the experiment were for column 4 (3.25 μmol/l) for column 5 (3.01 μmol/l) and for column 6 (3.45 μmol/l).

6.5.3.2.2 Nitrate levels Sand Columns

Nitrate levels (Table 6.8 and Figure 6.16 and 6.17) received the best reduction levels along the experiment.

Table 6.8: Nitrate levels Sand Columns

mmol/l	Pore Volume	Influent	Column 4 SMMS	Column 5 PMMS	Column 6 PSMS
Day 1	**1.5**	0.411	0.403	0.403	0.403
Day 2	**3.0**	0.442	0.424	0.354	0.428
Day 4	**6.0**	0.454	0.353	0.384	0.358
Day 7	**10.5**	0.392	0.236	0.256	0.27
Day 10	**15.0**	0.400	0.186	0.173	0.174
Day 13	**19.5**	0.401	0.156	0.138	0.179
Day 16	**24.0**	0.419	0.151	0.114	0.163
Day 19	**28.5**	0.401	0.131	0.152	0.140
Day 22	**33.0**	0.433	0.187	0.156	0.216
Day 25	**37.5**	0.418	0.136	0.175	0.192
Day 28	**42.0**	0.411	0.117	0.175	0.132
Day 31	**46.5**	0.422	0.191	0.166	0.211
Day 34	**51.0**	0.421	0.184	0.168	0.149
Day 37	**55.5**	0.426	0.175	0.214	0.129
Day 40	**60.0**	0.409	0.133	0.159	0.119
Day 43	**64.5**	0.407	0.156	0.166	0.104
Day 46	**69.0**	0.422	0.159	0.171	0.142
Day 49	**73.5**	0.412	0.142	0.147	0.136
Day 52	**78.0**	0.405	0.115	0.176	0.134
Day 55	**82.5**	0.403	0.096	0.115	0.103
Day 57	**85.5**	0.402	0.105	0.132	0.105
Day 60	**90.0**	0.399	0.094	0.094	0.079

At three columns the reduction levels were between 54-56% with the strongest reduction levels in column 4. The experimental period can be separated in 3 periods. The first period was from the start till day 20 which was a reducing period from the initial nitrate levels to average levels. The second period was from day 20 till day 36. Generally, was an increasing period with wide spread of results. Finally, the last period is from day 36 till the end that was a reduction period with the best results achieved in the end of experiment. This follows the pH and Conductivity and may be related to changes in the input water rather than changes in the denitrification behavior in the columns. The average nitrate levels were for columns 4 (0.183 mmol/l) for column 5 (0.190 mmol/l) and for column 6 (0.185 mmol/l).

6.5.3.2.3 Ammonium levels Sand Columns

Ammonium levels (Figures 6.16 and 6.17) were very low in these columns. At all the duration of experiment ammonium levels were >16.63 μmol/l. The experiment can be separated in 3 periods. The first one was till day 14. In that period there was wide spread of results without any specific trend. The second period was from day 14 till day 38. It was an increasing period and all the columns followed the same trend as the initial solution. It was noticed that column 5 had

higher levels than the initial solution and column 4 and 6 lower levels. The last period was from day 38 till the end. In that period every column followed different trend. This follows the pH and Conductivity and may be related to changes in the input water rather than changes in the denitrification behavior in the columns. In column 4 there was a reduction till day 50 and then an increase till the end of experiment. In column 5 there was an increase till day 50 and then a decrease till the end. Finally, in column 6 there was a continuous increase till end of the experiment. The average levels were for column 4 (7.01 μmol/l), for column 5 (10.12 μmol/l) and for column 6 (9.42 μmol/l).

6.5.3.2.4 TN levels Sand Columns

TN levels (Table 6.9 and Figures 6.16 and 6.17) as it is noticed in the other part of experiment followed the trend of nitrate levels. In these 3 columns the reduction levels were the highest with reduction in column 4 more than 54.5 % and average levels along the experiment (0.193 mmol/l) which was the column with the lowest levels.

Table 6.9: TN levels Sand Columns

mmol/l	Pore Volume	Influent	Column 4 SMMS	Column 5 PMMS	Column 6 PSMS
Day 1	**1.5**	0.422	0.415	0.415	0.415
Day 2	**3.0**	0.456	0.440	0.366	0.442
Day 4	**6.0**	0.467	0.368	0.398	0.376
Day 7	**10.5**	0.408	0.250	0.270	0.289
Day 10	**15.0**	0.413	0.200	0.186	0.192
Day 13	**19.5**	0.415	0.170	0.152	0.193
Day 16	**24.0**	0.429	0.159	0.123	0.173
Day 19	**28.5**	0.410	0.142	0.160	0.148
Day 22	**33.0**	0.441	0.195	0.164	0.223
Day 25	**37.5**	0.426	0.143	0.184	0.200
Day 28	**42.0**	0.419	0.125	0.185	0.140
Day 31	**46.5**	0.432	0.200	0.177	0.220
Day 34	**51.0**	0.432	0.193	0.180	0.159
Day 37	**55.5**	0.437	0.185	0.226	0.139
Day 40	**60.0**	0.418	0.142	0.173	0.132
Day 43	**64.5**	0.417	0.163	0.182	0.114
Day 46	**69.0**	0.432	0.166	0.187	0.152
Day 49	**73.5**	0.421	0.148	0.164	0.147
Day 52	**78.0**	0.414	0.123	0.193	0.145
Day 55	**82.5**	0.410	0.105	0.130	0.116
Day 57	**85.5**	0.409	0.114	0.144	0.121
Day 60	**90.0**	0.405	0.103	0.104	0.092

The reduction in column 5 was 52.12% with average levels 0.203 mmol/l and finally in column 6 the reduction was 53.5% with average levels 0.197 mmol/l. The best result with the highest reduction received in the last days of experiment.

6.5.3.3 TOC levels Sand Columns

In column 4 the first 4 days an increasing period existed with the highest value in day 4 (14.99 mg/l). Till the day 37 there was a reduction period with the lowest value (4.99 mg/l). An increasing period followed till day 52 and a reduction period till the end of experiment (Figure 6.18).

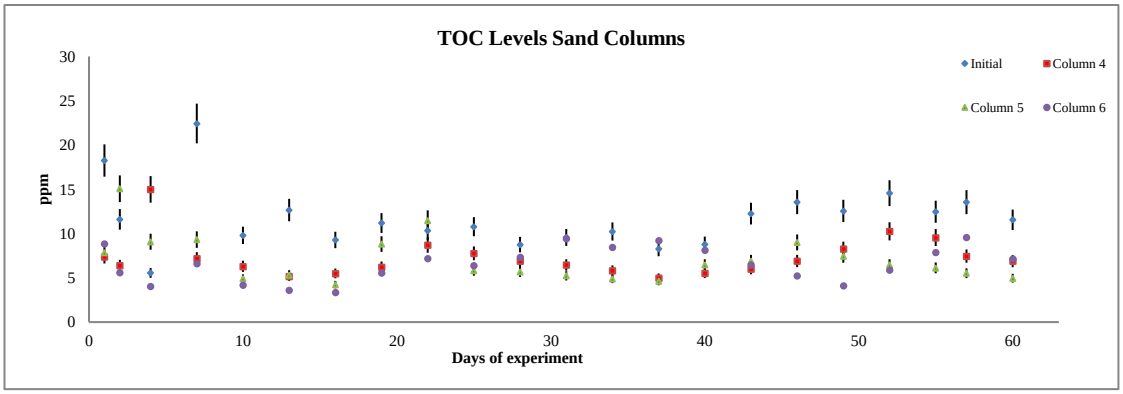


Figure 6.18: TOC levels Sand Columns

In column 5 the increasing and reduction periods were changing all the time. The increasing periods were between days 1-2, days 10-22, days 37-46. The reduction periods were between days 2-10, days 22-37 and days 46-60. In column 5 the lowest value noticed in the 16th day (4.23 mg/l) and the highest value in day 2 (15.06 mg/l).

In column 6 there were two increasing periods in days 16-31 and days 49-60 and two decreasing periods in days 1-16 and days 31-49. TOC levels in that column were lower than all columns with mulch substrate media with the lowest value in day 16 (3.37 mg/l) and highest value in day 31 (9.42 mg/l).

The TOC was measured to determine if the addition of perlite had any impact on the available carbon for denitrification. The error bar in these measurements (10%) does not affect the process. Results show that perlite does not interact with the TOC and therefore should not affect the denitrification reaction with organic substrate.

6.5.3.4 Phosphate levels Sand Columns

Phosphate levels were really low all duration of experiment (Figure 6.19). Significant levels noticed only in first 2 days. After that the levels were lower than 0.3 mg/l at all cases. Again, as

expected, perlite strongly removes phosphate from the system without detrimental effect on the denitrification process. It is also noticed that in column 4 where there is no perlite as substrate material, there is the same trend and the same reduction. This comes in agreement with the result in experiment 2 in chapter 4 where wheat straw also removes phosphate levels. Here in chapter 5 the mulch that used contains high levels of wheat straw and can force the reduction of phosphate levels.

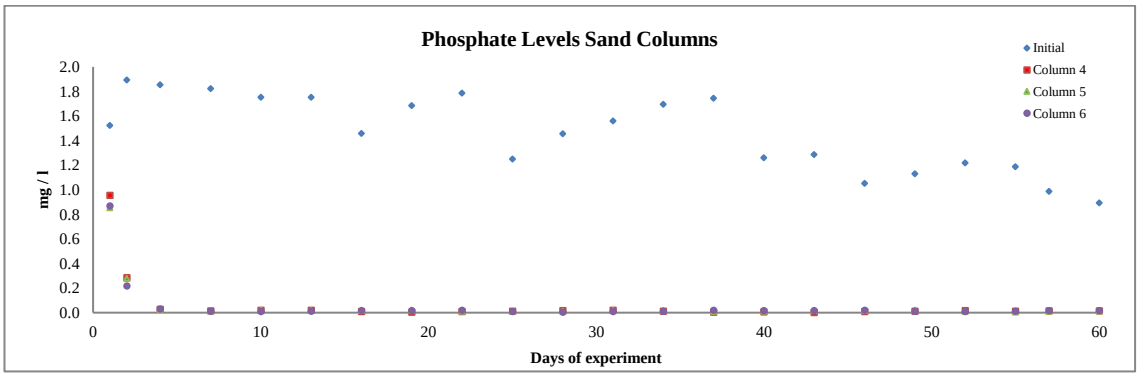


Figure 6.19: Phosphate Levels Sand Columns

6.6 Degradation rates

According to Figure 6.20 there is the detailed approach on degradation rates along the experiment at all columns. The λ values and half-life in columns are characteristics that help to understand the conditions and the microbial activity. The normalised degradation rates used to investigate in detail the difference between the experiments with different HRT and different flow rates. With normalized concertation VS Pore Volume there is a better description of instantaneous rate constants. The degradation rates focused on the stable phase that the process in stabilised and can be described according to kinetics laws. The degradation rates analysed using TN levels and not only NO_3-N, because the two measurements are very close at all measurements and with TN in the research analyse all nitrogen compounds that exist in the columns and participate in denitrification process.

In column 1, the degradation rates can be separated in smaller periods. There are 3 stable phases (PV 10-20, 35-40, 55-75) that are described by zero order kinetics and 3 reducing periods (PV 20-35, 40-50, 75-90) that described by first order kinetics. The changes in the trend along the experiment are a result of the influent groundwater that changed along the experiment and the time that the microbial activity demands to simulate the new environmental conditions. There is no problem with HRT that the denitrification process is completed without any problem and in

agreement with the whole process is the TOC levels that the system provides to the process. The degradation rates show that the reduction of TN in the system is more than 75% in the end of the experiment.

In column 2, the stable period can be separated again in smaller periods. There are 4 stable periods along the experiment described by zero order kinetics (PV 20-32, 45-55, 60-70, 70-85). There are also 2 reduction periods (PV 35-40, 85-90) and one increasing period (PV 10-20) that are described by first order kinetics. The degradation rates show that the reduction in the system can achieve reduction >75%.

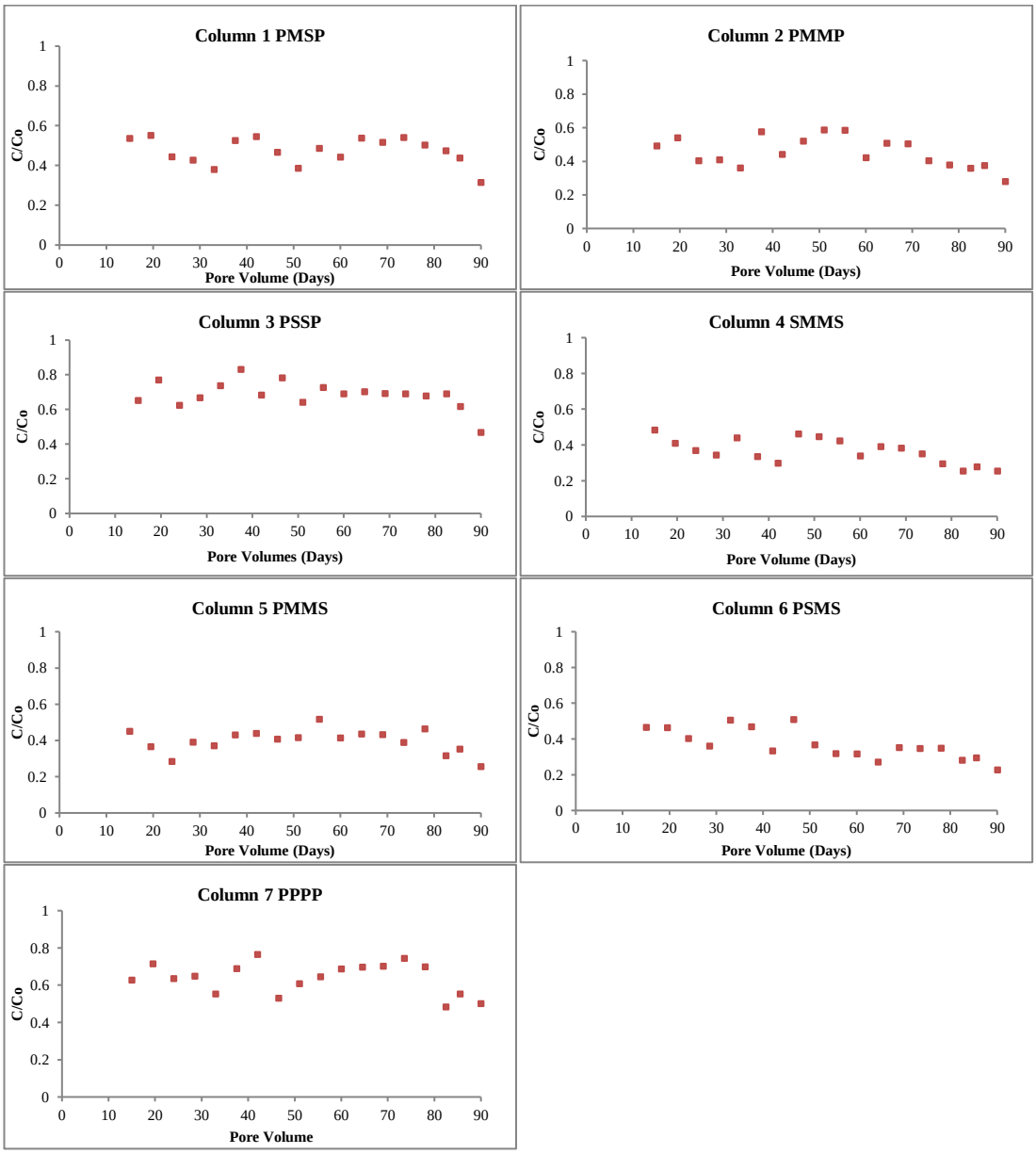


Figure 6.20: Degradation rates experiment sand, mulch and perlite

In column 3, there are lower degradation rates than the columns that contain one layer of mulch as substrate material. The reduction reaches the highest levels (45%) in the end of the experiment. The period can be categorized in smaller periods. There are 3 increasing periods (PV 10-20, 20-40, 40-50) and 1 decreasing period (PV 80-90) that are described by first order kinetics. There is also one stable period that can is described by zero order kinetics (PV 50-80).

In column 4, the reduction levels are increasing with the highest reduction (75%) in the end of the experiment. The experimental period characterized by 2 reducing periods (PV 10-30, 30-40) and 2 stable periods (PV 40-75, 75-90).

In column 5, the degradation rates are following the trend of all the columns with mulch as substrate materials. The reduction in that column achieved the highest levels (80%). In the duration of the experiment there are two reducing periods (PV 10-25, 80-90) that can be described by first order kinetics and one stable period (PV 25-80) by zero order kinetics.

In column 6, there are 2 stable periods (PV 10-20, 50-90) and 3 reducing periods (PV 20-30, 35-40, 45-50) that noticed along the experiment. The reduction in that column also achieved the highest levels (80%).

In column 7, it is the column that the only substrate material is perlite. The organic substrate material does not exist and the reduction is lower (50%). The experiment characterized by 2 increasing periods (PV 30-40, 45-50) which are described by first order kinetics and 3 stable periods (PV 10-30, 50-80, 80-90) which are described by zero order kinetics.

The kinetics in the columns can be described by Monod Kinetics, because the experiment is mentioned to the microbial activity that exists in the columns. According to the equation that describes the kinetics ($C=Co\ e^{-\lambda t}$) the only factor that is important to find is λ value and the half-life of experiment. In the adaptation lag phase is the phase that the reduction achieves levels more than 50%. The best reduction levels are noticed in columns that in the substrate material exist mulch. In that phase there is the fast reduction due to the carbon that exist in waste materials (mulch) that are used and the description of the adaptation lag phase fits better in 1st order kinetics.

In the stable phase, in the experiment that takes places is not easy to define only with one kinetic but is a combination of several smaller phases that described by zero and first order kinetics. The detailed approach described in previous paragraphs.

Table 6.10: λ values and $T_{1/2}$ values on adaptation lag phase and stable phase

Adaptation Lag phase		Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Days	λ_{min}	0.019	0.015	0.002	0.024	0.024	0.024	0.024
	λ_{max}	0.339	0.377	0.113	0.337	0.307	0.304	0.087
Hours	$T_{1/2\ min}$	54.01	48.52	161.97	54.33	59.56	60.24	210.65
	$T_{1/2\ max}$	939.11	1189.84	747.70	747.70	745.70	745.80	742.10

Stable phase		Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
$Days^{-1}$	λ	1.058	1.116	0.522	1.409	1.284	1.391	0.619
	SD	0.214	0.303	0.184	0.325	0.295	0.380	0.219
Hours	$T_{1/2}$	17.900	17.450	33.620	14.380	16.770	15.360	27.930
	SD	0.120	0.190	0.631	0.142	0.175	0.241	0.328

In Figure 6.20 and Table 6.10 there are λ values and half-lives of all the columns for the adaptation lag phase and stable phase. In the adaptation lag phase the λ values for control columns (3 and 7) are lower than all other columns and the half life time is also higher than other columns. This comes in agreement to all the results that the experiment provide for nitrogen compound, TOC levels and pH and conductivity. The same trend also continues in the stable phase. All the results are also in agreement with other studies that used column studies and investigated denitrification process (Aslan and Turkman, 2005; Robertson et al, 2010; Jing et al., 2010)

6.7 Discussion

The duration of experiment was 60 days, where the reaction of substrate material with nitrogen removal and phosphate removal can be described. Nitrogen levels do not meet the expected reduction during the experiment a reduction of 80% was not confirmed. In the last days it was noted that over the duration of experiments the reduction levels may have increased. According to the trends in the experiment and the dynamic that recorded, the reduction levels were expected to reach the highest levels in the next days and an experiment with duration more than 60 days would be more consistent for the microbial activity. The microbial activity exists in the columns and the results in this experiment are supported by the TOC measurements. The organic carbon is provided to the system. TOC remain in stable levels and it is important to provide that amount of carbon for long term period. With those conditions the microbial acidity is working and there is competition for all the processes that exist in columns. The advantage point is that these populations are not in fight to eliminate the organic carbon and can provide the denitrification process in long terms and the reduction of phosphate levels simultaneously. It is also important the reduction levels of nitrogen compounds. It is not noticed the reduction

that other studies (Su and Puls, 2007; Kasirajan and Ngouajio, 2012) noticed from the first days, especially in columns that exist mulch as substrate material, and it demands longer time period to achieve these reduction levels. There is an initial controversial condition that the denitrification bacteria demand more time to assimilate the environmental conditions; even they have all the preferable parameters under the best limits. It was also noticed in all columns, phosphate levels were almost close to zero, showing that perlite is a very good material for phosphate removal as mentioned by other researchers (Huett et al., 2005; Tanaka 2007; McLaughan and Al-Mashaqbeh, 2009; Moutsopoulos et al., 2011).

In Control Columns the reduction of nitrogen levels and more specific in nitrate levels was only between 28-31% in average levels and in absolute value the reduction in the last days achieved levels more than 53%. In combination with neutral pH and conductivity levels that were average and TOC levels that were noticeable, the results are promising to work in longer time period and under natural environment conditions. The advantage point was that not only nitrogen levels reduced but also phosphate levels that has a directly connection with the environment.

In Perlite Columns the reduction levels were higher than Control Columns as it was expected. The average reduction levels along the experiment was higher between 48-49%. The best reduction achieved in the end of experiment again reaching reduction levels > 73%. That was the point that the research focus on and perhaps high phosphate levels resulted in microbial competition and removal of phosphate with perlite helped to maintain the denitrifying bacteria, however as this project did not focus on the microbiology this hypothesis would need to be tested in future research. That was noticed due to the reduction levels that demand longer time periods to provide the best results. The pH levels are neutral lower than Control Columns but again in the gap limit to achieve the best denitrification process which is between pH 6.5 and 8.5 (Rivett et al., 2008). Conductivity levels were higher than Control Columns showing that microbial activity was on process. The supportive clue that the process was on progress was the TOC levels that in both columns were high. Finally, as it is noticed in the control column the reduction of phosphate levels was visible and promising for field application.

The last part was the part with Sand Columns. The average reduction levels of nitrogen compounds were the highest in those columns with average reduction levels along the experiment between 54-56%. This is happening due to the substrate materials that used in these columns and more specifically in the highest amount of mulch that used. The highest absolute reduction rate was achieved in the last days with levels > 78% at all columns. The pH levels were also neutral creating the best conditions for denitrification process. There was a range of

results in conductivity levels that was not showing that affect reduction rates, with higher levels in column 4 (424 μS/cm) and lower levels in column 5 (381 μS/cm). TOC levels were also higher than the other column, which also creates better condition for microbial activity in columns and provided carbon levels to continue the denitrification process. Finally, the reduction of phosphate levels again was noticed.

The hypothesis that was under investigation in Chapter 6 was successfully tested but with some points to consider. The reduction of nitrate levels as noted by other researchers (Gilbert et al., 2008, Schipper et al., 2010a,b) achieved acceptable levels but only in the end of experiment. Another point is the choice of the materials. It is important to choose and combine correctly the materials to achieve the results that expected. The combination of perlite with materials that contain various amount of carbon is the best choice. The layered design in contrast to homogenized designed is preferable, because is easier to handle out each substrate material and to add or remove quantities depending on the equilibrium of the system. The carbon source is concerning point and as other researchers mentions (Warneke et al., 2011, Feng et al., 2013). TOC on substrate material are the key to improve the progress of denitrification activity. Finally, the combination of perlite was significant because it is a new material for PRBs under investigation. Previous studies focused only in the removal of heavy metals and phosphorus, and it was not used in combination to removal of nitrogen compounds. For this experiment the hypothesis was successful. Perlite can be used in combination with other materials and provides very good results and the nitrogen and phosphorous removal rates that are promising at full scale and will be used it in future experiments.

For all columns, degradation rates follow the same trend. There was an initial adaptation lag phase that has duration 4-10 days and then there was a stable phase till the end of experiment. The half-lives received ranges between 14-33 hours which is in agreement with other studies (Robertson, 2010). The Retention Time (RT) at all the columns was between 4-10 hours which is much lower than half-lives times and in combination to TOC levels showing that microbial activity exists and forces the denitrification process along the experiment. Microaerophilic conditions are met in chapter 6 where after the denitrification process that take place in the columns, there are levels of nitrogen compounds along the experimental process. The reduction is achieving levels that are higher than 60% at all nitrogen compounds but still exist a countable amount of nitrogen compounds that is connected with the conditions that exist in columns. The DO measurement ensure that oxygen levels are very low close to zero but the activity that exists in denitrification process in this chapter indicate that there are oxygen levels (i.e. < 21% O_2; typically, 2-10% O_2).

The results for perlite, shows that it is a material that can be used in combination with organic substrates supporting the reduction of nitrogen compounds and further investigation is needed to find out if there are other organic waste substrates that can be used in combination that may enhance and support denitrification more. (More details for all measurements in this chapter exist in Appendix III.)

CHAPTER 7

BATCH EXPERIMENTS WITH HAZELNUT HUSK WASTES AND TEA WASTE MATERIALS

7.1 Introduction

The geographical focus of this research is the Mediterranean area and this chapter shows results of experiments using waste materials found in abundance throughout Greece and Turkey. Within the broad area of the Aegean Sea, Greece and Turkey are famous touristic areas and the huge biodiversity environment that exists there. The new experiment used local legume plants specifically tea waste materials and used hazelnut husk waste materials as a second substrate. These substrate materials were chosen because they are used for different purposes and there is a huge waste amount that is not used and burned producing air pollution. Additionally, that was an original research to find out how these materials react with denitrification process and if they can provide the suitable conditions for that. Finally, these waste materials come from the area that the research focus on, the broaden area of Greece and Turkey that the cultivations are the same. The experiments were separated into 3 three parts. The first batch experiments, the second one with columns studies with these new materials, and finally the last combined the perlite experiments with the new experiments.

The hypothesis that was under investigation here is that there new materials can be effectively combined to enhance the denitrification process and that removal rates can achieve acceptable levels. The hypothesis for batch experiments was to test conditions with very low oxygen levels which are unfavourable for denitrification process. Both materials were used (tea waste materials and hazelnut husk wastes) providing high carbon levels to accelerate denitrification process.

7.2 Substrate Materials

The substrate materials that used in this set of experiments described in details in Chapter 3. The first substrate material that used in was hazelnut husk wastes that received from local farms in Ordu (Middle Black Sea Region) after harvesting. The husk was cleaned of non-husk impurities, washed, dried at 105° C for 3 hours. The second substrate material that used was Tea

waste materials from the Caykur tea factory located in Cayeli Rize, the East Black Sea Region of Turkey.

7.3 Description of experiment

At first part of these experiments, batch tests were carried out. At this part, eight 100 ml glass flask were used. Batch tests (Figure 7.1) were carried out in triplicate to determine the capability of wastes to provide dissolved organic carbon and stimulate the activity of denitrifying bacteria. The glass flasks were filled with an equal part of reactive materials (about 20 cm^3). The measurement on the reactive materials was volumetric. The reactive materials were containing organic substrate and sand (inert material).

The organic materials that used in batch experiment were hazelnut husks and tea waste materials. The percentage of organic substrate in reactive material was 40, 60 and 100% (v/v).

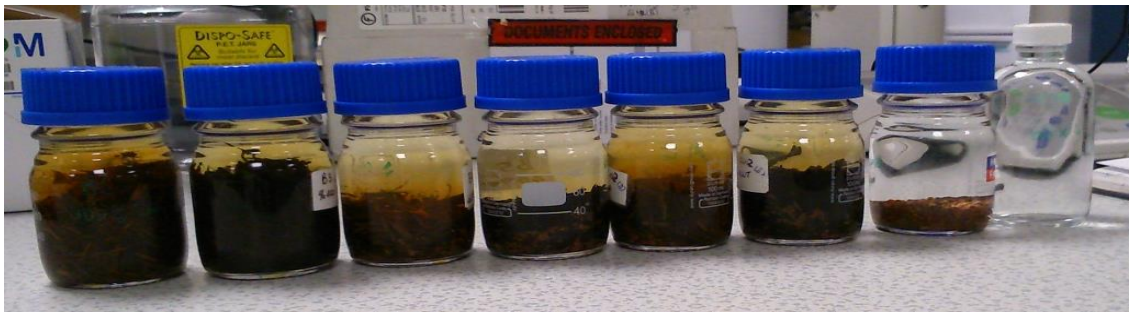


Figure 7.1: Batch experiment with TW

Mixtures consisting of organic residue and sand were carefully mixed to obtain homogeneous samples. They were not layered when the substrate materials insert in the flasks but with the addition of the solution and due to the specific density of each material finally in the samples days the result was as to be layered. Due to that fact the diffusion effect was visible especially in Tea flasks and all the measurements became under precision. Flasks with only sand and nitrate solution were also prepared for control. Each flask was filled with nitrate solution containing 32.2 mg/l NO_3-N and sealed to create as much anoxic conditions as it was possible. This was received by using stirrer and removing the amount of oxygen. All flasks remain in the stirrer for 10 minutes to achieve low oxygen levels and to remove all trapped oxygen in the substrate materials. All flasks were covered with aluminium foil to simulate dark conditions encountered in the aquifer and were kept at room temperature for 66, 132, 198 and 264 h at the initial experiment. In the first experiment tap water was the solution. All the experiments became in room temperature (20±2^{o}C). All measurements became in triplicate. The time that was the

conditions were changing and it was important to investigate in more details was 198 h. It was the critical time to see the changes in aqueous environment of flasks (change in nitrogen compounds concentration).

At the second part the solution media was local groundwater from Largs, UK (Figure 7.2). Groundwater was refrigerated at 4^{o}C from collection time till the application in experiments. The location of groundwater that received for the experiments was an agriculture area. The area was a grass area with extensive sheep grazing around. The collection point was from a spring that is connected with smaller torrents. The quality of groundwater depends on time period of the year due to environmental conditions and the agricultural activity of the area. The collection of groundwater for that experiment became in October that is typically to show the quality of water after the summer period. That is important because during the summer period the cultivations demand higher amount of water and additionally due to the livestock of the area the characteristics are changing. Another point is that period is the dry period and the level of water is lower than spring and are not affecting the quality of groundwater with the same way as in spring. Figure 7.2 shows the location of collection point.

Figure 7.2: Groundwater collection point Largs Scotland, UK (https://www.google.co.uk/maps)

After the experimental hours the aqueous solutions in flasks were passed through 0.45 μm cellulose syringe filters and then analysed for NO_3^-, NO_2^-, NH_4^+, TOC, pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO). The measurement for pH, ORP potential became with Mettle Toledo multimeter. DO measurement became with DO meter (Hanna). TOC analysed with TOC analyser Apollo. And finally all the measurements for nitrogen compounds became with IC chromatography (Metrohm 850). TN measurement became by the addition of all nitrogen compounds.

7.4 Results

Tap water used in find out the reaction of these materials with the water that is used for drinking purposes and it is disinfected to be capable for drinking purposes. The local tap water received and analysed. The groundwater as it is mentioned receives from Largs and also analysed before the application in the flasks. The detail results of solutions characteristics are shown in Table 7.1.

Table 7.1: Characteristics of Tap water and Groundwater

	pH	Conductivity µS/cm	Redox mV	TOC ppm	NO3-N µmol/l	NO2-N µmol/l	NH4-N µmol/l	PO4-P mg/l	TN µmol/l	K mg/l
Tap Water	7.55 ±0.14	360.86 ±42.58	234.97 ±62.87	2.69 ±1.38	2.09 ±1.05	1.02 ±1.48	0.50 ±1.01	1.63 ±1.27	3.61 ±1.58	1.22 ±0.58
Ground Water	7.52 ±0.08	399.41 ±21.06	146.95 ±17.84	20.35 ±1.68	5.07 ±2.05	2.78 ±0.54	1.18 ±1.26	3.53 ±0.67	9.03 ±1.75	2.55 ±0.95

7.4.1 Local Tap Water (TW) as solution media

In first part, the batch experiments ran with a repeating period of 66 hours. The addition of tap water in flask with the use of stirrer ensured the low oxygen levels that was under investigation in batch tests. There are repeating batch tests for 66, 132, 198 and 264 hours, to find out the reaction of the microbes in that environment and the effect of them in denitrification process. All experiments repeated in triplicate.

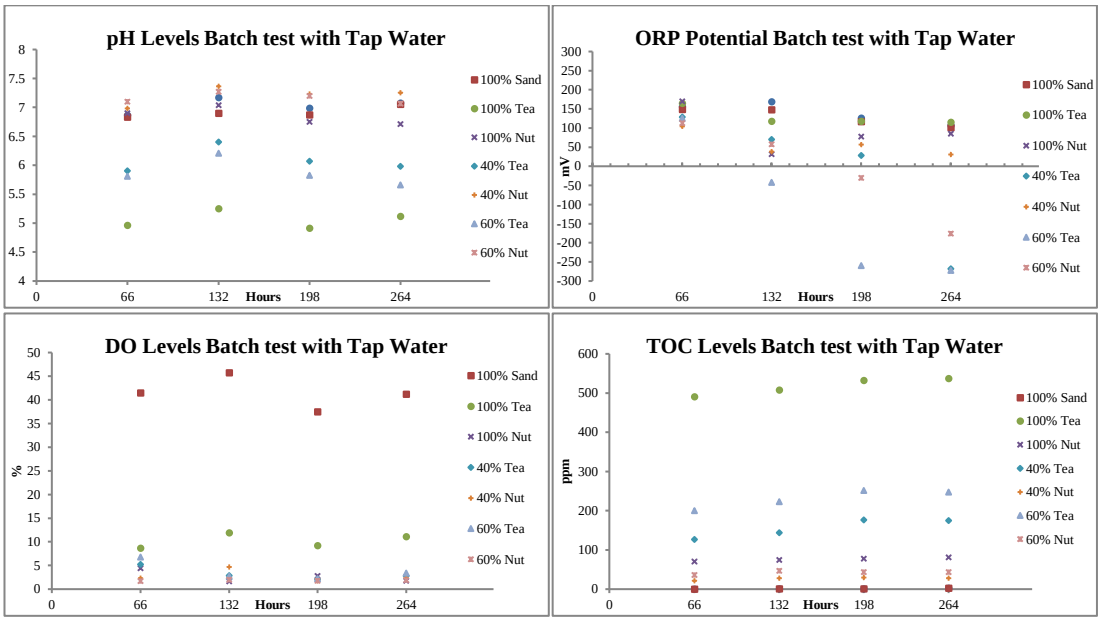


Figure 7.3: pH, redox potential, DO, TOC levels, batch experiment with TW

The pH levels at batch experiments are separated in the part of tea flasks that levels are much lower in contrast to hazelnut husk flasks that pH is higher. The values in Figure 7.3 shows that the changing period was 198 hours were the environment became again acidic. The lowest values noticed in the flask with 100% Tea (pH=4.91), the highest value achieved in 40% Nut flask (pH=7.36). Tea flasks achieved more acidic results in contrast to nut flasks that pH was in neutral zone and there were not a lot of changes even from control samples.

Redox potential (Figure 7.3) was an indicator to show the activity in flasks. It is noticeable at all bottles that the more the solution remained in flasks the lower values achieved. In the Tea experiment, the highest ORP potential was noticed in 100% Tea flask with the highest value in 66 hours (ORP= +164 mV). The lowest ORP values in Tea experiment were noticed in 60% Tea bottles with the lowest value at 264 hours (ORP= -272 mV). In the Nut experiment, the behaviour of flaks was different. The highest values were noticed in 100% Nut bottles in 66, 198 and 264 hours but in 132 the lowest value was noticed in that flask. The peak value was in 66 hours (+169 mV) which was the highest value at all experiment. The lowest value noticed in 66 hours in 40% Nut, in 132 hours in 100% Nut and in 198 and 264 hours in 60% Nut. The lowest value in the Nut experiment achieved in 264 hours (-176 mV).

DO levels (Figure 7.3) in batch test remained very low in Nut flasks with percentages DO < 4.5% saturation in all columns. DO levels are reducing along the time. This is happening at all columns. Tea columns have higher DO levels and especially in 100% Tea flask that was the

only column that DO levels were increasing and not decreasing. Finally, at Sand flask, DO were higher than any other material. DO levels were reducing more than 37% at all flasks.

TOC levels (Figure 7.3) were following the same direction in Tea and Nut flasks. Highest values were noticed in 100% Tea bottles at all cases, with the highest value (537.67 ppm) in 264 hours. In the Tea experiment the lowest values noticed in 40% Tea, with the lowest in 66 hours (126.66 ppm). In Nut experiment, TOC levels were much lower than Tea flasks. For the Nut experiment the same situation was noticed with 100% Nut bottles achieved the highest values and 40 % Nut bottles the lowest values. The highest value in the Nut experiment noticed in 100% Nut flask in 264 hours (81.04 ppm) and the lowest in 40% Nut flask in 66 hours (21.36 ppm) which was the lowest value at all experiment.

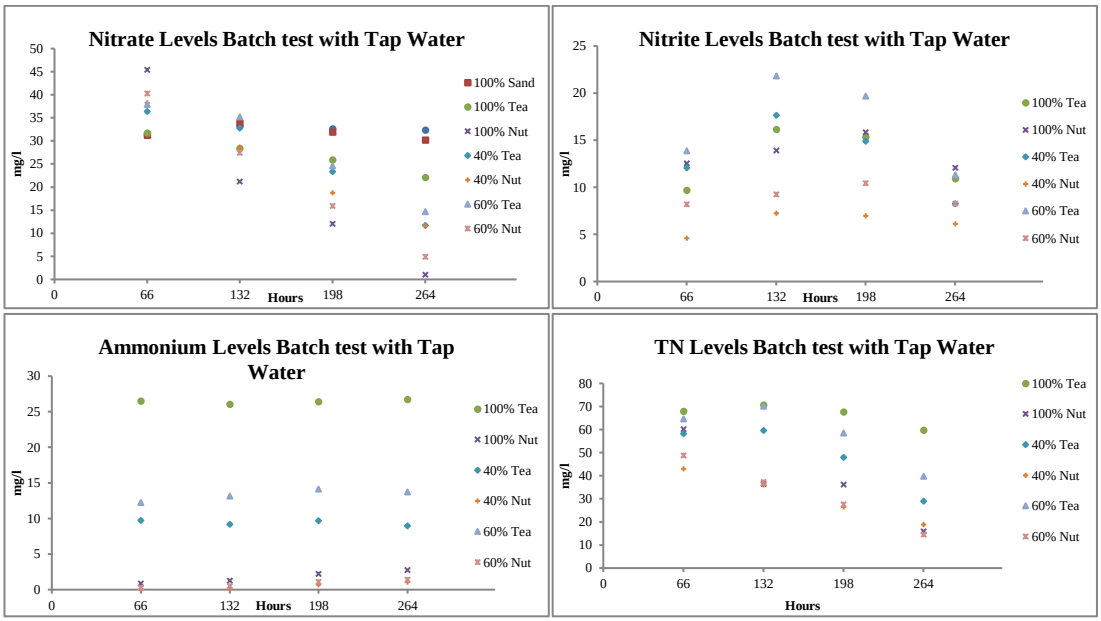


Figure 7.4: Nitrogen species with TW

Nitrate levels (Figure 7.4) were the part of experiment that research focus on with more details. The hypothesis of experiments was to find out the reduction levels of nitrates under not preferable for the process conditions (low oxygen levels, no lights connection). In the Tea experiment, it is shown that reduction. The levels of reduction achieved 75% but not in all bottles. The best decrease was achieved in 264 hours. In 66 and 132 hours in 40% and 60% Tea flask noticed an increase of nitrate levels which is something that is noticed at chapter 4 and from other researchers (Rivett et al., 2008). In 100% Tea flask the lowest value achieved from the first hours. The reduction was small and continued with the same way till the end of experiment but achieving only 25% reduction at total. In 100% flaks in 264 hours nitrate levels were 22.11 mg/l NO_3-N. In 40% Tea flask after 198 hours and at 264 hours the lowest value

was noticed and the highest nitrate reduction (75%) with NO_3=11.75 mg/l NO_3-N. The 60% Tea flask in 264 hours achieved reduction more than 55%. In the Nut experiment the reaction of denitrification bacteria was faster than the Tea experiment and more effective achieving reduction levels between 75-99%. In 66 hours the highest values were achieved not only at Nut experiment but at all experiment. The highest value was noticed in 100% Nut flask (45.39 mg/l NO_3-N). After that and 132, 198 and 264 hours the lowest values achieved in 100% Nut flask and highest values in 40% Nut flasks. The lowest value was received in 100% Nut in 264 hours (NO_3=1.06 mg/l) which was the lowest value at all experiment. The critical point at all experiments was 198 hours were the reduction was noticeable.

Nitrite levels (Figure 7.4) are noticeable at all days of experiments at all bottles. This was an indicator that denitrification process did not finish. In Tea experiment the highest values were noticed in 60% Tea flask at all the duration of experiment. The highest value in Tea experiment was noticed in 132 hours in 60% Tea (21.82 mg/l NO_2-N). In contrast the lowest levels were noticed in 100% Tea bottles till 132 hours and after that in 40% Tea bottles. The lowest value was noticed in 264 hours in 40% Tea (8.26 mg/l). In the Nut experiment, nitrite levels were lower than the Tea experiment. In all bottles the highest values were noticed in 100% Nut with the highest value in 198 hours (15.85 mg/l). The lowest values were noticed in 40% Nut with the lowest value in 66 hours (4.60 mg/l).

Ammonium levels (Figure 7.4) were noticeable especially in the experiment of Tea bottles. The noticeable in the Tea experiment was that ammonium levels remained stable along the experiment. The highest values achieved in 100% Tea flasks with the highest value in 264 hours (26.71 mg/l). The lowest values achieved in 40% Tea flasks with the lowest value in 264 hours (8.97 mg/l). In the Nut experiment, ammonium levels were much lower and at all flasks less than 3 mg/l. Again here as it was in Tea experiment the highest levels achieved in 100% Nut flasks with the highest value in 264 hours (2.73 mg/l). The lower levels achieved in 40% Nut flasks with the lowest level in 66 hours (0.06 mg/l).

Total nitrogen levels (Figure 7.4) were noticeable in the experiment with Tea bottles. At all the duration of experiment 100% Tea flasks achieved the highest values with the highest in 132 hours (70.64 mg/l). In the Tea experiment the lowest levels achieved in 40% Tea with the lowest value in 264 hours (28.99 mg/l). In contrast in Nut experiment the results were more complicated. Generally, TN levels were lower than Tea experiment. The highest levels noticed in 66 and 198 hours in 100% Nut flasks and 132 hours in 60% Nut and finally in 264 hours in 40% Nut. The highest values achieved in 66 hours in 100% Nut bottle (60.14 mg/l). In the other

side, the lowest values achieved in 40% Nut in 66, 132 and 198 hours and in 264 hours in 60% Nut flask which was the lowest value at all experiment (14.57 mg/l).

7.4.2 Groundwater (GW) as solution media

Groundwater solution received from the agricultural area outside of Largs UK and kept in 4 ºC till the time of experiment. At batch test (Figure 7.5) with groundwater the research focused on the 198 hours experiments because was the time that the changes were visible in experiment 1 with tap water.

Figure 7.5: Batch experiment with GW

The experiment run in triplicate and the result are shown below in comparison with the results from experiment1 for 198 hours results. The 198 hours selected because it was the time period that nitrogen compounds start reducing and especially in TN compounds that contains all other nitrogen compounds. Additionally, it was an indicator in that time period for the microbial activity in the flasks that denitrification process was working and could provide in the experiments the best results. The conditions of experiment were totally the same like the batch experiment with tap water. Groundwater used to simulate the conditions that exist in nature.

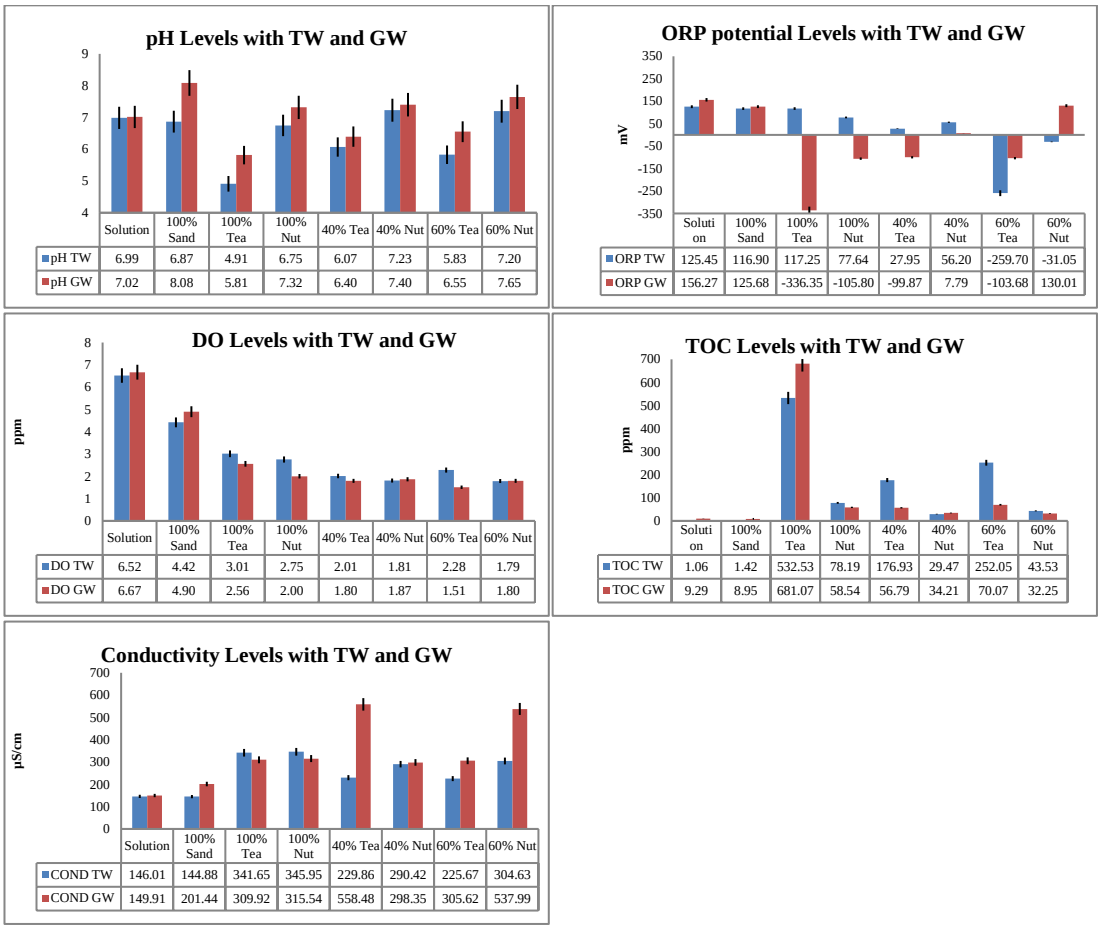


Figure 7.6: pH, redox potential, DO, TOC, conductivity levels, batch experiment with TW and GW (198 h)

The pH level in experiment with groundwater is higher than experiment with tap water (Figure 7.6). The values at tea flasks were lower than nut flasks. The lowest value noticed in 100% Tea flask (pH=5.81). The highest value noticed in the 60% Nut flask (pH=7.65).

Redox potential (Figure 7.6) in experiment with groundwater received values that were negative in all Tea flasks. In contrast, in tap water solution there were lower values except from 60% flasks. The most negative value in experiment with groundwater achieved in 100% Tea flask (-336 mV) and the highest value in 60% Nut flask (+130 mv).

DO levels (Figure 7.6) in groundwater flasks were slightly lower than flasks with tap water. This was noticeable at all flasks expect from flask with 40% Nut. The highest levels even in Tea even in Nut flasks were noticed in 100% bottles with 2.56 ppm and 2 ppm, respectively.

TOC levels (Figure 7.6) are also an indicator to show that microbial activity exists in flasks. This is important because the high TOC levels can create environmental conditions that can

stoke the system of denitrifier bacteria with the suitable amount of organic carbon that denitrification process demands. TOC levels were higher in experiment 1 expect from two flasks (100% Tea and 40% Nut). In flask with 100% Tea, TOC levels were the highest even in tap even in groundwater with the highest value in groundwater (681.07 ppm). At all cases Tea flasks achieved higher values than Nut flasks at both parts of experiment.

Conductivity levels (Figure 7.6) for experiment 2 were higher than experiment 1. The only two flaks that tap water flasks had higher conductivity were the bottles with 100% materials. The most noticeable difference received in 40% Tea flask with groundwater with conductivity 558.48 μS/cm which was the highest value at all experiment and in 60% Nut flask with groundwater with 537.99 μS/cm which was the highest value for the Nut flasks.

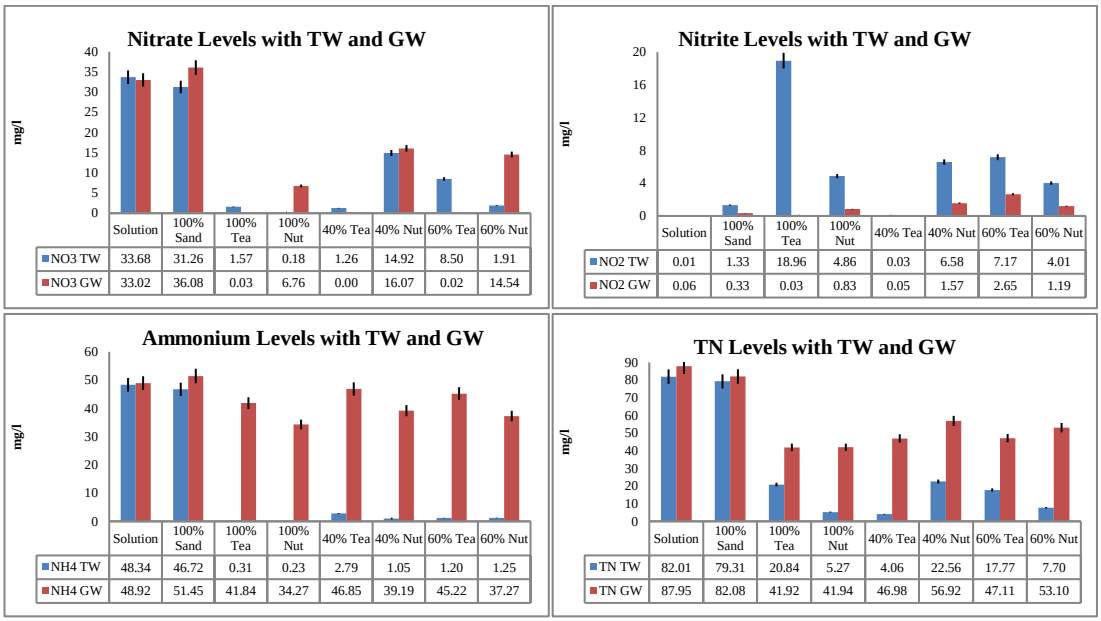


Figure 7.7: Nitrogen species TW and GW (198 h)

Nitrate levels (Figure 7.7) in experiment with groundwater were higher in Nut bottles and lower in Tea flasks. The noticeable was that in 8 days the reduction in Tea flaks was more than 99%. Again in contrast to experiment 1 nitrate levels were higher in experiment 2. At Nut flasks nitrate levels were much higher and reduction that achieved was between 50-75%. At all cases nitrate levels in Nut flasks with groundwater were higher tap water. The lowest value in the Nut experiment was notices in 100% Nut flask and the highest in 40% Nut.

Nitrite levels (Figure 7.7) at all cases were much lower than experiment 1. In Tea flasks the highest value achieved in 60% Tea (2.65 mg/l). That was also the highest value at all flasks. All Nut flaks had higher levels than Tea flasks except from 60%.

Ammonium levels (Figure 7.7) at experiment 2 were noticeable higher than experiment 1. At all flasks ammonium levels were more than 30 mg/l NH_4-N. Tea flasks achieve higher levels of ammonium than Nut flasks. The highest value achieved on 40% Tea flask (46.85 mg/l). The lowest value was in 100% Nut bottle (34.27 mg/l).

Total Nitrogen levels (Figure 7.7) in experiment 2 were much higher than experiment 1. The lowest values noticed in 100% flasks even with Tea even with Nut compounds. Total Nitrogen levels were higher than 40 mg/l at all flasks. The highest levels were noticed in 40% Nut flask (56.92 mg/l).

7.5 Discussion

The batch experiments were designed test to investigate the reaction of denitrification bacteria under low oxygen levels and without light connection conditions. The amount of oxygen that exists in flasks was the smallest that it can be achieved in the lab with the instruments that were available (stirrer). With those environmental conditions the researcher attempted to simulate an aquifer. Batch experiment with tap water was an initial approach to investigate the reaction of substrate media in these conditions during time. That was the reason that results received for 66, 132, 198 and 264 hours. With all this investigation the critical point for batch experiment in experiment 1 was 198 hours were nitrate levels started reducing on acceptable levels and also pH, redox and conductivity results supported that result. In groundwater solution there was a different approach of substrate materials with the solution. The pH results were more neutral that help denitrification process with microbial activity to react faster and to receive better results. TOC levels were also higher experiment 1, another indicator for better activity in bottles. Finally nitrate levels were investigated. In Tea bottles the reduction that was achieved was higher than in experiment 1; achieving levels more than 99%. In contrast in Nut bottles the groundwater solution did not work as well as in Tea bottles achieving reduction levels between 50-80%. The critical point for the in experiment with groundwater was the high levels of ammonium levels at all bottles.

Generally, the process of experiments with tap and groundwater and the results that received gave the motivation to investigate in details columns with the same substrate media. The point was to investigate not only in specific time but in long time period the reaction of materials in that environment and the effectiveness of them in removal of nitrogen compounds. More details about all measurements of Chapter 7 exist in Appendix IV.

The hypothesis that was under investigation was partially confirmed. Nitrate levels reduced in both substrate materials. The best reduction received in Tea bottles (more than 99% in GW and between 75-98% in TW). In Nut bottles the reduction did not achieve these reduction levels with the best results in TW (55-99%) and in GW (45-95%). For nitrate levels hypothesis confirmed. For TN levels there is a depending situation. In TW solution the reduction achieved levels between 35-90%. In contrast in GW there was no reduction but in contrast increase of TN levels between 30-55%. This happened due to high ammonium levels. Further investigation for that experiment is required. The time period for GW was not enough to finish totally denitrification process. To support our results, samples for microbial analysis were undertaken and results obtained through q-PCR method (Chapter 4) proved that denitrifying bacteria exist in both substrate materials bottles.

According to the results that provided from the q-PCR analysis and for denitrification process the main issue was to focus on the nirK and nirS reductases. The nirK and nirS genes were useful targets for PCR primers to detect communities of denitrifying bacteria in samples from batch test flasks. 20ml of each sample was filter and countered the communities of denitrifying bacteria. The results are providing in Table 7.2.

Table 7.2: nirK and nirS genes in 20ml filtered sample Batch Experiment

log[(nirK/S) /ml]	**NO_3 solution**	**100% sand**	**100% Tea**	**100% Nut**	**60% Tea**	**60% Nut**	**40% Tea**	**40% Nut**
nirK	3.77	2.27	0.91	3.72	3.21	3.85	2.26	2.86
nirS	2.50	2.97	3.86	3.93	2.01	3.82	1.17	3.60

The results of q-PCR that are provided support the hypothesis that denitrifying bacteria exist in the flasks. As it is mentioned with that measurement detect the community that exist without knowing if these are active or not. In combination with the results that received at the chapter, the hypothesis confirmed and the colonies are active.

With batch experiments providing the results that expected for total new materials in denitrification process, the next step was to investigate in longer term column experiments to evaluate these processes under groundwater flow conditions.

CHAPTER 8

COLUMNS EXPERIMENT WITH TEA WASTE MATERIALS AND HAZELNUT HUSK WASTES

8.1 Introduction column experiments

The experiments in Chapter 7 used tea waste materials and hazelnut husk waste in batch tests gave results that showed reduction of nitrates through denitrification process under conditions that are not preferable for the process.

Here, column flow experimental approach for these two materials was continued as longer time-scale experiments. The question under investigation was if these two waste materials can be successfully sustained denitrification process in columns to reduce nitrogen levels in long term experiments. The substrate materials are chosen in combination with the research that took place in Samos Island and it is connected with the area of East Mediterranean area. The two substrate materials can provide high levels of organic carbon that can enhance denitrification process. The approach was the same as the previous column experiments (Chapters 5-6) and the solution that used was in the Experiment 1 was tap water (TW) and in Experiment 2 was groundwater (GW) from suburban area of Largs, outside from Glasgow.

The new hypothesis was to find out if these substrate materials work effectively in longer term experiment. It is the first time these materials are used in columns experiments. From the results found in Chapter 7, it is expected nitrate removal of more than 80% and a removal of TN levels more than 50% as the initial hypothesis.

Lab-scale barrier system was set up using eight PVC columns of 52.5 cm length and 5 cm internal diameter. These columns were filled with identical reactive materials, two of the columns were filled with 40% (v/v) organic substrate and 60% (v/v) sand, two with 60% (v/v) organic substrate and 40% (v/v) sand and two with only sand (100%). All materials were placed as saturated materials into the columns full with water to avoid the presence of trapped air. The porosity of reactive mixtures was determined approximately from the volume of displaced water. The columns were fed with a synthetic groundwater (0.511mmol/l NO_3^--N) in up-flow mode. Two different flow rates were applied using two multichannel peristaltic pumps. The hydrodynamic characteristic, flow rate and hydraulic retention time of columns were

investigated with a tracer test using 1000 mg/l chloride solution. The determined flow rates and retention times were approximately 42 mL d^{-1} and HRT 3.25 d for the slow columns and 84 mL d^{-1} and HRT 6.10 d for the fast columns, respectively. All columns were sealed to obtain anoxic conditions and covered with aluminium foil to avoid light penetration. Experiments were carried out at room temperature (20±2ºC). Effluents were taken four to five times a week and analysed for NO_3^-, NO_2^-, NH_4^+, PO_4^-, TN and TOC.

8.2 Experiments with tea waste materials and hazelnut husk wastes

The experiment with columns (Figure 8.2.1) is separated in two main parts. The first one is the experiment 1 with columns flowing with tap water as solution media and experiment 2 with groundwater as the solution. The experiment 1 was running for 51 days. The experiment 2 with groundwater was running for 31 days.

The stability of flow rate insured with peristaltic pumps (Ismatec). Two pumps were used in the experiment one in the part with fast flow rate and one with the slow flow rate. The tubes had the same diameter as well as the connections from initial tanks until columns. They had also the same length to reduce any loose from the transfer of solution media in columns from initial bottles that used as initial tanks.

Figure 8.2.1: Design of column experiments with tea and nut

8.3 Results Experiment 1 with Tap Water solution

Tap water was used from the laboratories in Civil and Environmental Engineering department. No treatment applied before the use of water in the experiment. Initial measurement for initial levels of all anions, cations, pH, conductivity, redox potential and TOC received. Average results (±SD) are given in the Table 8.3.1. All anions and cations measured with IC. More details are given in Appendix V.

Table 8.3.1: Results experiment with TW (all results received after (N=3 replicates) triplicate analysis)

		Influent	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
pH	mean	7.55	7.59	7.44	7.44	7.21	7.27	7.53	7.39	7.36
	SD	0.14	0.24	0.22	0.28	0.39	0.25	0.24	0.26	0.26
	% change		0.54	-1.43	-1.44	-4.51	-3.61	-0.20	-2.09	-2.44
Conductivity µS/cm	mean	360.86	349.91	401.93	617.06	494.98	357.79	430.56	404.92	381.92
	SD	42.58	134.05	110.81	87.35	74.29	57.10	64.81	59.47	143.07
	% change		-3.03	11.38	71.00	37.17	-0.85	19.32	12.21	5.84
Redox mV	mean	234.97	212.71	233.44	207.03	197.38	209.63	218.60	208.68	223.78
	SD	62.87	52.99	58.36	35.61	38.47	35.26	46.45	43.10	51.75
	% change		-9.47	-0.65	-11.89	-16.00	-10.78	-6.96	-11.19	-4.76
TOC ppm	mean	2.69	3.24	3.50	38.72	52.36	7.62	6.82	7.90	4.45
	SD	1.38	1.22	1.46	17.28	32.44	5.70	1.51	1.65	1.25
	% change		20.41	29.89	1336.87	1843.27	182.97	153.07	193.25	65.10
NO3 mmol/l	mean	511.09	372.83	348.12	0.06	0.17	0.63	0.14	0.22	0.69
	SD	14.23	74.59	35.93	0.22	0.30	1.31	0.30	0.58	1.79
	% change		-27.05	-31.89	-99.99	-99.97	-99.88	-99.97	-99.96	-99.86
NO2 mmol/l	mean	1.02	3.97	89.46	1.15	1.15	1.29	1.25	1.51	1.81
	SD	1.48	6.24	122.14	1.46	1.31	1.33	1.29	1.69	1.49
	% change		288.11	8653.72	12.28	12.38	25.98	22.11	47.50	77.40
NH4 mmol/l	mean	0.50	1.70	2.56	0.72	7.34	1.75	2.34	2.66	1.00
	SD	1.01	2.43	2.96	1.70	8.80	2.47	3.05	3.93	1.43
	% change		242.68	417.36	45.81	1381.94	253.78	371.85	436.33	102.02
PO4 mg/l	mean	1.63	12.82	17.66	12.58	10.99	10.95	13.68	11.49	22.87
	SD	1.27	9.27	4.85	18.62	11.11	12.71	13.93	12.25	11.31
	% change		688.12	985.78	673.04	575.40	573.27	741.08	606.52	1305.48
TN mmol/l	mean	512.61	378.50	440.15	1.93	8.66	3.67	3.72	4.38	3.51
	SD	13.36	75.99	119.22	2.27	9.33	4.22	3.72	4.98	3.42
	% change		-26.16	-14.14	-99.62	-98.31	-99.28	-99.27	-99.14	-99.32
K mg/l	mean	31.87	15.43	19.31	14.92	16.31	18.06	13.44	16.61	17.96
	SD	1.60	9.42	9.84	9.59	8.36	12.13	7.87	7.95	11.50
	% change		-51.59	-39.42	-53.18	-48.83	-43.35	-57.83	-47.90	-43.67

Tap water was used in that experiment and not distilled or deionized water due to the microbial activity that exist in the solution. Tap water is the water that is used from drinking purposes and can be found everywhere. Additionally, tap water contains anions and cations and there is the characterization of water (hard-soft). Another point is the choline disinfection that is applied to the water to kill all biological toxins that may exist.

8.3.1 pH and conductivity levels

Figure 8.3.1 shows the results of pH levels with tap water solution. All the detailed results are in Appendix V.

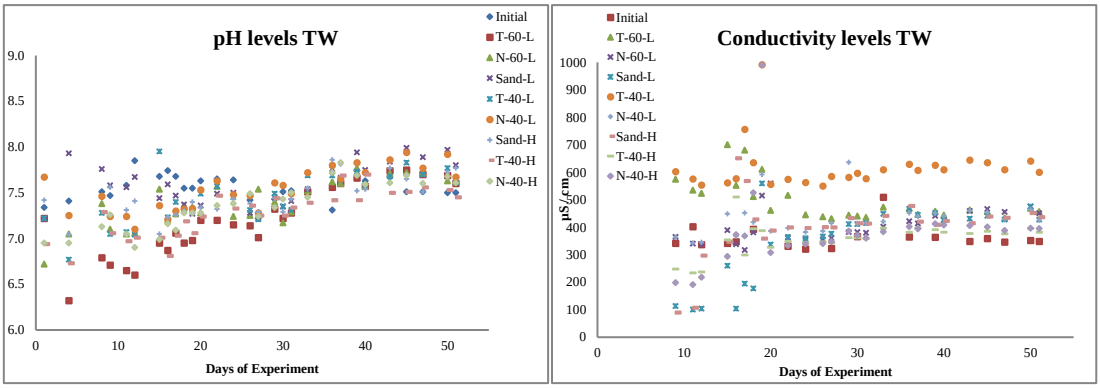


Figure 8.3.1: pH and conductivity levels in tea and nut columns with TW

The pH levels at columns almost follow the same trend. They are categorized in 3 parts depending on the substrate materials of each column: Sand columns, Tea columns and Nut columns. Sand columns are working as control columns because the only material is sand.

Sand Low Flow column was the column with the highest pH levels in experiment (pH=7.99). Sand High column follows the trend of Sand Low column again with high pH at all the duration of experiment.

Tea columns have the lowest pH levels than any other columns. T-60-L column was the column with the lowest levels of pH of all experiments. There were 19 days that pH kept below 7 with the lowest value measured in day 4 (6.32). After that period with acidic pH there was an increasing period till day 45 were the highest pH received and till the end of the experiment there was a reduction period. The average pH for all duration of experiment was pH=7.21. In T-40-L column pH was higher than T-60-L column. The highest value received in day 15. After that there was a reduction period till day 27 and then again an increasing period till day 45. That is Tea column with the highest pH and the highest average (pH=7.44). Finally, is T-40-H column. The flow rate was higher than other two columns. In that column there were many days with pH lower than 7 and pH remained in average values (pH= 7.27). The highest pH received in day 40 (pH=7.70).

The last part is the part with Nut columns. In Nut columns pH levels were higher than Tea columns. In N-60-L column pH except from day 1 remained in neutral area with the highest value in day 39 (7.77). In N-40-L column pH was higher than all other Nut columns and the average value along the experiment was pH=7.53. The highest value noticed in day 45 (7.94). It is also noticeable the lowest pH levels received not the first four days but at day 12 (7.10). Finally, N-40-H column was the Nut column with the lowest average pH (7.36). The highest pH received in day 39 and at the first 15 days there were periods with pH lower than 7.

In Figure 8.3.1 there are the results of all conductivity levels with tap water solution. Conductivity levels had wide range of results in experiment 1. In Sand columns there were very low conductivity levels especially in first days of experiment. In Sand Low column the lowest value received in day 16 (104.01 μS/cm). The next period was a period with the highest values following by a stabilized period till the end of experiment. The average conductivity in Sand Low column was 349.91 μS/cm. That was the column with the lowest average conductivity levels. In Sand High column the lowest conductivity value observed in day 1 (89.70 μS/cm). The conductivity was increasing till day 16 where the highest value observed (651.32 μS/cm). After that there was a reduction period till day 27 and till the end of experiment conductivity was increasing.

In Tea columns conductivity levels were the highest than all other columns. In contrast to Sand columns conductivity levels in Tea columns with low flow rate were much higher from day 1. In T-60-L column, the highest value observed in day 15 (699.87 μS/cm). After that there was a reduction period till day 27 (431.71 μS/cm) and then till the end of the experiment an increase period but with stable way. The average conductivity in column was 494.98 μS/cm. In T-40-L column conductivity levels were the highest. In this column the lowest value observed in day 12 which was really high (553.36 μS/cm). The experiment continued with an increasing period till day 19 were the highest conductivity noticed in all columns (991.82 μS/cm). After that, till the end of experiment there was a stable period with conductivity levels between 550-650 μS/cm. The average conductivity was 619.28 μS/cm. Finally, T-40-H column was reacting with a totally different way. Conductivity was much lower than other Tea columns. The lowest value observed in day 11 (232.81 μS/cm), which was the lowest level for Tea columns. In day 16 the highest conductivity observed (509.46 μS/cm). The average conductivity for that column was 357.79 μS/cm.

In Nut columns conductivity levels were more stable than other two categories with Sand and Tea. In Nut columns it was noticed the same approach as Tea columns. The levels in low flow

rate columns were higher than column high flow rate. In N-60-L column there was an initial low period till day 17 where the lowest conductivity level observed (316.98 μS/cm). The highest value observed in day 20 (559.53 μS/cm) and average levels along the experiment were 404.92 μS/cm. In N-40-L column conductivity levels were higher than all Nut columns. The highest value observed in day 29 (636.19 μS/cm). The average conductivity along the experiment was 430.56 μS/cm. Finally, in N-40-H column conductivity levels were lower than low flow rate experiment. Average conductivity levels on that column were 381.92 μS/cm.

8.3.2 Redox potential and TOC levels

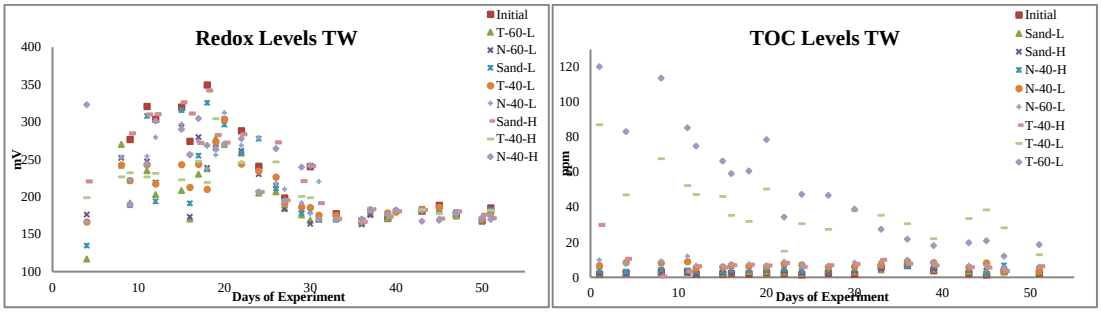


Figure 8.3.2: Redox Potential and TOC levels in tea and nut columns with TW

In Figure 8.3.2 are plotted the redox potential results. Another indicator for microbial activity of columns and the environment inside them was redox potential. Redox potential in all columns follows almost similar trend which was expected due to tap water solution when spiked. The peak period at all columns was noticed between days 15-20. In Sand columns the environment in columns reacted with the same way in low and high flow rate. In both columns the highest value observed in day 18 with redox potential in Sand Low column (+325 mV) and in Sand High (+342 mV) respectively. Average redox potential in Sand Low column was +212 mV and in Sand High column +233 mV.

In Tea columns redox potential levels were lower than Sand and Nut columns. In T-60-L column, the lowest redox potential observed. The highest levels noticed in day 19 (+278 mV) and the average potential in column was +197 mV. This was the lowest from all columns. In T-40-L column redox potential levels follow the same route like T-60-L column. The highest value observed in day 20 (+303 mV) and the average value was +207 mV. In T-40-H column redox potential increased till day 19 (+304 mV). Then there was a decreasing period till day 36 and the last part was a stable period. The average potential in that column was +209 mV.

In Nut columns redox potential was higher than Tea columns. As it is noticed in Tea columns the lowest levels observed in low flow rates and more specifically in N-60-L column. The highest levels in low flow rate observed the same day (day 20) for N-60-L column (+303 mV) and for N-40-L column (+312 mV). It is also noticeable that the lowest levels observed not in the start of experiment but in day 36 at both of columns. The average redox potential for N-60-L was +208 mV and for N-40-L was +218 mV. In N-40-H column the average potential was the highest than any other column (+223 mV). The attitude of column follows the other two Nut columns with the highest value observed in day 17 (+304 mV) and the lowest in day 45 (+168 mV).

At all columns the ORP levels remain I positive levels and after day 30 between 150-200 mV which are not anaerobic conditions. The main outcome of that was conditions in the columns cannot become anaerobic with the design that is proposed and there is always an amount of oxygen that exists along the experiments.

Total Organic Carbon (Figure 8.3.2) is very important indicator for the environment in columns because is the carbon source for denitrification process. It is the source for denitrification process, the source to create microbial colonies that accelerate the whole process. Column system is separated in three categories depending on substrate material of each column. Sand columns were the columns with the lowest TOC levels, with average values in Sand Low column (3.25 ppm) and in Sand High column (3.47 ppm). The highest TOC levels in both columns observed in day 36 of experiment.

In Tea columns TOC levels were higher than all other columns. The organic materials of tea waste product create high levels of organic compounds. In T-60-L column the highest levels of TOC observed with average levels of 50.63 ppm at all the duration of experiment. In T-40-L column TOC levels were also high but not as high as T-60-L column. The average value was 38.17 ppm. In T-40-H column TOC levels were much lower with average value to be reduced only in 7.85 ppm.

In Nut columns TOC levels were lower than Tea columns. In N-60-L there was the highest amount of TOC levels in Nut columns. The average TOC was 7.96 ppm. In N-40-L column TOC levels were lower than previous column. In both columns the highest TOC observed in day 36. In N-40-H, TOC levels were even lower and the average value was 4.47 ppm.

8.3.3 Nitrogen species

All nitrogen species are shown in Figure 8.3.3 along the experiment. The separation is based on the substrate material that used and the flow rate of tap water (same HRT). The details results and the analysis of the results are described in next paragraphs and more details are given in Appendix V. The analysis of nitrogen compounds were measured with ion chromatography.

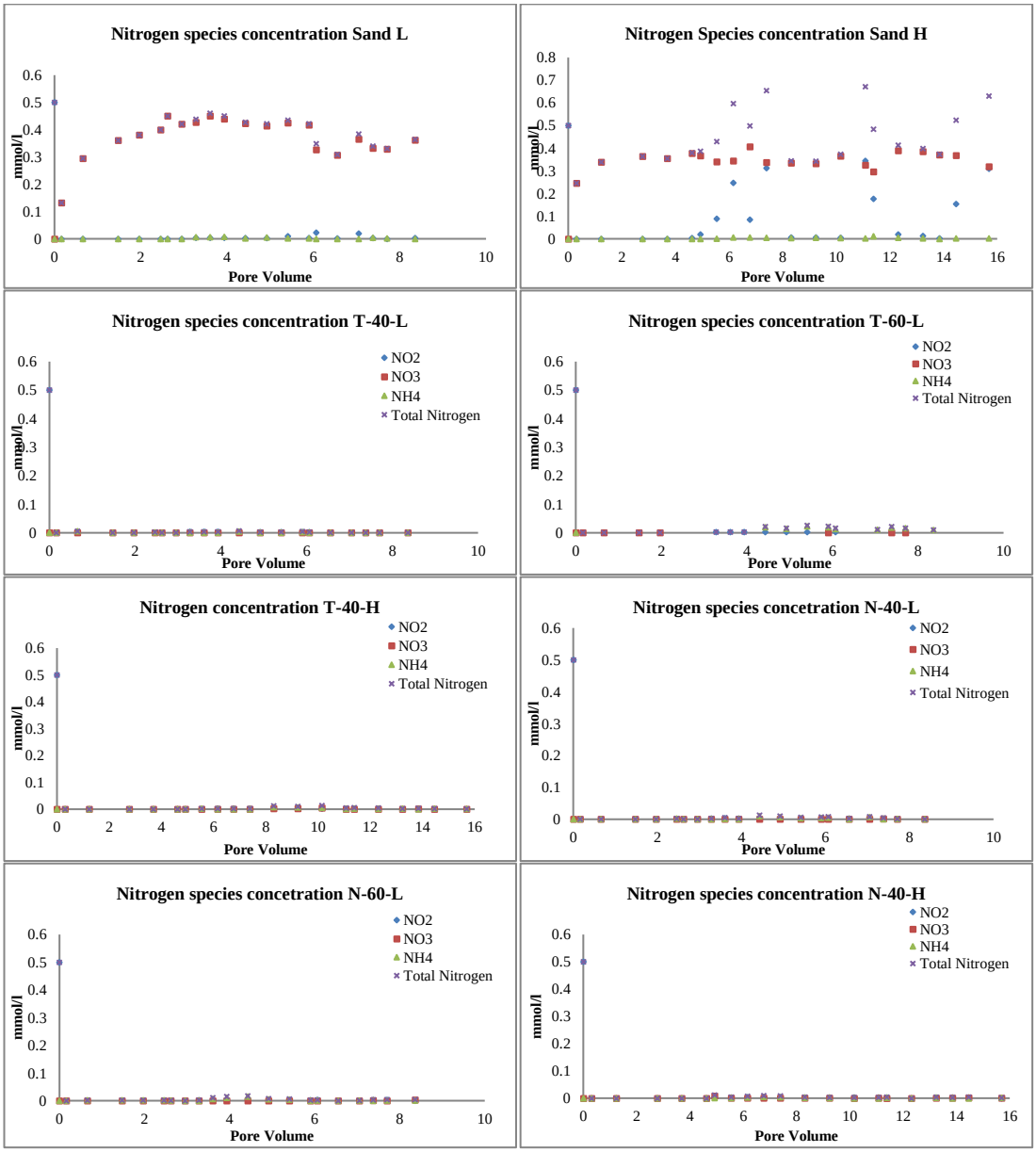


Figure 8.3.3: Nitrogen Species experiment with TW

As it is visible the separation of the experiment is in two phases. The first one is the adaptation lag phase that is between 1-4 days (PV 0-1). Then there is the stable phase till the end of experiment. In sand columns the level of NO3-N and TN are not reduced as it is noticed in all

tea and nut columns. In Sand L column there is an increasing phase till PV=6 and then a stable period. In Sand H column the spread of results is noticed. Except from the NO_3-N levels that are higher there are also NO_2-N levels that are noticed and affect also TN levels. That is happening due to the changes in the influent solution and in Sand H column the HRT that is used is not enough to assimilate all nitrogen compounds.

8.3.3.1 Nitrate levels

All experiments focus on the reduction of nitrates. In Figure 8.3.3 are the results of nitrate levels with tap water. Specifically, the experiment with tap water solution is separated in 3 categories depending on the substrate material of each column. The initial KNO_3 amount that spiked in tap water was standard to achieve the same initial concentration. The average initial concentration was 0.511 mmol/l (32.2 mg/l) nitrate. In Sand columns the reduction as it was noticed in previous experiments (Chapters 5 and 6) was not in the levels that expected but there was reduction. In Sand columns the reduction even in low even in high flow rate achieved the same reduction levels (30%).

In Tea columns the reduction achieved levels more than 99.8% at all columns. The best reduction is noticed in T-40-L column and the worst in T-40-H column where the retention time in column was smaller.

In Nut columns the reduction was also achieving levels more than 99.8%. The best reduction as it happened in Tea columns observed in N-40-L column and the worst in N-40-H column.

8.3.3.2 Nitrite levels

Nitrite levels are another nitrogen compound that was measured along the experiment. It is also an indicator that the reaction for denitrification process is under way or finished. In Figure 8.3.3 there are the results of nitrite levels. In Sand columns nitrite levels were higher than all other columns. Specifically, in Sand Low column the average nitrite levels were 3.91μmol/l. In Sand High column nitrite levels were higher than all other columns and the average levels were 89.46 μmol/l.

In Tea columns nitrite levels were really low, lower than Sand and Nut columns. At all columns the average levels were < 1.30 μmol/l. Again here the best results observed in T-40-L column.

In Nut columns nitrite levels were very low, slightly higher than Tea columns. At all columns the average levels were < 1.80 μmol/l. The lowest level noticed at N-40-L column and the highest in N-40-H column.

8.3.3.3 Ammonium levels

The ammonium (Figure 8.3.3) levels were really low at all columns. In Sand columns the ammonium levels were low enough. In Sand Low column the average levels were 1.70 μmol/l and in Sand High column 2.56 μmol/l.

In Tea columns ammonium levels were also low. The lowest levels observed in T-40-L column with average level along the experiment 0.72 μmol/l and the highest in T-60-L column with 7.34 μmol/l.

In Nut column the noticeable thing is that ammonium levels were higher in low flow rate column. The highest levels observed in N-60-L column (2.66 μmol/l) and the lowest in N-40-H column (1.01 μmol/l).

8.3.3.4 Total Nitrogen levels

The summation of the experiment is total nitrogen levels (Figure 8.3.3). In that part is the detail analysis of all nitrogen compounds (nitrate, nitrite and ammonium levels). This answers the hypothesis question. The initial solution after the addition of KNO_3 had average TN levels 0.513 mmol/l (32.2 mg/l). The reduction in Sand columns was not something that was expected and the results were not promising. These comes in agreement with the experiments in chapter 6 (lab scale barrier) and also in agreement with other studies (Soares et al., 1991; Andres and Chrysikopoulos, 2008) that find out the same results. The reduction reached levels between 15-25%. In Sand Low Flow column, the reduction was more effective than Sand High Flow column. The average TN values for these columns were for Sand Low 0.378 mmol/l and for Sand High 0.440 mmol/l. This result was also in agreement with the other experiments in chapters 4 and 5, which contain sand columns.

In Tea columns the reduction levels were more than 99% at all columns. That was important because the hypothesis to use that substrate material was correct. In Tea columns the lowest levels of TN compounds noticed in T-40-L column (1.93 μmol/l). In contrast the highest level

noticed in T-60-L column (8.66 μmol/l). It is important that even in low even in high flow rate the reduction achieved the same levels. In T-40-H column the average TN levels were 3.67 μmol/l.

In Nut columns the reduction levels were also more than 99%. At all columns the average levels were 4.01 μmol/l. It is again important that even the second substrate material works perfectly to reduce nitrogen levels.

8.3.4 Phosphate and potassium levels

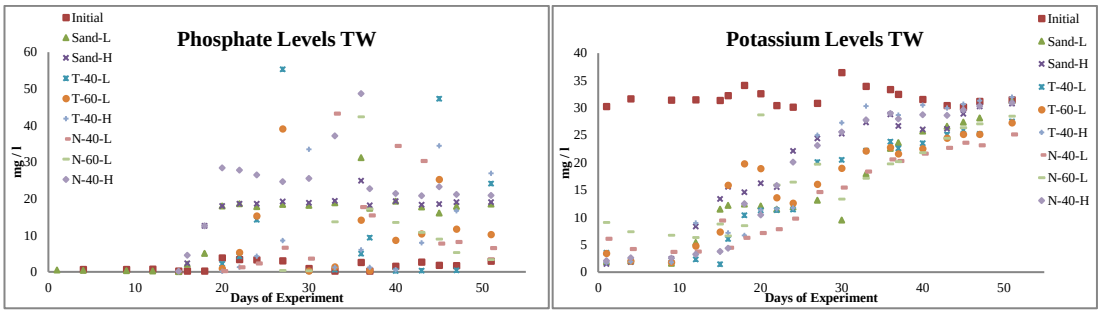


Figure 8.3.4: Phosphate and potassium levels in tea and nut columns with TW

Phosphate levels are the warning part of the experiment (Figure 8.3.4). In all columns it is noticed that phosphate levels were increasing along the experiment. It was something that was not expected and it is important point for further investigation. It is important that there is an increase in phosphate levels at all the columns even in sand columns something that was not noticed in the previous experiments in Chapter 6 and 7. It is important that the duration of the experiment is longer than the previous experiments. The phosphate levels can affect the denitrification process because there are different procedures that took place in the columns and it is possible to be competitive with denitrification process. Another point that is important is the HRT in this experiment. The HRT times are the highest that are used and the environment in the columns is possible to create the ideal conditions for the one process (denitrification) and not preferable conditions for phosphate compounds. It is important that the phosphate levels increased after day 10 of the experiment, showing that there is an initial period that supports the previous statements.

Sand columns were the columns that phosphate levels were visible from the first days of experiment. The highest value in both Sand columns observed in day 36 with 31.15 mg/l in Sand Low column and 24.86 mg/l in Sand High column.

In Tea columns phosphate levels were visible after day 20. In both columns with low flow rate the highest levels observed the same day (day 27), in T-40-L (55.35 mg/l) and in T-60-L (39.06 mg/l). The average values were in T-40-L (12.57 mg/l) and in T-60-L (11.18 mg/l). In T-40-H column average levels were lower than other Tea columns (10.95 mg/l). The highest value noticed in day 45 (34.40 mg/l).

In Nut columns phosphate levels were in the same range with Tea column at low flow rate. In N-40-H phosphate levels were higher and double than any other column. It is also important than in low flow rate phosphate levels noticed after the 20 day of experiment. The average levels in N-40-L were 13.08 mg/l and in N-60-L were 11.19 mg/l. The highest value in N-40-L column observed in day 33 (43.52 mg/l) and in N-60-L column in day 36 (42.25 mg/l). In N-40-H column, there was a totally different condition. Phosphate levels remained high at all the duration of experiment with double concentration than other columns and with average value 22.86 mg/l. The highest value noticed in day 36 (48.64 mg/l) which was the highest value at all columns.

The potassium levels (Figure 8.3.4) that measured in this experiment was indicator for the KNO_3 that was spiked in the initial solution. Due to the molecular structure (KNO_3) which is 1:1 molar it is important to ensure that nitrate levels exist in the solution. Except from that, it is important to notice that the potassium was absorbed at all the columns with different way till the point that every column cannot assimilate more amount of potassium. It is noticed that there is a change in K^+ as a flow tracer. The adsorption of the potassium levels is important because the substrate materials can assimilate only a specific amount. The K^+ does not affect the HRT that remains stable at all the duration of the experiment and there are no ion exchange effects due to K^+. The changes in K^+ concentrations follow the phosphate levels, after day 10 of experiment, and it is possible to have a connection between these two compounds.

8.3.5 Degradation rates

As it is noticed in the previous chapters the degradations rates are following the reduction rates in experiment. The separation of experiment can become in two phases. The two phases were noticed in Chapter 8. The first one from day 1 till day 4 (PV 0-1) and the second one from day 4 till the end of experiment. The initial phase because the reduction is very fast cannot be described so all the approach is after PV=1. The degradation rates are described the changes in the NO_3-N compounds. As it is noticed in Chapters 5-6 there is a description of instantaneous

constant rate for the specific time period with specific HRT (which is stable at all experimental time) and in specific PV. The Pore Volume (PV) is the total volume of the volume multiplied by the porosity of it and it is the total volume of water in the column at any time.

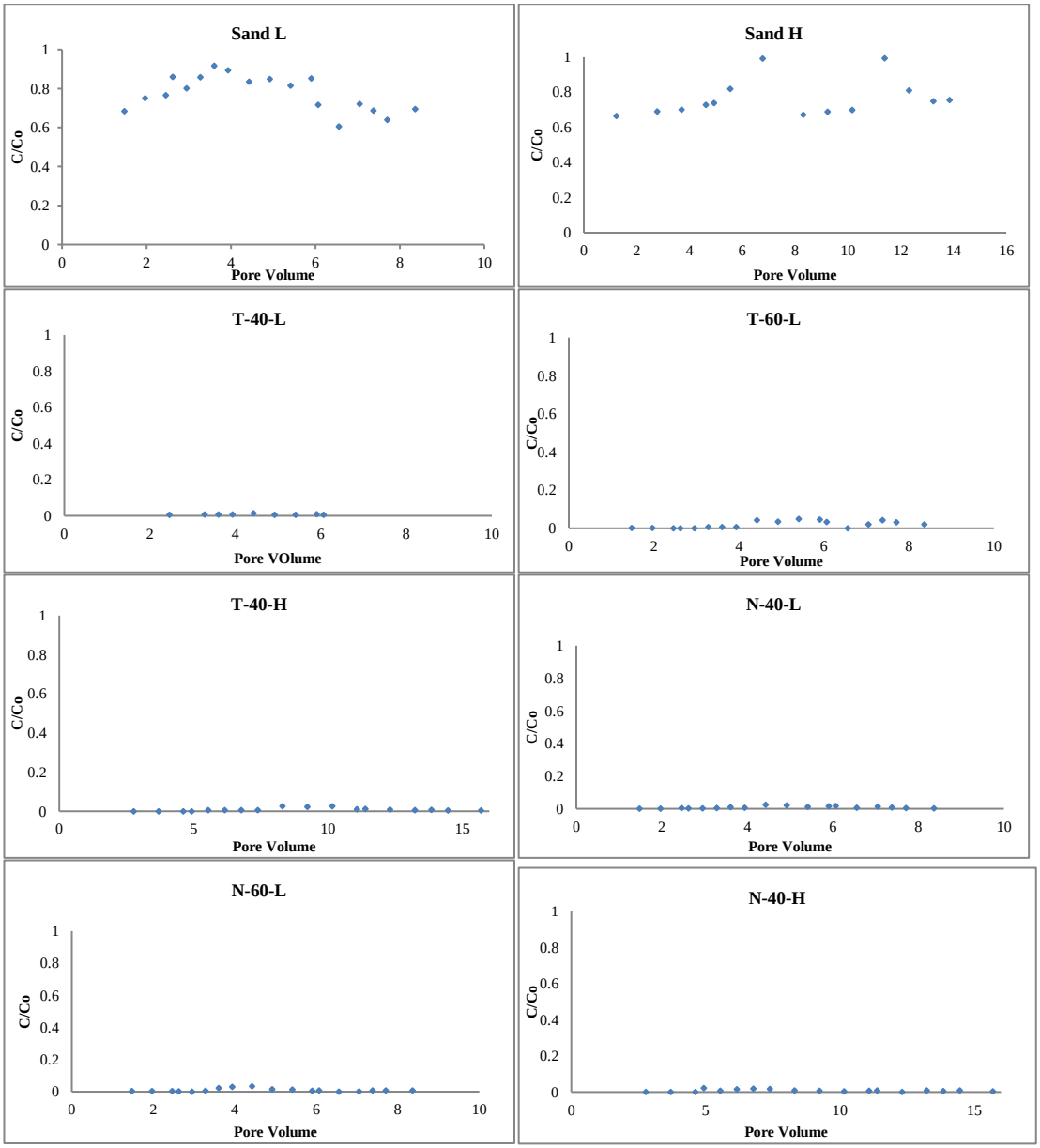


Figure 8.3.5: Degradation Rates experiment with TW

The degradation rates for NO_3-N are shown in Figure 8.3.5. The initial phase has duration from 1-4 days (PV 0-1) and in Tea and Nut columns less than one day. The degradation and reduction of nitrogen compounds is so fast that cannot be described.

Table 8.3.2: λ values, half-life and retention time experiment with TW

Adaptation Lag Phase	Sand L	Sand H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
λ min ($days^{-1}$)	0.090	0.125	0.000	0.000	0.001	0.001	0.001	0.001
λ max ($days^{-1}$)	0.223	0.229	0.000	0.000	0.020	0.020	0.020	0.020
T1/2 min (hours)	74.490	73.430	0.000	0.000	0.020	0.020	0.020	0.020
T1/2 max (hours)	184.950	132.570	0.000	0.000	0.050	0.050	0.050	0.050

Stable Phase	Sand L	Sand H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
λ (days)	0.043	0.044	0.410	0.541	1.152	0.733	0.725	1.144
SD	0.020	0.074	0.061	0.351	0.209	0.301	0.303	0.649
T1/2 (hours)	451.170	518.780	10.320	4.080	8.040	16.630	17.090	8.040
SD	11.162	107.119	0.443	0.494	0.233	0.381	0.361	0.230
RT (hours)	23.230	428.640	2.080	10.580	2.180	7.750	4.650	3.090
SD	1.544	40.502	0.280	1.352	0.183	0.423	0.404	0.223

The adaptation lag phase of experiment cannot be described in detail way any of the kinetics law due to the high velocity of the reaction and the λ-values and half-lives are indicative. Especially in Tea and Nut columns that approach was not possible.

In stable phase (PV=1-end), the approach is more consistent especially in Tea and Nut columns. In sand columns the degradation rates have different trends. In Sand L column there is an increasing period (PV 1-3), a stable period (PV 3-6) and two decreasing periods (PV 6-7, 7-8). The increasing and decreasing periods can be described by first order Monod Kinetics and the stable period from zero order kinetics. In Sand H column the trend is different. There are 3 stable periods (PV 1-5, 8-11, 13-15) which are following zero order kinetics and an increasing period (PV 5-7) and a decreasing period (PV 11-13). The λ-values in these columns are much lower than Tea and Nut columns (Table 8.3.2) and the half life time is much higher than other Tea and Nut columns. The changes in the trends along the experiments are affected by the changes in the influent solution. The reduction that is noticed in NO_3-N in Sand columns was less than 40%, which was a low reduction. This reduction is in agreement with other researchers that used sand as substrate material (Soares et al., 1991; Aslan, 2005).

In Tea columns the degradation rates provide to the system reduction levels more than 99% at all columns. The degradation rates can be described by zero order kinetics and the small changes in that are noticed are results of the changes in the influent solution. The λ-values are increasing with the percentages of tea in the columns and with the flowrate (Table 8.3.2). The half-life is decreasing with the different way than λ-values. The half-lives are in agreement with

other researchers (Robertson et al., 2010; Jing et al., 2010) that used mulch in column experiments ($T_{1/2}$=14.8 hours - 27 days) and achieving even lower half-lives. This is very promising because the system in the preferable conditions can provide to denitrification process the entire suitable environment and can be more competitive (better results) than other substrate materials that have already been investigated.

Finally Nut columns can provide the same degradation rates as Tea columns. The reduction at all columns (NO_3-N) is more than 99%. The initial phase cannot be described because the speed of reaction is really fast. The stable phase can be described by zero order kinetics and as it is noticed in Tea columns the small changes along the experiment caused to the changes in the influent solution. The λ-values are higher than Tea columns in higher HRT, in low HRT they are in the same levels. It is also noticed that here there is no change for λ-values in higher percentage of nut in the columns. The half-lives are higher than Tea columns (Table 8.3.2) and it is noticed that the lower levels are noticed in N-40-H column. The half-lives and λ-values are in agreement with other studies (Robertson et al., 2010; Jing et al., 2010) and Nut is another promising substrate material that can be used in denitrification process.

8.4 Results Experiment 2 with groundwater solution

At the second part of the column experiments the solution media was changed to groundwater. Groundwater was collected from suburban area outside Largs, UK and kept until the day that used (less than 1 month) in 4 ^{o}C to keep all active and alive microbial activity in water. Groundwater was collected in October 2013. The HRT in columns remained the same. Peristaltic pumps and connections remained the same. The conditions of experiment were the same as before with only change in influent solution. There was an initial adjustment period of one week to create the suitable conditions from tap water to groundwater solution. In that period there was the replacing of the water from tap water to groundwater in the columns by flushing water in the columns. The adjustment of the initial conditions became analysing the water solution that was coming out from the columns. The details results are given in Appendix V. The initial conditions in contrast to the tap water in columns were higher pH, conductivity and TOC levels that provided by Groundwater and lower Redox levels. Groundwater was measured directly after the collection day to analyse all characteristics.

Table 8.4.1: Results experiment with GW (all results received after (N=3 replicates) triplicate analysis)

		Influent	**Sand-L**	**Sand-H**	**T-40-L**	**T-60-L**	**T-40-H**	**N-40-L**	**N-60-L**	**N-40-H**
pH	mean	7.52	7.92	7.63	7.70	7.64	7.58	7.86	7.71	7.60
	SD	0.08	0.13	0.11	0.16	0.23	0.11	0.16	0.16	0.08
	% change		5.38	1.46	2.51	1.60	0.82	4.57	2.63	1.10
Conductivity µS/cm	mean	399.41	499.69	500.18	638.81	483.53	439.44	485.03	496.95	451.58
	SD	21.06	34.13	20.60	20.09	16.88	34.91	16.47	20.95	23.83
	% change		25.11	25.23	59.94	21.06	10.02	21.44	24.42	13.06
Redox mV	mean	146.95	149.65	151.08	145.01	141.48	146.98	151.49	148.48	147.93
	SD	17.84	16.33	18.01	21.76	19.80	19.42	17.08	18.59	17.53
	% change		1.84	2.81	-1.32	-3.72	0.02	3.09	1.04	0.66
TOC ppm	mean	20.35	12.15	14.83	77.22	44.26	21.53	17.64	22.26	15.94
	SD	1.68	2.63	4.40	25.27	9.10	2.99	2.24	3.24	2.98
	% change		-40.30	-27.13	279.52	117.50	5.82	-13.31	9.39	-21.67
NO_3^- µmol/l	mean	516.32	287.28	388.28	0.00	0.15	1.65	0.38	0.48	12.83
	SD	18.23	104.59	63.74	0.00	0.29	3.00	0.61	0.56	18.31
	% change		-44.36	-24.80	-100.00	-99.97	-99.68	-99.93	-99.91	-97.52
NO_2^- µmol/l	mean	2.78	3.35	143.71	0.00	1.55	2.49	0.66	1.00	1.59
	SD	0.54	0.82	170.01	0.00	1.33	7.20	1.15	1.29	1.25
	% change		20.31	5062.16	-100.00	-44.35	-10.46	-76.39	-64.10	-43.03
NH_4^+ µmol/l	mean	1.18	1.46	1.39	0.23	10.17	1.37	0.46	0.25	0.24
	SD	1.26	3.13	2.24	0.74	19.70	2.33	0.75	0.52	0.57
	% change		24.19	17.69	-80.38	762.01	15.94	-61.39	-79.20	-79.88
PO_4^{2-} mg/l	mean	3.53	11.65	6.89	11.57	12.89	12.77	7.64	11.13	10.89
	SD	0.67	11.17	6.41	12.16	17.85	15.85	4.84	7.50	11.62
	% change		229.56	94.93	227.33	264.78	261.46	116.11	214.81	208.07
TN µmol/l	mean	520.28	292.10	533.38	0.23	11.86	5.51	1.49	1.73	14.65
	SD	18.88	105.00	120.94	0.74	19.30	9.36	1.91	1.54	18.87
	% change		-43.86	2.52	-99.96	-97.72	-98.94	-99.71	-99.67	-97.18
K^+ mg/l	mean	32.16	29.95	29.82	27.82	27.69	30.64	26.46	29.31	30.50
	SD	1.58	1.40	0.84	1.10	1.75	1.35	1.84	1.22	0.80
	% change		-6.90	-7.29	-13.51	-13.90	-4.75	-17.74	-8.86	-5.17

All the average results from experiment with tap water are given in Table 8.4.1. The detailed approach of the results is given in the following paragraphs and Appendix V.

8.4.1 pH and conductivity levels

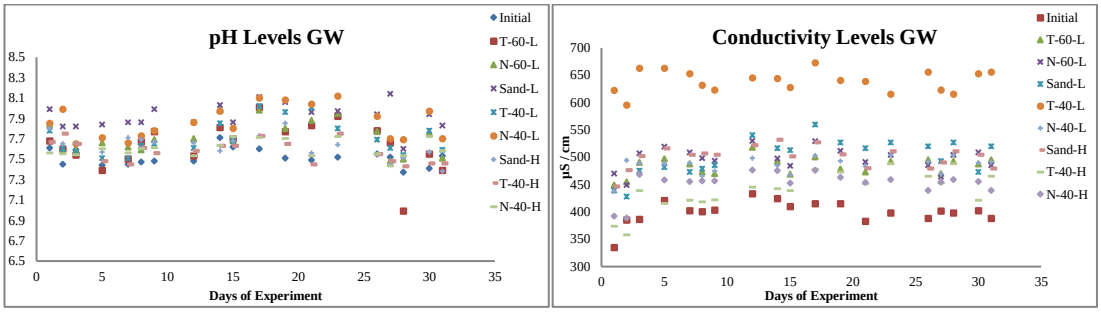


Figure 8.4.1: pH and conductivity levels in tea and nut columns with GW

The pH levels (Figure 8.4.1) along part 2 of experiment were higher than experiment 1. Groundwater pH that was spiked in columns had the same range of pH as with tap water. Generally, at all columns the results were slightly higher than tap water experiment.

The Sand columns as it was expected, the highest pH levels observed. Sand Low column was the column with the highest pH at all experiment with average pH=7.92. In Sand Low column, the pH levels were lower than all low flow rate columns but again the highest in high flow rate part. The average pH was pH=7.63.

In T-60-L column pH levels were higher than experiment 1 and remaining neutral at all the duration of experiment. The average pH in column was 7.64 with the highest value observed in day 17 (8.01) and the lowest in day 28 (6.99). In T-40-L column the range of pH was even smaller than T-60-L column and the average pH levels along the experiment were pH=7.70. The highest value observed in day 17 (8.02) and the lowest was noticed in day 5 and day 7 (7.51). Finally, in T-40-H column, the pH range was even smaller than other two Tea columns. The average pH was the lowest (7.58) of Tea columns which was not expected. The highest value observed in day 2 (7.75) and the lowest in day 28 (7.43). That column was the column with the lowest average pH along the experiment.

In Nut columns pH follows the same route as it is noticed at all columns. The pH levels were higher than experiment with tap water. In N-60-L column pH remained neutral at all the duration of experiment with average pH=7.71. The highest value observed in days 14 and 17 (7.98) and the lowest value in day 27 (7.48). In N-40-L column pH levels were higher than all Nut columns. The average pH=7.86 was the highest in columns with Tea and Nut. In column

there was a period more than 5 days that pH was higher than 8 and the highest value observed in day 23 (8.12). The lowest pH observed in day 3 (7.65). In N-40-H column as it is noticed in Tea experiment the lowest pH levels observed here. The average pH along the experiment was pH=7.60. The range was also here small with the lowest value at all the duration of experiment to receive in day 27 (7.43) and the highest value (7.72) noticed in days 15, 23 and 30.

Conductivity levels at experiment 2 of experiment were higher than experiment 1 (Figure 8.4.1). This is happening due to the solution of experiment that the initial conductivity levels of groundwater were higher than tap water. In Sand columns conductivity levels were much higher than experiment 1. Conductivity levels were really close to both Sand columns close to 500 μS/cm. In Sand Low the average conductivity was 495.7 μS/cm, with the lowest value observed in day 2 (427.8 μS/cm) and the highest in day 17 (559.9 μS/cm). In Sand High column the range was smaller and the average conductivity levels were 500.2 μS/cm. The lowest value observed in day 1 (446.9 μS/cm) and the lowest in day 14 (531.9 μS/cm).

In Tea columns conductivity levels in low flow rate columns were higher than in high flow rate columns. In T-60-L column the average conductivity due to the higher percentage of substrate material was lower than T-40-L column with average levels 483.5 μS/cm. The highest conductivity observed in day 12 (518.4 μS/cm). In T-40-L column conductivity levels were the highest than any other column. The average levels along the experiment were 638.8 μS/cm and the highest value noticed in day 17 (672.4 μS/cm). In T-40-H column, the lowest value was noticed for all the experiment. The average conductivity levels were 439.4 μS/cm with the lowest value in day 2 (357.7 μS/cm) and the highest in day 14 (531.9 μS/cm).

In Nut columns conductivity levels were higher than tap water solution experiment. In that part conductivity followed the attitude of Tea columns with lower levels in high flow rate and higher levels in low flow rate. In N-60-L column average levels were 496.9 μS/cm and it is noticeable in that column that the lowest levels did not receive in the first days but in day 27 (464 μS/cm). The highest levels achieved in day 12 (530 μS/cm). In N-40-L column conductivity levels were lower than N-60-L column with average conductivity 485 μS/cm. Finally, in N-40-H column the lowest conductivity was observed in Nut columns with average conductivity levels 451.6 μS/cm, which were the lowest levels at those columns.

8.4.2 Redox potential and TOC levels

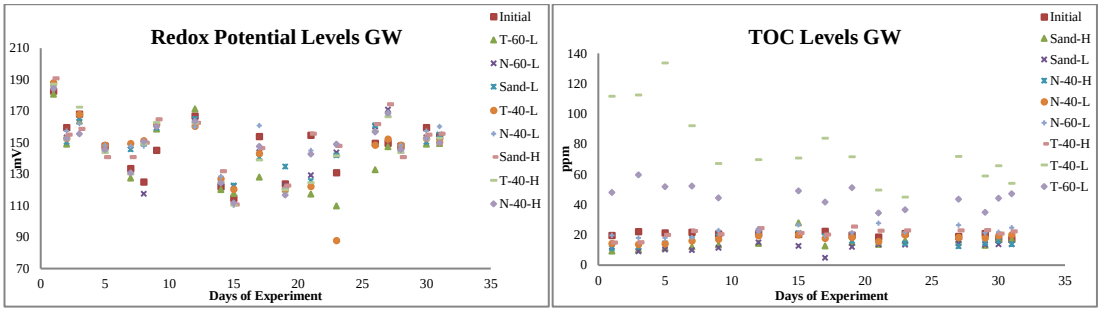


Figure 8.4.2: Redox potential and TOC levels in tea and nut columns with GW

Redox potential levels (Figure 8.4.2) were much lower than experiment 1. In Sand columns redox potential was very close at both columns. In Sand Low column the average Redox potential was +149 mV and in Sand High column was +151 mV. In both columns the highest value observed in day 1 (Sand High=+187 mV, Sand Low=+191 mV) and the same was happening with the lowest value in day 15 (Sand High=+122 mV, Sand Low=+111 mV).

In Tea columns redox potential was lower than other columns. In T-60-L column redox potential was the lowest than all other columns with average redox levels along the experiment +141 mV. The lowest value noticed in day 23 (+110 mV). In T-40-L column redox potential levels were slightly higher with average levels +145 mV. Finally, in T-40-H column the highest redox potential observed for Tea columns with average levels +147 mV.

In Nut columns redox levels were higher than Tea part of experiment. In N-60-L column the lowest levels of redox potential observed with average levels +148 mV. In N-40-L column the highest levels of redox potential in Nut columns observed (+151 mV), which was also the highest levels at all experiment. Finally, in N-40-H column the levels were really close to T-40-H column and average levels in that column was +147 mV.

As it is noticed in the experiment 1 in the chapter, the redox potential levels cannot reach anaerobic conditions and all the time are between 110-190 mV. In contrast to Tap water solution that redox levels are lower but again aerobic. This shows that the system cannot become anaerobic. This is important for the microbial activity that exists in the columns. Denitrifier bacteria are separated in two categories depending on the environment that exist. The aerobic denitrifier bacteria that are met in the experiment are from the gene *Thiobacillus* and *Pseudomonas*.

In Figure 8.4.2 there are visible all TOC levels of experiment with groundwater. TOC levels in experiment 2 are much higher than experiment 1. This was noticed at 6 of 7 columns. This is

expected because groundwater as initial solution contains higher levels of organic carbon than tap water. In Sand columns TOC levels were lower than other columns. In Sand Low column, the average TOC levels were 12.15 ppm with the highest levels in day 12 (15.07 ppm) and the lowest in day 17 (4.92 ppm). In Sand High column, TOC levels were higher than Sand Low column with average TOC level 14.83 ppm. The highest TOC level noticed in day 15 (28.09 ppm).

In Tea columns, TOC levels were higher than all the columns in experiment. The low flow rate columns had higher levels of TOC. In T-60-L column, the average TOC levels were 44.26 ppm with the highest value in day 3 (59.81 ppm) and the lowest value in day 15 (22.98 ppm). In T-40-L column, TOC levels were higher than all columns with average levels 77.22 ppm and the highest value in day 5 (133.70 ppm). The lowest value noticed in day 23 (45.06 ppm). Finally, in T-40-H column, TOC levels were much lower than other two columns. The average TOC levels were 21.53 ppm with the highest value in day 19 (25.72 ppm).

In Nut columns, TOC levels were lower than Tea columns. As it is noticed at Tea columns TOC levels were higher in low flow rate columns. In N-60-L column, the average TOC levels were 22.26 ppm which was the highest in Nut columns. The lowest value observed in day 5 (18.04 ppm) and the highest in day 21 (27.79 ppm). In N-40-L column, TOC levels were lower with average TOC levels 17.64 ppm. The highest value noticed in days 15 and 31 (20.18 ppm). In N-40-H column, TOC levels were the lowest in Nut columns with average TOC (15.94 ppm).

8.4.3 Nitrogen species groundwater solution

As it is noticed in part 1 the nitrogen species receive reduction in the first 3 days of experiment (PV=0-1). The detailed results are given in Figure 8.4.3.

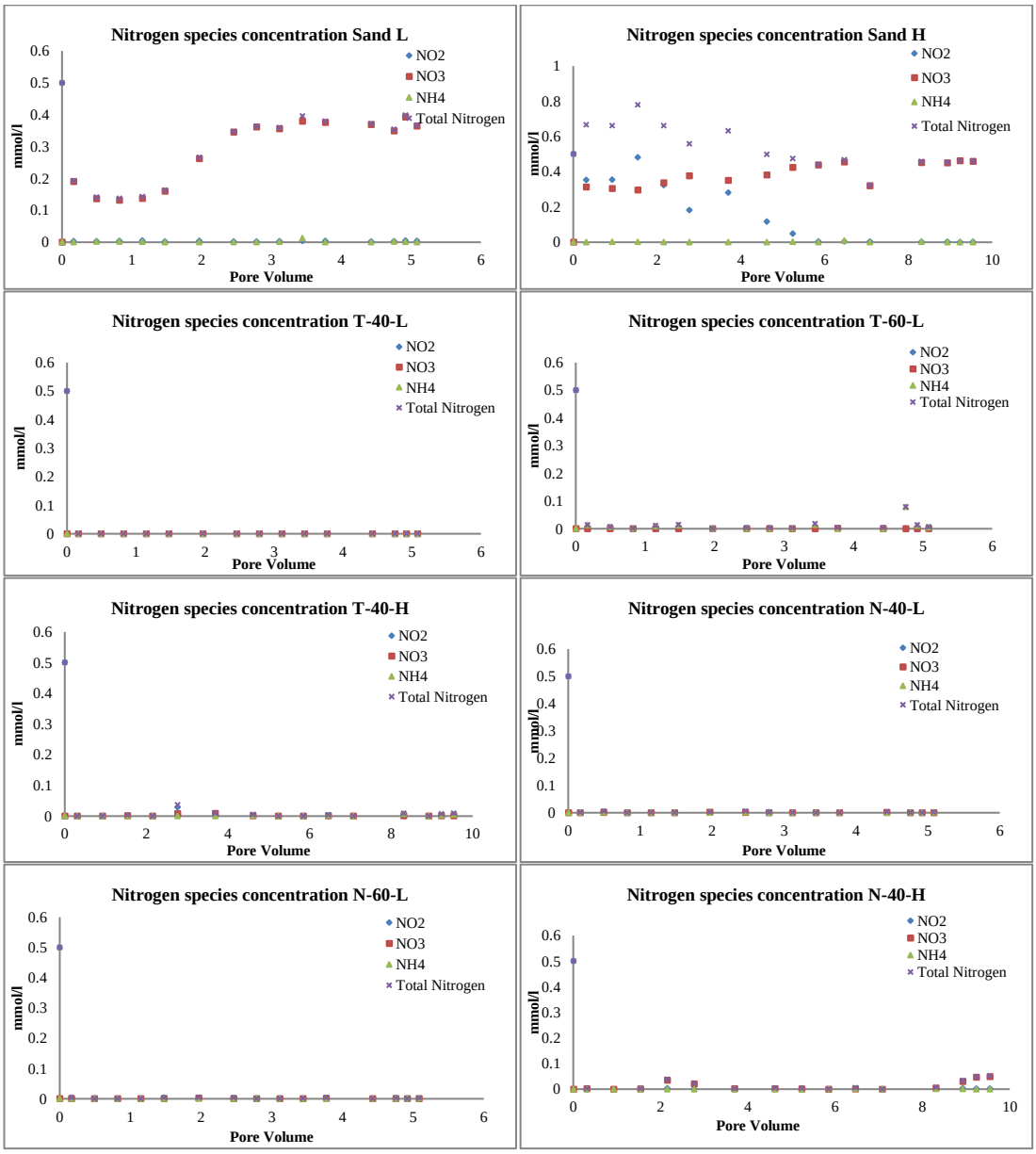


Figure 8.4.3: Nitrogen species experiment with GW

All nitrogen species along the experiment are visible in Figure 8.4.3. In Sand Columns the reduction is not achieving the levels of reduction than expecting but it is in agreement with all the previous sand columns that the reduction remained between 30-55%. In Sand Low column it is noticed an initial reduction in TN and NO_3-N till PV=1.5 and then an increase till the end of the experiments. The final reduction was almost 40%. In Sand H column there is until PV=6 where there are important levels of NO_2-N and NO_3-N, showing in first instance that the HRT is not enough to assimilate the nitrogen compounds in the system. The reduction levels in the end of the experiment reach levels 35-40% (NO_3-N and TN). In the Tea and Nut columns there is a totally different approach. At all columns the reduction levels reach 99% showing that these two

substrate materials can work in denitrification process. The detailed analysis of the figure is given in next paragraphs.

8.4.3.1 Nitrate levels

The solution in the experiment was groundwater that received from the suburban area outside of Largs, UK. The initial solution analysed and spiked with KNO_3 achieving the same initial nitrate levels. All the results are visible in Figure 7.4.3. The average initial concentration along the experiment was 0.516 mmol/l (32.2 mg/l). In Sand columns at it was noticed before the reduction levels were lower than other columns. In Sand High Flow column, the reduction achieved levels of 25%. In Sand Low Flow column, the reduction levels were higher and achieving reduction levels 45%.

In Tea columns the reduction was the highest than all other columns. At all columns the reduction was more than 99.6%. The best reduction observed at T-40-L column. The general approach was that the low flow rate columns received higher reduction levels and lower amount of nitrate. In T-40-H level received the highest nitrate levels in Tea columns.

In Nut columns the reduction levels at all the columns were more than 97%. In N-40-L and in N-60-L column the reduction rate were higher achieving levels more than 99.9%. The lowest nitrate levels observed in N-40-L column.

8.4.3.2 Nitrite levels

Nitrite levels (Figure 8.4.3) were low in that experiment. In Sand columns nitrite levels were higher than other columns with Tea and Nut. More specifically the levels in Sand High column were the highest with average nitrite levels 143.72 μmol/l. In Sand Low column the levels were much lower (3.35 μmol/l).

In Tea columns nitrite levels were really low. In T-40-L column was the highest reduction, where no nitrite levels observed at all the duration of experiment. The highest level noticed in T-40-H column with average nitrite levels 2.49 μmol/l.

In Nut column nitrite levels were lower than other columns. The low flow rate contained lower levels than high flow rate and the lowest average value along the experiment observed in N-40-L column (0.66 μmol/l).

8.4.3.3 Ammonium levels

Ammonium levels (Figure 8.4.3) remained very low in experiment with groundwater solution. In Sand columns the amount of ammonium in both columns was almost the same. It was slightly higher in Sand Low column with 1.47 μmol/l than Sand High column (1.46 μmol/l).

In Tea columns, there were the two limits of ammonium levels. In T-40-L column, ammonium levels were the lowest that noticed along the experiment with average levels (0.23 μmol/l). In T-60-L column, there were the highest average levels (10.17 μmol/l). In T-40-H column, ammonium levels reduced again with average value (1.37 μmol/l).

In Nut columns, ammonium levels remained in the lowest point. In contrast to Tea columns in Nut columns ammonium levels were lower in N-60-L (0.25 μmol/l) and N-40-H (0.24 μmol/l) column. In N-40-L column the highest average ammonium levels observed but again were really low (0.46 μmol/l).

8.4.3.4 Total Nitrogen levels

All Total Nitrogen results are visible in Figure 8.4.3. Total Nitrogen levels as it is noticed before were following the attitude of nitrate levels. In Sand columns there was reduction. In Sand Low column the reduction was 43%. In Sand High column there was no reduction but increase 2.5%. That was the only column that increase on nitrogen levels was noticed.

In Tea columns, the reduction was more than 97% at all columns. The best reduction achieved in T-40-L column with reduction 99.9%. The smallest reduction observed in T-60-L column with 97.7%.

In Nut columns, the reduction was noticed again more than 97% at all columns. The best results achieved in low flow rate column with reduction in N-40-L (99.7%) and in N-60-L column (99.6%). In high flow rate the reduction was slightly smaller and achieved reduction levels 97.1%.

8.4.4 Phosphate and potassium levels

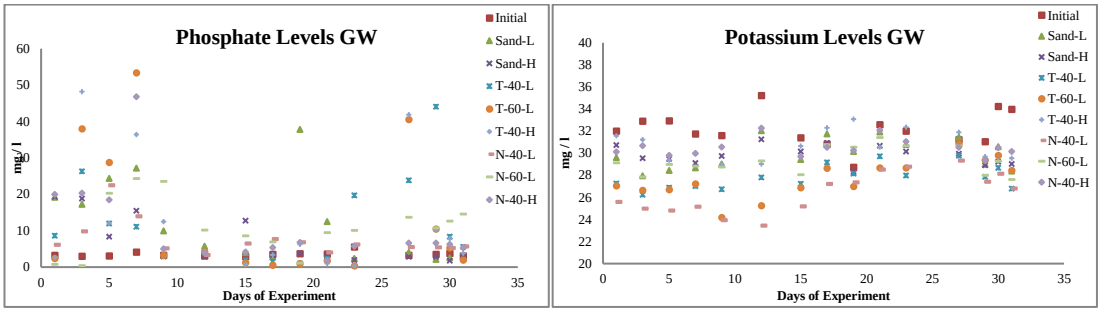


Figure 8.4.4: Phosphate and potassium levels in tea and nut columns with GW

Phosphate levels (Figure 8.4.4) were a critical observation from the experiment again. In the initial solution of groundwater contains levels between 3-4 mg/l. At Sand columns, phosphate levels were increased in the first 20 days and after that there was a reduction period achieving levels which were in the same amount as in the initial solution.

In Tea columns there were much higher levels in all Tea columns and phosphate levels increased more than the initial solution about 15 times the source water.

In Nut columns phosphate level were again noticeable and again higher than the initial solution. That was the critical observation for the whole experiment, even in part 1 even in part 2. It is clear the substrates provide a source of phosphate, either as contamination (dust) or as part of the waste materials themselves. Therefore, further study with perlite to reduce the phosphate is recommended.

The initial phosphate levels remain stable along the experiment and the changes noticed in the columns.

Potassium levels (Figure 8.4.4) were stable at all the duration of experiment at all columns. Additionally, there is nothing that adsorbed the amount of potassium in the columns and the amount remained stable. Again even in Figure 8.3.4 experiment 1 of this chapter are higher than other columns almost at all the duration of the experiment. This is also an adjustment for the KNO_3 that is added in the influent solution. Due to the molar ratio 1:1 can be described the initial concentration of nitrate levels that insert into the solution. The K^+ levels are between 24-32 mg/l.

8.4.5 Degradation rates

The degradation rates at the experiment with groundwater as solution are shown in Figure 8.4.5. The degradation rates are described according to the NO_3-N and describe the instantaneous rate constants as it is described in the previous experiments. As it is noticed at the previous chapters, all the experiments are separated in two phases. The phase one is the adaptation lag phase with duration 1-4 days (PV=0-1) which cannot be described by the kinetics due to the very fast speed of the reaction. The second phase is the stable phase till the end of the experiment.

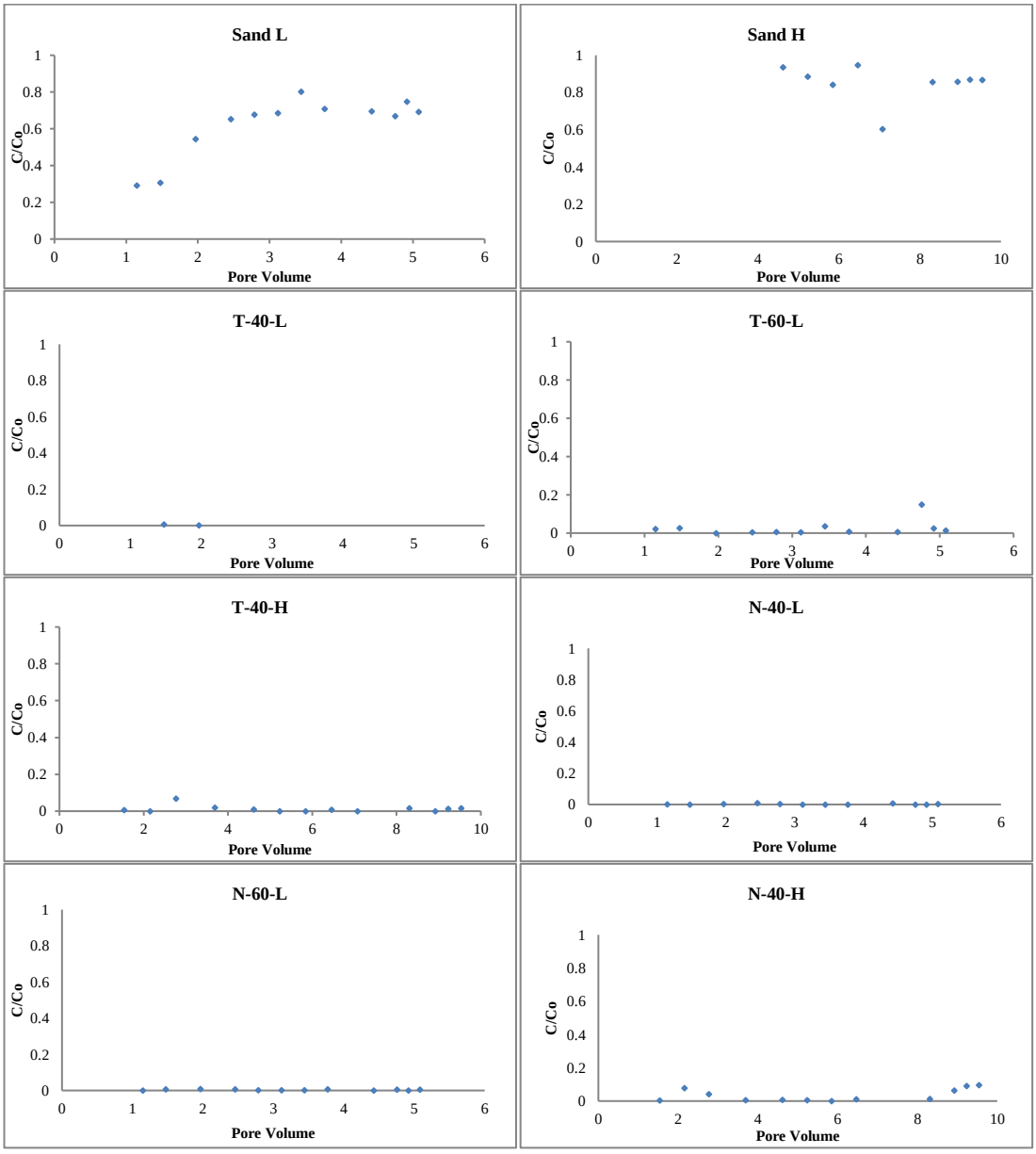


Figure 8.4.5: Degradation rates experiment with GW

The speed of reduction is so fast that cannot be described by a specific kinetic law in adaptation lag phase. In sand columns there is a different approach than Tea and Nut columns. The degradation rates cannot be described by only one kinetic law and are separated in smaller time periods. In Sand L column there are two stable periods (PV 1-1.5, 2.5-5.5) which are following zero order kinetics and an increasing period (PV=1.5-2.5) which is following first order kinetics. In Sand H column there is a decreasing period (PV 4-7) and a stable period (PV7-10).

In all other columns with Tea and Nut the stable phase follows the zero order kinetics. The small changes along the experiment caused due to the changes in the influent groundwater.

Table 8.4.2: λ values, half-life and retention time experiment with GW

Adaptation Lag Phase	Sand L	Sand H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
λ min	0.160	0.005	0.000	0.607	0.001	0.008	0.008	0.008
λ max	0.218	0.010	0.000	0.948	0.008	1.093	0.841	1.824
T1/2 min	70.11	0.010	0.000	17.480	0.050	0.080	0.030	0.080
T1/2 max	99.77	0.500	0.000	27.380	10.500	22.720	19.710	19.710

Stable Phase	Sand L	Sand H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
λ (days)	0.085	0.039	0.163	0.638	0.934	0.744	0.996	1.150
SD	0.055	0.042	0.383	0.264	0.419	0.217	0.185	0.484
T1/2 (hours)	217.900	276.810	2.300	23.680	7.530	12.570	15.090	12.400
SD	4.768	13.046	0.256	0.455	0.287	0.351	0.281	0.301
RT (hours)	17.830	36.880	2.250	23.490	2.540	10.390	2.830	10.050
SD	1.568	2.959	0.364	1.667	0.303	2.822	2.607	0.892

The λ values, half-life and retention time (Table 8.4.2) in the columns are characteristics that help to understand the conditions in the columns and the microbial activity.

The adaptation lag phase of experiment cannot be described in detail by the kinetics law due to the high velocity of the reaction and the λ-values and half-lives are indicative. Especially in Tea and Nut columns that approach was not possible.

In stable phase (PV=1-end), The λ-values in Sand columns are much lower than Tea and Nut columns (Table 8.4.2) and the half life time is much higher than other Tea and Nut columns and higher than tap water solution. The changes in the trends along the experiments are affected by the changes in the influent solution. The reduction that is noticed in NO3-N in Sand columns

was less than 40%, which was a low reduction. This reduction is in agreement with other researchers that used sand as substrate material (Soares et al., 1991; Aslan, 2005).

In Tea columns the degradation rates provide to the system reduction levels more than 99% at all columns. The degradation rates can be described by zero order kinetics and the small changes in that are noticed are results of the changes in the influent solution. The λ-values are increasing with the percentages of tea in the columns and with the flowrate (Table 8.4.2). The half-life is decreasing with the different way than λ-values. The half-lives are in agreement with other researchers (Robertson et al., 2010; Jing et al., 2010) that used mulch in column experiments. This is very promising because the system in the preferable conditions can provide to denitrification process. The results are even better than tap water solution in experiment 1 of this chapter.

Finally Nut columns can provide the same degradation rates as Tea columns. The reduction at all columns (NO3-N) is more than 99%. The initial phase cannot be described because the speed of reaction is really fast. The stable phase can be described by zero order kinetics and as it is noticed in Tea columns the small changes along the experiment caused to the changes in the influent solution. The λ-values are higher than Tea columns in higher HRT, in low HRT they are in the same levels. It is also noticed that here there is no change for λ-values in higher percentage of nut in the columns. The half-lives are higher than Tea columns (Table 8.4.2). The half-lives and λ-values are in agreement with other studies (Robertson et al., 2010; Jing et al., 2010) and Nut is another promising substrate material that can be used in denitrification process.

8.5 Discussion

The new materials added in the column experiments worked perfectly in the denitrification process. The reduction of nitrogen levels observed were higher than 85% at all cases with both substrate materials (Tea waste materials and Hazelnut Husk wastes). The only concerning point noticed was the high phosphate levels that created at all the duration of the experiment with groundwater.

The degradation rates that observed in the experiment were noticeable. The fast reduction in the nitrogen levels in the first day of experiment cannot be described from any kinetic law in Monod kinetics. The TOC levels are noticeable in both parts and in combination with half-lives and retention time; it is visible that from the first day the microbial activity is working during

the duration of experiment. The activity is more effective with groundwater solution than tap water due to the smaller half-lives and retention times that calculated.

The reduction in nitrogen levels achieved levels more than 95% at all cases with tea columns achieving the best results. The point that was a disadvantage to the design of the experiment was the high amount of phosphate compounds in the end. That amount noticed in the part with groundwater where the initial concentration was noticeable more than 5 mg/l at the initial solution. All that results showed excess phosphate levels. The idea was to create an ultimate system that can reduce not only the nitrogen compounds but also the phosphate levels of the whole system. Therefore, there is a need to evaluate perlite as an additional substrate in the system.

CHAPTER 9

COLUMNS EXPERIMENT WITH TEA WASTE MATERIALS, HAZELNUT HUSK WASTES AND SEQUENTIAL PERLITE COLUMN

9.1 Introduction

After three months of experiments with the tap water and groundwater in the columns it was noted that higher levels of phosphate were exiting the columns, thus a new experiment was devised to investigate where the system can be improved. The reduction of nitrogen levels was acceptable and more promising than expected. The point of concern was the amount of phosphates in the output of columns. Especially in the part of where the solution was groundwater the phosphate levels were noticeably high. That was the hypothesis to be tested in this experiment, to combine TN reduction more than 90% and phosphate reduction more than 70%.

Previous experiments (Chapter 6), shown the use of perlite to reduce phosphate levels. Additionally, perlite has a good reaction with nitrogen compounds. The combination of waste tea materials, hazelnut husk and perlite was the next step in research. Perlite as it was noticed in previous experiments (Chapter 6) has the property to absorb heavy metals and phosphate compounds. For that reason, perlite was chosen to use as a media for reduction/absorption of phosphates.

The design of the experiment (Figure 9.1.1) was kept the same as the previous experiment in Chapter 8. The investigation of columns focused on longer duration for the selected substrate materials. The columns of previous experiment continued from the last experiment with tea, nut and perlite with groundwater solution to keep alive the microbial environment in them.

The new part in experimental design was the addition of a second sequential column in the output of existing columns with only substrate material, perlite. The sequential columns start in the end of the previous reactor barriers and it is connected with a tube from the output point of the initial columns to the input point for the perlite columns. The sequential reactor is necessary due to the levels of phosphate that noticed in the experiments with tap and groundwater in the last Chapter 8. It is important to find out the reaction of the system in long term experiments and as it is noticed the trends of PO_4-P were increasing. For the denitrification process this

additional perlite reactor was not important, but for the combination of two processes that approach is demanding. Furthermore, except from the removal of phosphate levels in high levels perlite can reduce the remaining nitrogen compounds even lower if there is not acceptable reduction in the first part of reactors (Tea and Nut Columns).

The determined flow rates and retention times were approximately 42 mL d^{-1} and 3.25 d for the slow columns and 84 mL d^{-1} and 6.10 d for the fast columns, respectively, the same as the previous experiment without perlite columns. The stability of the initial flowrate was ensured by peristaltic pump (Ismatec). All columns were sealed to obtain anoxic conditions and covered with aluminium foil to avoid light penetration. The determination of flowrate in the first columns became as it was described in Chapter 7. The determination of the second 'perlite' columns became volumetrically with the quantity of solutions that come out from the system in specific time period. For the stability of flowrate in the second columns along the experiment analysis of output quantity became at least once per week.

The columns with perlite had length 20 cm and internal diameter 5 cm. The columns filled in with 34 gr of perlite. Perlite was received from local stores and it had diameter less than 1cm all at parts. The connection between the two columns was made by tube (tygon) with diameter 5.2 mm that was not reducing the speed of water at the output of first columns. The stability of the speed measured also at the output of perlite columns to insure that flow rate was the same at all experiment (once per week). The design of the columns is shown in Figure 8.1.1 where the initial columns with Tea, Nut and Sand substrate material are the longer columns and the columns with perlite are the shorter columns.

The duration of experiment was 98 days and samples were taken 3-5 times per week. The temperature of the experiment was stable room temperature (20±2ºC). All columns were protected with foil from light penetration. All samples were analysed immediately for pH, redox potential and conductivity and stored at 4 ºC till the analysis time in IC Chromatography and TOC analyser.

Figure 9.1.1: Design of column experiment with tea, nut and perlite

With this design of experiment, nitrogen treatment was achieved in first part and in second part the reduction of phosphates achieved. The design of experiment was chosen due to perlite properties. Perlite can absorb phosphate compounds, heavy metals and an amount of nitrogen. It was more effective to achieve the highest levels of nitrogen treatment first and then to reduce phosphate levels and any other compound that probably exist.

Table 9.1.1: Results of experiment with sand, tea, nut columns and sequential perlite column (all results received after (N=3 replicates) triplicate analysis)

		Influent	**Sand-L**	**Sand-H**	**T-40-L**	**T-60-L**	**T-40-H**	**N-40-L**	**N-60-L**	**N-40-H**
pH	mean	7.71	7.56	7.44	7.87	7.41	8.06	8.07	7.97	7.89
	SD	0.30	0.22	0.13	0.19	0.19	0.26	0.32	0.23	0.22
	% change		-1.99	-3.57	2.08	-3.89	4.43	4.63	3.35	2.28
Conductivity µS/cm	mean	276.98	347.10	328.50	273.87	250.35	247.24	293.35	242.08	325.24
	SD	68.86	72.34	59.43	36.04	53.93	50.90	68.46	55.48	83.87
	% change		25.31	18.60	-1.12	-9.61	-10.74	5.91	-12.60	17.42
Redox mV	mean	168.47	171.82	171.75	169.38	171.83	177.85	168.64	172.35	170.44
	SD	30.41	30.64	32.64	34.18	36.92	27.21	39.12	34.94	39.99
	% change		1.99	1.94	0.54	1.99	5.57	0.10	2.30	1.17
TOC ppm	mean	6.63	6.26	7.13	8.92	11.84	8.22	6.56	7.05	7.68
	SD	3.99	4.22	3.22	5.07	5.45	4.74	3.57	3.40	3.39
	% change		-5.52	7.57	34.61	78.64	24.04	-1.07	6.41	15.90
NO3 µmol/l	mean	514.79	390.45	398.48	0.28	0.93	1.63	3.71	0.11	194.82
	SD	19.98	54.74	25.20	0.33	2.16	5.89	7.90	0.29	44.74
	% change		-24.15	-22.59	-99.94	-99.82	-99.68	-99.28	-99.98	-62.16
NO2 µmol/l	mean	1.28	2.54	0.45	1.24	0.25	0.51	0.32	1.71	13.67
	SD	1.37	2.38	0.38	2.99	0.28	1.44	0.80	10.33	22.76
	% change		98.21	-65.05	-3.04	-80.19	-60.19	-75.19	33.98	968.65
NH4 µmol/l	mean	8.33	3.26	3.75	6.45	9.11	12.04	20.30	1.71	10.17
	SD	21.61	4.50	5.50	8.93	12.12	20.67	23.10	3.92	17.77
	% change		-60.89	-54.98	-22.60	9.26	44.41	143.55	-79.45	22.07
PO4 mg/l	mean	10.87	1.29	1.34	0.31	0.16	0.97	1.50	0.60	1.99
	SD	1.91	1.51	1.10	0.55	0.22	0.99	1.17	1.29	2.05
	% change		-88.13	-87.65	-97.14	-98.51	-91.11	-86.17	-94.51	-81.71
TN µmol/l	mean	524.40	396.25	402.67	7.98	10.28	14.17	24.32	3.54	218.66
	SD	29.17	53.93	26.85	9.08	12.41	24.68	29.49	10.76	65.94
	% change		-24.44	-23.21	-98.48	-98.04	-97.30	-95.36	-99.33	-58.30
K mg/l	mean	30.34	28.11	29.45	29.13	31.11	29.57	28.06	27.62	28.39
	SD	1.43	2.85	2.44	2.21	2.68	2.28	3.39	3.07	3.69
	% change		-7.35	-2.92	-3.97	2.55	-2.52	-7.50	-8.94	-6.42

All average results of experiment are described in Table 9.1.1 and the analysis of results is given with all details in next paragraphs. The results are analysed all direct and indirect measurements. More detailed results are given in Appendix VI.

9.2 Results

9.2.1 pH and conductivity levels

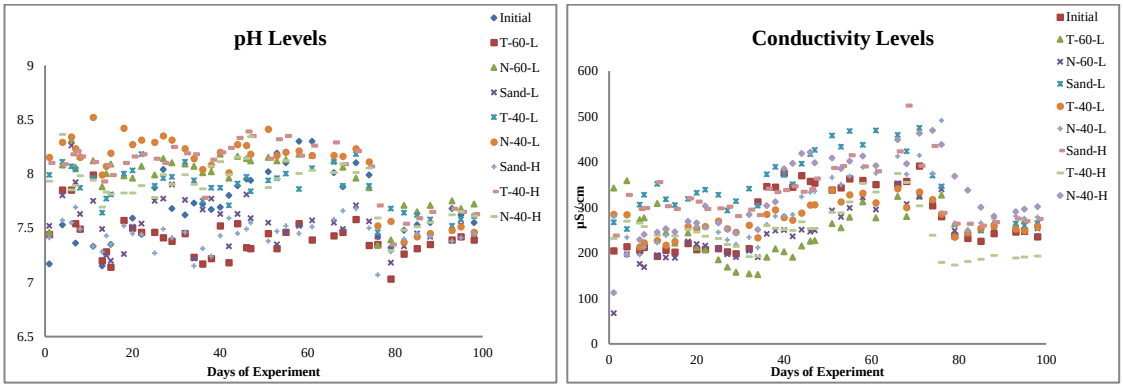


Figure 9.2.1: pH and conductivity levels in tea, nut and perlite columns

The pH in experiment is comparable with the previous experiment that the solution media was the groundwater from the same collection point. The duration of experiment (Figure 9.2.1) is totally different with 98 days to observe the reaction of the system in a time period more than 3 months. In Sand columns pH levels were lower than other columns. More specific in Sand L flow column the average pH was 7.56. In Sand H flow column pH levels were lower than Sand L flow column with average pH=7.44. In that column the range was smaller with the highest value in day 7 (7.69).

In Tea columns, pH was higher than Sand columns and lower than Nut columns. T-60-L column was the column with the lowest pH along the experiment (7.41). The highest value observed in day 11 (7.99) and the lowest in day 79 (7.03). In T-40-L column, average pH was 7.87 with the highest value in day 22 (8.18) and the lowest value in day 76 (7.46). In T-40-H column, the noticeable was the high average pH with pH=8.05 with the majority of days pH higher than 8. The highest levels noticed in day 46 (8.39).

In Nut columns, the pH range was smaller than any other column and the results were between 7.8 and 8.0. In N-60-L column, pH was close to 8 with average levels pH=7.97. The highest value observed in day 6 (8.31) and the lowest in day 76 (7.34). In N-40-L column, the highest

value than other two nut columns noticed and also the highest from all columns. The average value was 8.07 and the highest value achieved in day 11 (8.52). Finally, in N-40-H, column pH levels were lower than other columns with average pH=7.89.

In that experiment conductivity levels (Figure 9.2.1) were much lower than conductivity levels in first experiment with groundwater (Chapter 8). Conductivity levels started from low stable levels until day 30. From that day until day 70 there is an increase in Conductivity levels and after that there was a reduction period until the end of the experiment. The changes that noticed in conductivity noticed also in the measurements that are following in the next paragraphs. The changes in the levels are a result of the influent solutions that is spiked in the columns. The changes in the initial conditions of groundwater affect the EC, and more specifically in that time period there is the change of initial groundwater that is used that collected in the autumn and the second higher EC levels groundwater that received from the same area in the spring time period (February collection). The change in EC from day 70 until the end of experiment is caused to the stored groundwater, which is not spiked immediately from the collection date.

In Sand columns there was an approach that was the same in low flow and high flow rate. In Sand L flow column, conductivity is slightly higher than Sand H flow column. The average conductivity levels for Sand L flow column were 347.10 μS/cm. There was an increasing period till day 71 (474.61 μS/cm) and then a decreasing period till the end of experiment.

In Tea columns, conductivity levels were following the same way as Sand columns. In T-60-L column, the initial conductivity reached high levels with the highest conductivity observed in day 4 (358.82 μS/cm), then there was a reduction period till day 34 (152.62 μS/cm) and till the end of the experiment there was an increasing period. The average conductivity levels at this column were 250.35 μS/cm. In T-40-L column, conductivity levels were the higher than other two Tea columns. The average conductivity along the experiment was 273.87 μS/cm. The experiment was starting with a reduction period till day 13 (216.90 μS/cm), then there was an increasing period till day 66 (340.95 μS/cm) and a final decreasing period till the end. In T-40-H column, the lowest conductivity values were noticed in Tea columns. The average conductivity levels were 247.24 μS/cm. The highest conductivity levels noticed in day 66 (374.04 μS/cm).

In Nut columns, conductivity levels were higher than Tea and Sand columns. In N-60-L column, conductivity was the lowest in Nut columns. The average conductivity levels were 242.08 μS/cm and the lowest levels observed in day 8 (168.36 μS/cm) and the highest in day 76 (339.92 μS/cm). In N-40-L column, conductivity was higher than N-60-L column with average

conductivity levels 293.35 μS/cm. The highest value achieved in day 76 (491.26 μS/cm). Finally, in N-40-H column there was an increasing trend till day 74 (469.47 μS/cm) and then a reduction period till the end of experiment. This column was the column with the highest average conductivity levels in experiment with 325.24 μS/cm.

9.2.2 Redox potential and TOC levels

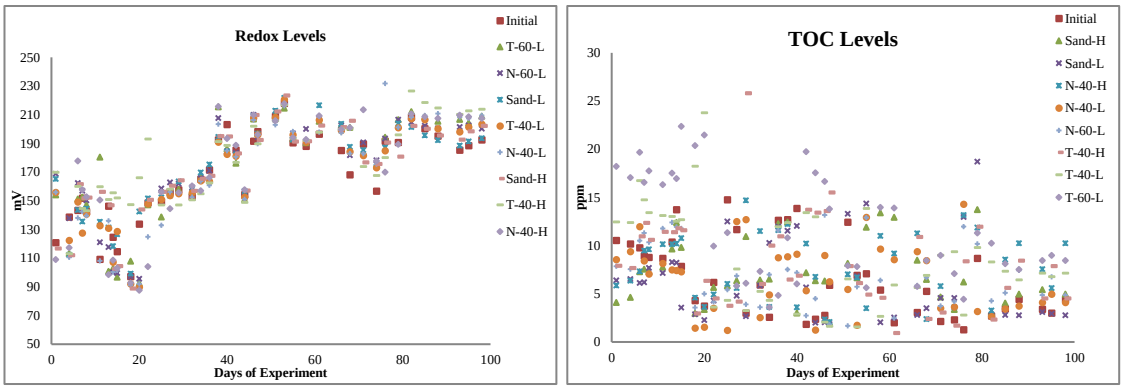


Figure 9.2.2: Redox Potential and TOC levels in tea, nut and perlite columns

Redox potential levels in experiment (Figure 9.2.2) with groundwater solution and perlite columns were very stable. There were increasing and reduction periods at all columns but the average levels are really close to initial solution redox potential. More specifically in the initial solution, the average redox potential along the experiment was +170 mV. At the influent solution as it is noticed in the EC there are changes along the experiment. In redox potential there is an initial stable period till day 30. When the influent groundwater is changing the redox potential is increasing. The increasing period is the result of new groundwater that used that was collected immediately before the application. For that reason, there is the increasing period till day 50. Thereafter there is a decreasing period till day 80 of experiment when there is the stabilization of the new influent solution and finally till the end of the experiment a stable period. It is characteristic that at all the changes of redox potential there is a change in the influent solution. At the last part of experiment the solution is the same as it used in day 30, with the only change that is stored in 4^{o}C till application time. The changes in the influent solution changed the redox potential to more positive redox levels. This was not affected the system, in contrast due to the environmental conditions that created the microbial activity assimilate those conditions and delivered the best results in denitrification process.

In Sand columns, the trend was the same, even in Low Flow and high flow rate. In both columns the lowest redox potential observed in day 18, in Sand Low Flow +99 mV and in Sand

High Flow 88.98mV. From that point till day 53 where the highest values observed, there was an increasing period. The highest levels for Sand Low Flow column were +221 mV and for Sand High Flow column +223 mV. Till the end of experiment a reduction period existed. The average redox potential levels were for Sand Low Flow +171 mV and for Sand High Flow +171 mV.

In Tea column, redox potential levels had wider range of results than Sand columns. In Low Flow rates there was a similar trend along the experiment. In T-40-L column the average redox potential was 169.83 mV and in T-60-L is +171 mV. In high flow rate higher levels observed (T-40-H=+177 mV). In Low Flow rate columns there was an initial reduction period for redox potential till day 20 where the lowest values observed for both of the columns (T-40-L=+89 mV and T-60-L=+92 mV). The highest values observed for T-40-L column in day 53 (+220 mV) and for T-60-L column in day 38 (+215 mV). In T-40-H column redox potential followed totally different trend than Low Flow rate columns. The lowest value observed in day 4 (+113 mV) much higher than the lowest values in Low Flow rate columns. After that, followed an increasing period till day 82 that the highest redox potential observed (+226 mV).

In Nut columns, redox potential was following the trend of Tea columns in Low Flow rate with average values along the experiment very close to initial solution. In Low Flow rate the lowest average value observed in N-40-L column (+168 mV) and in contrast in N-60-L column the highest average levels (+172 mV) observed. In N-40-L column the lowest value observed in day 18 (+91 mV) and the highest in day 76 (+231 mV). In N-60-L column the lowest value observed in day 20 (+95 mV) and the highest in day 46 (+210 mV). Finally, in N-40-H column, redox potential levels were similarly with Low Flow rate columns with average redox potential levels (+170 mV). The lowest value observed in day 20 (+87 mV) and the highest in day 53 (+217 mV).

TOC levels (Figure 9.2.2) were much lower than the previous experiment with groundwater (Chapter 8). Groundwater that used was from the same collection point. In Sand columns, TOC levels were two times lower than Chapter 8. It is also noticed in TOC results the change in influent groundwater. The initial groundwater that was collected in autumn and used in the first part of the experiment (until day 30) had higher levels of organic carbon (10ppm). Those levels are reducing to the half (5 ppm) from day 30 till the end of the experiment where the new groundwater from the same area used and collected in spring. That change does not affect the system because the substrate materials can deliver the amount of organic carbon to complete successfully denitrification process along the experiment.

In Sand High Flow column, TOC levels were higher than Sand Low Flow. The average levels were in Sand High Flow (7.13 ppm) and in Sand Low Flow (6.26 ppm). In both columns the highest levels achieved in day 79 (Sand High Flow=13.73 ppm, Sand Low Flow=18.74 ppm).

In Tea columns, TOC levels were again much lower than Chapter 8. These columns were the columns with the highest TOC levels in that experiment. In T-60-L column, TOC levels were the highest than any other column. The average TOC levels were 11.84 ppm with the highest value observed in day 15 (22.39 ppm). In T-40-L column, the average TOC levels were 8.92 ppm. The highest levels observed in day 20 (23.77 ppm) and the lowest in day 47 (1.63 ppm). In T-40-H column, the lowest levels of Tea columns were noticed with average levels 8.22 ppm. The highest value observed in day 47 (15.54 ppm) and the lowest in day 61 (0.96 ppm).

In Nut columns, TOC levels were lower than Tea columns. In N-60-L column, the average levels were 7.05 ppm with the highest levels in day 55 (12.93 ppm). In N-40-L column, the lowest levels of TOC observed from all columns. The average levels were 6.55 ppm with the highest value in day 76 (14.32 ppm) and the lowest in day 25 (1.21 ppm). In N-40-H column finally, TOC levels were higher than other two Nut columns. The average TOC levels were 7.68 ppm with the highest value in day 29 (14.72 ppm).

9.2.3 Nitrogen species

Nitrogen species for that experiment are showing in detailed in Figure 9.2.3. The reduction of nitrogen species is visible at all columns.

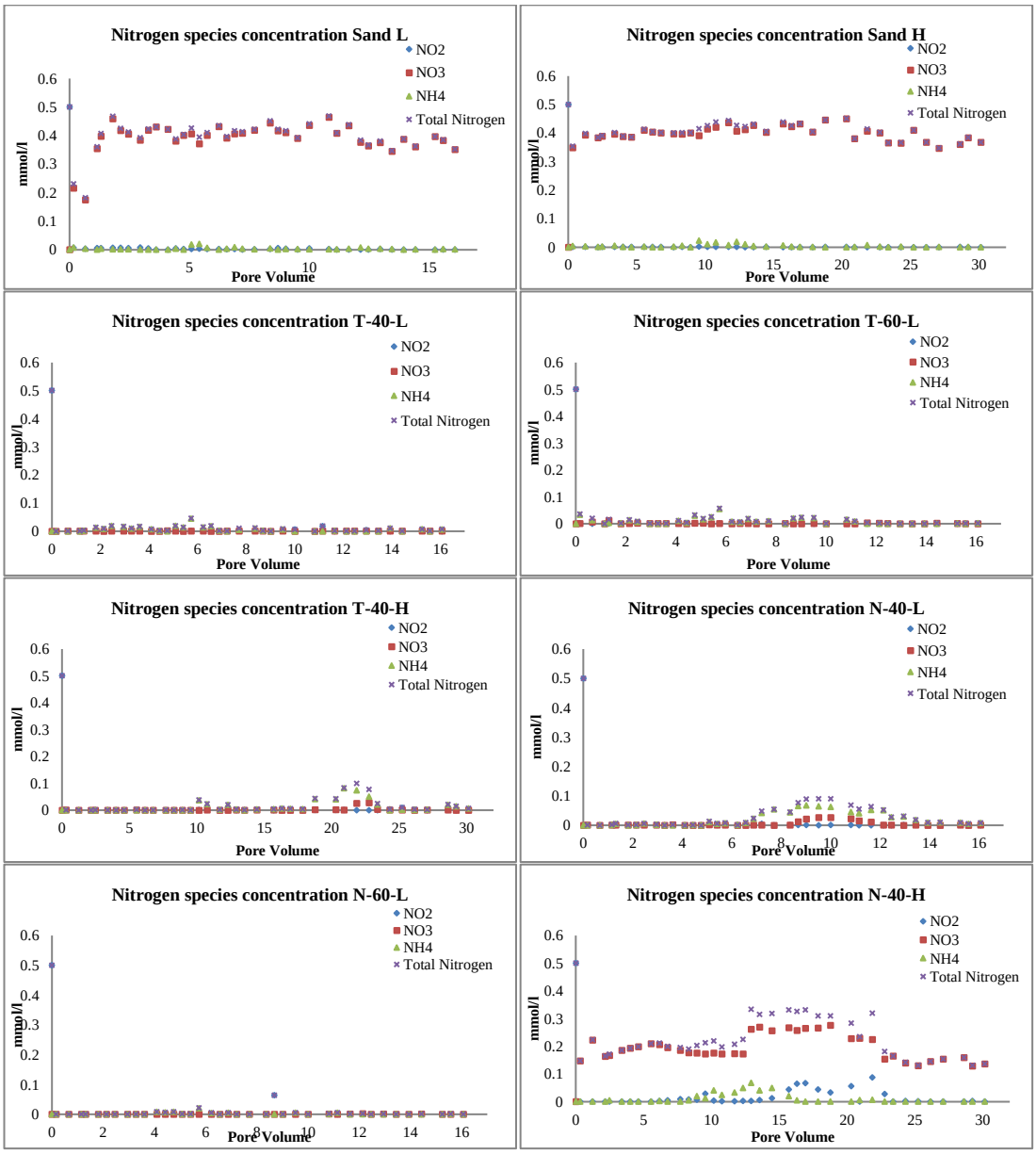


Figure 9.2.3: Nitrogen species in tea, nut and perlite columns

The experimental period is separated in two phases. The first one from day 1 till day 4 (PV=0-1) where the reduction observed and the second one from day 4 until the end of experiment which is the stable phase. The sand columns cannot reduce the NO_3-N and TN levels as can Tea and Nut columns can. The reduction in those columns is in low levels (30-40%). The noticeable result that received in that experiment was for N-40-H column, that the reduction levels were not following the reduction levels of other 2 Nut columns and the result of Chapter 8. The reduction levels remained between 60-80% that was something that was not expected.

Some changes that are noticed in T-40-H, N-40-L and N-40-H after PV=8, are characteristic of the changes in the influent groundwater. Because the reaction on Tea columns and the

establishment of microbial activity works better, the change is noticed in high flow rate Tea column between PV=18-23. The assimilation of the new solution was fast accepted by the column system and the results continue to deliver the best reduction in the column. In N-40-L column the same changes noticed between PV=6-12. In that column the assimilation of the new solution demands longer time period and finally delivers again the same reduction levels in nitrogen compounds. The changes in groundwater affected by the percentage of Nut levels, because in N-60-L column, those changes did not notice. Finally, in N-40-H column, the HRT time as it is noticed at all the duration of the experiment is not enough to deliver the best reduction as it is noticed in Chapter 8 and other Nut columns. Again here the changes in influent groundwater are more noticeable than any other column. This affected capability of the system between PV=12-22 and again remain in the initial levels after that time period.

9.2.3.1 Nitrate levels

Nitrate levels (Figure 9.2.3) along the experiment are the most important compounds for research. The initial concentration on groundwater solution was kept stable using KNO_3. The initial concentration was 514.79 μmol/l nitrate (32.20 mg/l). In Sand columns as it was noticed in all previous experiments, the reduction levels were very low flow. In this experiment Sand L column achieved reduction levels 24.15%, and Sand H column 22.53% only.

In Tea columns, the reduction levels achieved the highest levels with reduction at all columns more than 99.6%. In low flow rate columns, the reduction was higher and in T-40-L column achieved reduction more than 99.94% with average nitrate levels 0.28 μmol/l. In T-60-L column, the reduction was slightly lower achieving reduction levels 99.82% (0.93 μmol/l). In high flow rate column, the reduction levels were again very high and very close to Low Flow rate columns (reduction more than 99.68% and average levels 1.63 μmol/l).

In Nut columns, the trend of columns was changing. The low flow rate columns were achieving reduction more than 99.2%. More specifically in N-40-L column, the reduction was 99.27% with average nitrate levels 3.71 μmol/l. The best reduction was noticed in N-60-L column with reduction more than 99.97%. That was the best reduction along the columns. In N-40-H column with high flow rate a really different result observed. In the previous experiment (Chapter 8), the reduction in the same column achieved levels more than 97%. In that experiment the reduction remains at 62.15% and a really noticeable amount of nitrate remained through column

treatment. The average nitrate levels were 194.82 μmol/l. It was 17 times more than Chapter 8 in the same column with groundwater solution.

9.2.3.2 Nitrite levels

Nitrite levels (Figure 9.2.3) measured also in that part of experiment. In general approach nitrite levels were very Low Flow at all columns. In Sand columns, Sand L column had higher levels of nitrite with average levels 2.54 μmol/l. In Sand H column nitrite levels were lower (0.45 μmol/l).

In Tea columns, nitrite levels were again very low with average values in T-40-L (1.24 μmol/l), in T-60-L (0.25 μmol/l) and in T-40-H column (0.51 μmol/l). It was very similar with nitrate levels that were very Low Flow in all Tea columns.

In Nut columns, the approach was similar with nitrate levels again. In low flow rate columns nitrite levels were lower than the high flow rate. The average levels were in N-40-L column (0.32 μmol/l), in N-60-L column (1.71 μmol/l) and in N-40-H column (13.67 μmol/l).

9.2.3.3 Ammonium levels

Ammonium levels (Figure 9.2.3) were low but in contrast to nitrite levels higher than them. Sand columns observed the lowest levels at all columns. In Sand Low Flow column, the average ammonium levels were 3.26 μmol/l and in Sand High Flow column 3.75 μmol/l.

In Tea columns, ammonium levels were higher than Sand columns and lower than Nut columns. The lowest levels observed in low flow rate columns. More specifically in T-40-L column, the average ammonium levels were 6.43 μmol/l, which were the lowest levels in Tea columns. In T-60-L column, the average levels were 9.11 μmol/l. In T-40-H column the reduction levels were slightly higher than low flow rate with average ammonium levels 12.03 μmol/l.

In Nut columns, ammonium levels were higher than other columns. The column with the lowest ammonium levels was N-60-L column with average levels 1.71 μmol/l. In N-40-L column in contrast the highest levels observed. The average ammonium levels in that column were 20.30 μmol/l. Finally, in N-40-H the average ammonium levels were 10.17 μmol/l.

9.2.3.4 Total Nitrogen levels

TN levels (Figure 9.2.3) in experiment were reduced at all columns. The reduction in separated again according to substrate media of each column. In Sand columns, the reduction levels were not as successful as it was expected. The reduction in Sand H column achieved 23.21% and in Sand L column 24.43%. These were the columns with the smallest reduction.

In Tea columns, the reduction levels were higher than all other columns. In low flow rate the reduction levels were higher than high flow rate column. In detail, the reduction on T-40-L column was more than 98.47% and in T-60-L more than 98.03%. Finally, in high flow rate the reduction levels were slightly lower and in T-40-H column the reduction achieved levels more than 97.29%.

In Nut columns, the reduction levels were following the flow rates. In low flow rates the reduction was high. In N-60-L column the reduction was the highest from all columns with reduction more than 99.32%. In N-40-L column, the reduction was very high again achieving levels more than 95.36%. Finally, in N-40-H column, the reduction that noticed was not in the same levels as other columns. It is noticeable that the reduction was only 58.30% showing that flow rate is also an important indicator for the reduction of TN.

9.2.4 Phosphate and potassium levels

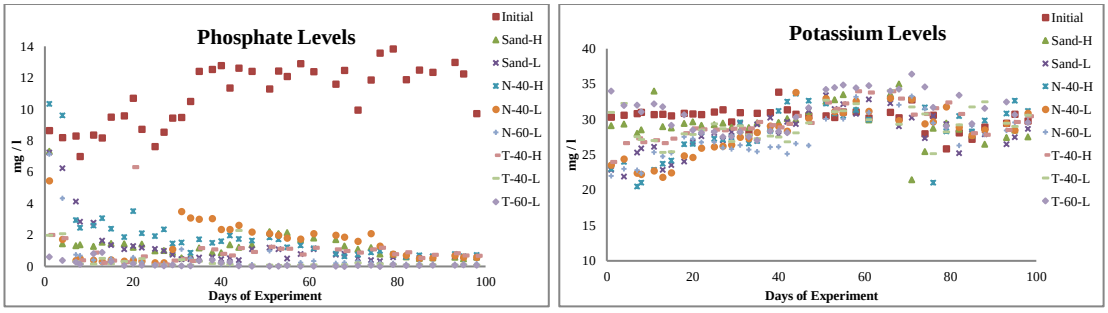


Figure 9.2.4: Phosphate and potassium levels in tea, nut and perlite columns

Phosphate levels (Figure 9.2.4) were the problem that it noticed in the previous experiment and it was a very concerning point. In experiment with perlite columns the hypothesis also focused on reduction of phosphate levels. In this experiment, results that observed were the expected, with reduction of phosphate levels at all columns. All columns achieved reduction more than 70%. At the influent solution as it is noticed at all the measurements in this chapter there is a change in phosphate levels from day 30 till the end of the experiment that the groundwater

solution was changed. As it is noticed in the Figure 8.2.4 the change in the solution does not affect the capability of the system to absorb the phosphate levels almost at all columns. The reduction continued with the same trend and did not affect. The only column that there is a gap in day 30 but afterwards the reduction levels reached the same levels as the other columns was the N-40-L column. That insured the design of the system was correct and can provide the expected result that expected and confirm the hypothesis of this experiment.

In Sand columns, the reduction was in the same levels in both columns with reduction in Sand H column 82.5% and in Sand L column 83.15%.

In Tea columns, the results were very good with the best reduction rates. In T-60-L column the reduction was 97.9% and it was the column with the best reduction at the experiment. In T-40-L column, the reduction was again in high levels with reduction more than 95%. Finally, in T-40-H column, the reduction was smaller than other Tea columns achieving reduction levels 87.4%.

In Nut columns, reduction was noticeable but not as high as Tea columns. In N-60-L column, the reduction that was noticed was the best in Nut columns. The reduction achieved levels 92.2%. In N-40-L column, the reduction was lower but again the levels achieved levels more than 80.4%. Finally, in N-40-H column, the reduction was the smaller than any other column. The reduction achieved levels 74%.

Potassium levels (Figure 9.2.4) were an indicator for initial concentration that was added in groundwater solution. It is the molecular structure that helps us for this. At all columns potassium levels were stable along the experiment and there was spread between 25-35 mg/l due to the absorption from the substrate materials. The change in the influent solution was also noticed in K^+ measurement because from day 30 and afterwards there is a higher spread of results.

9.2.5 Degradation rates

The degradation rates are analysed according to the NO_3-N levels and described the microbial activity that exist in the columns. The description of degradation is based on instantaneous constant rate for specific time period (PV) between each measurement. It is characteristic that the initial phase where the reduction achieved cannot be described by the kinetics because the speed of the reaction was very fast.

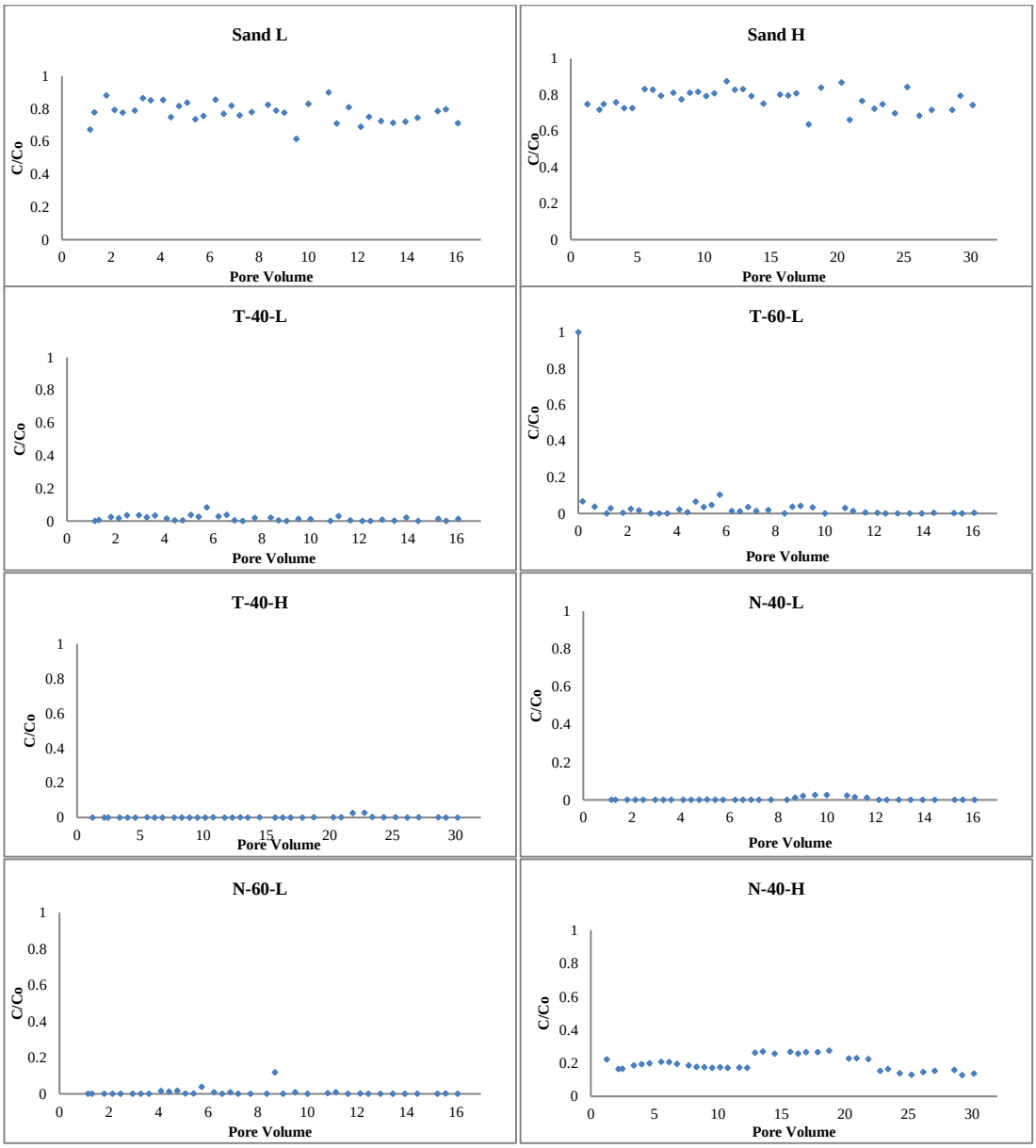


Figure 9.2.5: Degradation rates

The degradation rates (Figure 9.2.5) contain an initial adaptation lag phase (PV=0-1) and then there is a stable phase till the end of experiment. The stable phase is not the same for all the columns. In sand columns the reduction levels are not as high as the other columns and there is a spread of results at all the duration of the experiment. In Sand L column there are two increasing phase (PV 1-2, 9-11) and two stable periods (PV 2-9, 11-16). The stable periods can be described by zero order kinetics. In Sand H column there are 3 stable periods (PV 2-6, 6-12, 24-31). Between PV12-24 there are 3 decreasing periods.

In Tea columns there is the same trend at all columns. After the initial period there is a stable period till the end of the experiment that can be described by zero order kinetics. Small spread

of results does not affect the system and the microbial activity and noticed in the time period that the influent groundwater was changed. The reduction level at all columns is more than 99%.

In Nut columns the trend that is noticed in Tea columns is also characteristic in low flow columns with reduction levels more than 99%. In N-40-H there is a change in the approach. There are 3 stable periods that can be described by zero order kinetics and in that columns is more characteristic the changes in the water solution. In N-40-H column the reduction levels are between 60-80%.

Table 9.2.1: λ values, half-life and retention time

Adaptation Lag Phase	Sand L	Sand H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
λ min	0.136	0.090	1.391	0.445	2.052	0.080	1.669	0.265
λ max	0.176	0.123	1.489	0.541	3.153	1.141	1.670	0.392
T1/2 min	94.340	134.980	11.170	30.750	5.300	0.090	9.940	42.390
T1/2 max	122.340	185.190	11.950	37.360	8.100	14.570	9.950	62.110

Stable Phase	Sand L	Sand H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
λ (days)	0.042	0.082	0.794	0.844	1.663	0.619	1.133	0.279
SD	0.013	0.024	0.256	0.336	0.851	0.248	0.393	0.084
T1/2 (hours)	426.140	221.800	21.140	22.080	11.640	27.860	13.920	65.710
SD	7.186	2.741	0.355	0.410	0.315	0.658	0.400	1.007
RT (hours)	20.160	7.320	18.180	23.160	10.050	11.800	16.510	6.090
SD	1.201	0.596	1.274	1.423	0.676	1.084	1.397	0.594

According to Figures 9.2.5 and Table 9.2.1, there is the detailed approach on the degradation rates along the experiment at all the columns. The λ values, half-life and retention time in the columns are characteristics that help to understand the conditions in the columns and the microbial activity.

In sand columns the λ values as it is noticed in the previous experiments (Chapter 8) remain in low levels with the lowest prices and the half-lives are the highest than any other column. This comes in agreement with the results that analysed and with other studies (Jing et al., 2010) that similar results were received.

In Tea columns the λ values are lower and very close in low flow rate that is not noticed in Chapter 8 and higher in T-40-H column. This also affected the half-lives that are very close to

low flow rate columns and higher than T-40-H column. At all cases the results are in agreement with other studies but with different substrate materials in columns (Aslan and Turkman, 2005).

In Nut columns there is totally different trend. In N-40-H column due to low reduction of NO_3-N and TN the λ value is the lowest than Nut columns and the half-life the highest. All the results are in agreement with the previous paragraphs and the results that received. In N-40-L column the results for low flow rate columns are the lowest for λ value and the highest for half-life. All of the results are also in agreement with other studies (Robertson et al, 2010) and provided results that other studies noticed with different substrate materials.

9.3 Discussion

The experiment columns with Tea and Nut as substrate materials were the most important and most detailed experiment that took place as part of this Thesis. Here two important parameters were taken into account. The first experiment (Chapter 8) was separated in two parts depending on solution that used. The first part was with tap water solution and the second one was with groundwater solution. Finally, the second experiment (Chapter 9) was with groundwater solution and additional perlite column to combine the knowledge from previous experiments.

In the first experiment (Chapter 8) with tap water solution, the whole system was new and it was running for an initial period of 10 days only with water to create the suitable conditions. As it happened before in previous experiments (Chapters 5 and 6), specific amount of KNO_3 was spiked to receive an initial concentration of nitrates in the tap water. The initial concentration of tap water was 32.20 mg/l (0.512mmol/l). The duration of experiment was 51 days.

In first experiment (Chapter 8) with groundwater solution, the procedure that followed was the same. The column system was running for 10 days with groundwater to create the suitable conditions and the environment for denitrification process. Groundwater observed for a rural area outside from Glasgow, Largs where ships are grazing there and there is also an industrial activity in broader area. The collection point was next to a small pond. Groundwater solution before spiked into the columns analysed to find out the initial concentration of anions and cations. Groundwater till the time of experiments remained in 4^oC temperature. Potassium nitrate added to the initial groundwater solution to achieve initial concentration similar with tap water solution (0.51l mmol/l). The duration of experiment with groundwater solution was 31 days.

The second experiment (Chapter 9) was a combination of the previous experiments with groundwater and the previous experiment that perlite as substrate material was used. The observed increases of phosphate levels, force the research to use materials that absorb phosphate compounds. For that reason, additional perlite columns added to the system. The liquid media that used in experiment was groundwater solution with the same procedure as it was mentioned before. The groundwater from the site area collected for the start of previous experiment (October) and the second part of groundwater in the first month of that experiment (February). Due to weather conditions during the winter and different trend of flora and fauna of the area there were some changes that were visible in results of experiment. The duration of experiment was 98 days because with the previous experiment the effectiveness of the substrate material was observed for sorter time period. The initial concentration was again the same (0.512 mmol/l) at all experiment.

9.3.1 Comparison between tap water and groundwater solution experiments

This is the first part of experiments (Chapter 8) that the only change in the system was the solution media that used. Tap water used from the Laboratory of Civil and Environmental Engineering in Strathclyde University. Groundwater collected from rural area outside of Largs.

In initial solutions, there are differences in characteristics of each solution. The pH at both solutions is almost the same with neutral character and pH=7.5. Conductivity is higher in groundwater for about 40 μS/cm than tap water. Redox potential is much lower in groundwater solution with average redox potential along the experiment 146.95 mV, in contrast to tap water that the average redox potential was 234.97 mV. Another difference that is noticeable in two solutions is TOC levels. In tap water TOC levels are 7 times lower than groundwater solution and with average levels along the experiments for tap water 2.77 ppm and for groundwater 20.35 ppm. In Nitrogen compounds, the levels of nitrate nitrite, ammonium and Total Nitrogen are the same due to specific amount of potassium nitrate that spiked to achieve the same initial nitrate concentration. Another point that was very important was phosphate levels. In tap water, the average levels where lower about 2 times than in groundwater. The average phosphate levels in tap water were 1.53 mg/l and in groundwater 3.53 mg/l. In groundwater it was expected to be higher due to the weather and ground conditions that exist in the area and affect the characteristics of solution. Another point was the cultivation of the area and the additions of any fertilizers that possible exist.

The Sand columns were used as control columns. The only media that used was sand which meshed and only sand particles less than 0.66 cm used. The Sand columns were two. The first one is with Low Flow rate and the second one with high flow rate. In Table 9.3.1 are concentrated all details from experiment and the differences between tap and groundwater.

Table 9.3.1: Sand columns average details (all results received after (N=3 replicates) triplicate analysis)

	Sand Low Flow				Sand High Flow			
Results	**Tap Water**		**Groundwater**		**Tap Water**		**Groundwater**	
	SD		***SD***		***SD***		***SD***	
pH	7.59	*0.24*	7.92	*0.13*	7.44	*0.22*	7.63	*0.11*
Conductivity (µS/cm)	+349.91	*134.05*	+499.72	*34.13*	+401.93	*110.81*	+500.21	*20.60*
Redox Potential (mV)	212.70	*52.99*	149.70	*16.33*	233.40	*58.36*	151.10	*18.01*
TOC (ppm)	3.25	*1.22*	12.15	*2.63*	3.47	*1.46*	14.83	*4.40*
K (mg/l)	12.93	*9.42*	29.95	*1.40*	17.82	*9.84*	29.82	*0.84*
PO4 (mg/l)	12.82	*9.27*	11.64	*11.17*	17.66	*18.62*	6.89	*6.41*
NO3 (µmol/l)	372.83	*74.59*	287.28	*104.59*	348.12	*35.93*	388.28	*63.74*
NO2 (µmol/l)	3.97	*6.24*	3.35	*0.82*	89.46	*122.14*	143.72	*170.01*
NH4 (µmol/l)	1.70	*2.43*	1.46	*3.13*	2.56	*2.96*	1.39	*2.24*
TN (µmol/l)	378.50	*75.99*	292.10	*105.00*	440.15	*119.22*	533.38	*120.94*

In both columns, pH with tap water solution was lower than groundwater solution. The same was also happening with conductivity levels that were lower in tap water. Redox potential was lower in groundwater solution columns and higher in tap water. TOC levels were lower in tap water about 4 times. Additionally, the levels in Sand Low Flow column were lower than Sand High Flow column. In phosphate levels there was an increase in all columns. It is noticeable that phosphate levels were higher in tap water. The lowest levels met in Sand High Flow solution with only 6.89 mg/l average value along the experiment. In Nitrogen compound there was more detailed investigation. In Sand Low Flow column, the trend was difference than Sand High Flow column. Nitrate levels were higher in tap water solution. The same was happening with all nitrogen compounds. In Sand High Flow column, the opposite was happening. Nitrogen levels were higher at groundwater solution part. Expect for that it is noticeable in both solution significant nitrite levels that existed showing that denitrification process did not finish and was still on process. Another noticeable point in Sand High Flow column with groundwater solution there was no reduction in Total Nitrogen but an increase of 2%.

The next was Nut columns (Table 9.3.2). The hazelnut husk wastes were the only substrate material in percentages with sand.

Table 9.3.2: Nut columns average details (all results received after (N=3 replicates) triplicate analysis)

	N-40-L				N-60-L				N-40-H			
Results ***SD***	**TW**		**GW**		**TW**		**GW**		**TW**		**GW**	
pH	7.53	*0.24*	7.86	*0.16*	7.39	*0.26*	7.71	*0.16*	7.36	*0.26*	7.60	*0.08*
Conductivity (µS/cm)	430.56	*64.81*	485.01	*16.47*	404.92	*59.47*	496.90	*20.95*	381.92	*143.1*	451.61	*23.89*
Redox Potential (mV)	+218.60	*46.45*	+151.5	*17.08*	+208.7	*43.10*	+148.5	*18.59*	+223.8	*51.75*	+147.90	*17.53*
TOC (ppm)	6.82	*1.51*	17.64	*2.24*	7.90	*1.65*	22.26	*3.24*	4.47	*1.25*	15.94	*2.98*
K (mg/l)	13.44	*7.87*	26.46	*1.24*	15.11	*7.95*	29.31	*1.22*	17.96	*11.50*	30.50	*0.80*
PO4 (mg/l)	13.68	*13.93*	7.63	*484*	11.19	*12.25*	11.12	*7.50*	22.86	*11.31*	10.89	*11.62*
NO3 (µmol/l)	0.14	*0.30*	0.38	*0.61*	0.22	*0.58*	0.48	*0.56*	0.69	*1.79*	12.83	*18.31*
NO2 (µmol/l)	1.25	*1.29*	0.66	*1.15*	1.51	*1.69*	1.00	*1.29*	1.81	*1.49*	1.59	*1.29*
NH4 (µmol/l)	2.34	*3.05*	0.46	*0.75*	2.66	*3.93*	0.25	*0.52*	1.00	*1.43*	0.24	*0.57*
TN (µmol/l)	3.72	*3.72*	1.49	*1.91*	4.38	*4.98*	1.73	*1.54*	3.51	*3.42*	14.65	*18.87*

The results for the Nut columns was following Sand columns for pH, Conductivity, TOC levels where tap water levels were lower than groundwater levels. The pH levels were also neutral and the highest levels observed in N-40-L column. Redox potential was lower in groundwater solution. This was happening for all Nut columns. TOC levels were lower in tap water at all columns about 3 times than the part with groundwater. Phosphate levels were in Nut columns a concerning point in experiment. Phosphate levels were higher than expected and at all columns higher in tap water experiment. The last was nitrogen compounds. For all columns the reduction was more than 98%. It was noticed that levels were higher in groundwater experiment in high flow rate and in tap water experiment in Low Flow rate. The highest levels of nitrogen compound were visible in N-40-H column in experiment with groundwater.

The last substrate material, Tea waste materials from Turkey. It was the substrate material that was expected to provide the best reduction rates (Table 9.3.3).

Table 9.3.3: Tea columns average details (all results received after (N=3 replicates) triplicate analysis)

	T-40-L				T-60-L				T-40-H			
Results ***SD***	**TW**		**GW**		**TW**		**GW**		**TW**		**GW**	
pH	7.44	*0.28*	7.70	*0.16*	7.21	*0.39*	7.64	*0.23*	7.27	*0.25*	7.58	*0.11*
Conductivity (µS/cm)	619.29	*87.35*	638.81	*20.09*	494.98	*74.29*	483.51	*16.89*	357.79	*57.10*	439.42	*34.91*
Redox Potential(mV)	+207.00	*35.61*	+145.40	*21.76*	+197.40	*38.47*	+141.50	*19.80*	+209.60	*35.26*	+147.10	*19.42*
TOC (ppm)	38.17	*17.28*	77.22	*25.27*	50.63	*32.44*	44.26	*9.10*	7.83	*5.70*	21.53	*2.99*
K (mg/l)	13.43	*9.59*	27.82	*1.20*	14.66	*8.36*	27.69	*1.75*	16.56	*12.13*	30.63	*3.85*
PO4 (mg/l)	12.57	*18.62*	11.57	*12.16*	10.98	*11.11*	12.89	*17.85*	10.95	*12.71*	12.77	*15.85*
NO3 (µmol/l)	0.06	*0.22*	0.00	*0.00*	0.17	*0.30*	0.15	*0.29*	0.63	*1.31*	1.65	*3.00*
NO2 (µmol/l)	1.15	*1.46*	0.00	*0.00*	1.15	*1.31*	1.55	*1.33*	1.29	*1.33*	2.49	*7.20*
NH4 (µmol/l)	0.72	*1.70*	0.23	*0.74*	7.34	*8.80*	10.17	*19.70*	1.75	*2.47*	1.37	*2.33*
TN (µmol/l)	1.93	*2.27*	0.23	*0.74*	8.66	*9.33*	11.86	*19.30*	3.67	*4.22*	5.51	*9.36*

In Tea part of experiment, pH levels in tap water experiment were lower than groundwater. For all columns pH levels were neutral between 7.2 and 7.7. Conductivity levels were depending on columns. The highest levels noticed in T-40-L column. The highest levels noticed in groundwater experiment. In T-60-L column, conductivity levels were lower than T-40-L column at about 120-60 μS/cm. This was the only column where the highest conductivity was noticed in tap water experiment. Finally, in T-40-H, there are the lowest conductivity levels and again tap water experiment observed the lowest values. For Redox potential, the results were the same receiving lower values in tap water experiment and the difference were between 50-65 mV. TOC levels were a main indicator for microbial activity that existed in the columns. For all Tea columns, TOC levels were higher than all other columns, showing that microbial activity exists. In T-40-L column, TOC levels the highest in groundwater solution. The lowest levels observed in T-40-H column. In T-60-L was the only column where TOC levels were higher in tap water solution. Phosphate levels were noticeable at all columns and at the same levels in both experiment with tap and groundwater. Phosphate levels were between 10.5 and 12.8 mg/l. That is an important observation for the research and it was an issue to solve in the last experiment.

9.3.2 Comparison between groundwater solution experiments

The last part of research used only groundwater as the solution and it was the closest simulation of true environmental conditions. At the last experiment, perlite columns were added due to high phosphate levels that forced to investigate this parameter in detail. In the last part groundwater used but in the first experiment collected in autumn with different weather conditions and for the second experiment groundwater collected in spring. In Table 9.3.4 there are the details for the initial solutions

Table 9.3.4: Initial groundwater solutions characteristics (all results received after (N=3 replicates) triplicate analysis)

		Initial solution			
Results	***SD***	**GW perlite**		**Groundwater**	
pH		7.71	*0.30*	7.52	*0.08*
Conductivity (μS/cm)		275.72	*68.86*	399.41	*21.06*
Redox Potential (mV)		+170.57	*30.41*	+146.95	*17.84*
TOC (ppm)		6.60	*3.99*	20.35	*1.68*
K (mg/l)		30.30	*1.43*	32.16	*15.80*
PO4 (mg/l)		10.66	*1.91*	3.53	*0.67*
NO3 (μmol/l)		514.79	*19.98*	516.32	*18.23*
NO2 (μmol/l)		0.08	*1.37*	2.79	*0.54*
NH4 (μmol/l)		8.33	*21.61*	1.18	*1.26*
TN (μmol/l)		524.40	*29.17*	520.28	*18.88*

The solution collected from the same spring and differences were not initially expected. In groundwater that was collected in autumn pH levels were lower than spring. Conductivity levels were higher in autumn samples and it is noticeable that the difference was about 125 μS/cm, showing that different microbial activity exists in groundwater solution. Redox potential was higher in spring samples at about 25 mV. This is another indicator for different activity in solutions. The main difference noticed in TOC levels. The amount of organic carbon in autumn samples was 3 times higher than spring samples. The autumn TOC levels reached levels of 20.35 ppm that is a very important clue to find out differences in experiments. Phosphate levels were higher in spring experiment for about 3 mg/l and with a significant amount of 10 mg/l. Finally, nitrogen levels remain in same levels due to addition of specific amount of potassium nitrate. The only difference that is noticed was in ammonium and nitrite initial levels. In autumn samples, nitrite levels were higher than spring samples and the opposite happened with ammonium levels which were lower in spring groundwater.

Sand columns were used as control columns. The most important part was the duration of experiments. The details for Sand columns are in Table 9.3.5.

Table 9.3.5: Sand columns average characteristics, experiments with GW (all results received after (N=3 replicates) triplicate analysis)

	Sand Low Flow				Sand High Flow			
Results	**GW perlite**		**Groundwater**		**GW perlite**		**Groundwater**	
SD		***0.22***		***0.13***		***0.13***		***0.11***
pH	7.56		7.92		7.44		7.63	
Conductivity (μS/cm)	347.10	*72.34*	499.72	*34.13*	328.51	*59.43*	500.21	*20.60*
Redox Potential (mV)	+171.80	*30.64*	+149.70	*16.33*	+171.70	*32.64*	+151.10	*18.01*
TOC (ppm)	6.26	*4.22*	12.15	*2.63*	7.13	*3.22*	14.83	*4.40*
K (mg/l)	28.11	*2.85*	29.95	*1.40*	29.45	*2.44*	29.82	*0.84*
PO4 (mg/l)	1.29	*1.51*	11.64	*11.17*	1.34	*1.10*	6.89	*6.41*
NO3 (μmol/l)	390.45	*54.74*	287.28	*104.59*	398.48	*25.20*	388.28	*63.74*
NO2 (μmol/l)	0.15	*2.38*	3.35	*0.82*	0.03	*0.38*	143.72	*170.01*
NH4 (μmol/l)	3.26	*4.50*	1.46	*3.13*	3.75	*5.50*	1.39	*2.24*
TN (μmol/l)	394.86	*53.93*	292.10	*105.00*	402.67	*26.85*	533.38	*120.94*

Sand columns have the same results in almost all characteristics. There were no significant differences even in both flow rates. The only part that the difference was an issue was on nitrogen levels and more specific in experiment without perlite columns.

In Sand Low Flow column pH levels were higher in first experiment without perlite columns and were close to pH=8. Also conductivity levels were 150 μS/cm higher in autumn experiment. TOC levels were double in first experiment reaching average levels of 12 ppm. The only characteristic that was higher in second experiment with perlite column was Redox potential that average levels were higher for about 20mV. With all that initial characteristics nitrogen compounds levels were expected. In first part, with sand column due to higher TOC levels and

in combination with higher conductivity the removal rates of nitrates were better, receiving reduction in average levels 44% in contrast to second experiment with perlite columns that reduction levels were only 25%.

In Sand High Flow column there was the same results as Sand Low Flow column. More specific, pH levels were lower in both columns and again here pH was lower in experiment with perlite with the lowest average levels with pH=7.44. Conductivity levels remained in same levels with Sand Low Flow column. In Sand High Flow columns, the differences between two experiments in conductivity levels were 170 μS/cm. The lowest average value observed in experiment with perlite (328.51 μS/cm). Redox potential was at the same levels as in Sand Low Flow columns with higher levels in experiment with perlite. TOC levels were following again the same trend as in Sand Low Flow columns. TOC levels in first experiment were double than experiment with perlite with average levels 14.83 ppm. In nitrogen levels there was the main difference than Sand Low Flow columns. In nitrate compounds the reduction that was noticed was 22% at both experiments. The concerning point was in first experiment nitrite levels that were really high in contrast to last experiment. These levels show that denitrification process was under the way and retention time that groundwater remained in column was not enough to achieve a complete treatment. With that nitrite levels Total nitrogen were increased and this was the only column that there was an increase in Total Nitrogen levels for 3%.

Finally, phosphate levels in both experiments reacted with opposite effect. In first experiment phosphate levels increased with very high average levels along the experiment. In second experiment the reduction of phosphate levels was noticeable and the reduction was more than 85% at all columns.

The main part of experiment was the use of new substrate materials. The first one was hazelnut husk wastes. In Table 9.3.6 there are all detailed characteristics of hazelnut columns.

Table 9.3.6: Nut columns average characteristics, experiments with GW (all results received after (N=3 replicates) triplicate analysis)

	N-40-L				N-60-L				N-40-H			
Results / ***SD***	**GW perlite**		**GW**		**GW perlite**		**GW**		**GW perlite**		**GW**	
pH	8.07	*0.32*	7.86	*0.16*	7.97	*0.23*	7.71	*0.16*	7.89	*0.22*	7.60	*0.08*
Conductivity (μS/cm)	293.35	*68.46*	485.01	*16.47*	242.55	*55.48*	496.90	*20.95*	325.24	*83.87*	451.61	*23.83*
Redox Potential (mV)	+168.60	*39.12*	+151.5	*17.08*	+172.3	*34.94*	+148.5	*18.99*	+170.4	*39.99*	+147.9	*17.53*
TOC (ppm)	6.55	*3.57*	17.64	*2.24*	7.05	*3.40*	22.26	*3.24*	7.68	*3.39*	15.94	*2.98*
K (mg/l)	28.06	*3.39*	26.46	*1.84*	27.62	*3.07*	29.31	*1.22*	28.39	*3.69*	30.50	*0.80*
PO4 (mg/l)	1.50	*1.17*	7.63	*4.84*	0.59	*1.29*	11.12	*7.50*	1.98	*2.05*	10.89	*11.62*
NO3 (μmol/l)	3.71	*7.90*	0.38	*0.61*	0.11	*0.29*	0.48	*0.56*	194.82	*44.74*	12.83	*18.31*
NO2 (μmol/l)	0.02	*0.80*	0.66	*1.15*	0.25	*10.39*	1.00	*1.29*	0.70	*22.76*	1.59	*1.25*
NH4 (μmol/l)	20.30	*23.10*	0.46	*0.75*	1.71	*3.92*	0.25	*0.52*	10.17	*17.77*	0.24	*0.57*
TN (μmol/l)	24.03	*29.49*	1.49	*1.91*	2.06	*10.76*	1.73	*1.54*	205.67	*65.54*	14.65	*18.87*

The trend of columns depending in the initial groundwater solution and similar results observed.

In N-40-L column, in both experiments pH levels increased from the initial solutions. The increase in both experiments was in same magnitude. It was an increase of average pH for 0.35 at both experiments. The highest levels observed in experiment with perlite (8.07) creating an environment which was more basic than it was expected but in limitation levels for the best denitrification process. Conductivity levels also increased in compare to initial solutions. The increase in first experiment was higher (85 μS/cm) than the part with perlite columns (18 μS/cm). Redox potential levels remained almost in the same levels like initial solutions but with a slightly reduction at both experiments. The highest average levels observed in experiment with perlite (168.6 mV). TOC levels in both experiments reduced from initial solutions. As it was noticed the first experiment TOC levels are about 3 times higher than experiment with perlite and with average levels 17.64 ppm. Nitrogen levels were the most important part of experiment. Nitrate levels reduced in both experiment more than 99%. The best reduction noticed in first experiment but the duration of the experiment was something that should take into account. In experiment with perlite there was a small but noticeable amount of ammonium 20 μmol/l in contrast to experiment without perlite (0.46 μmol/l). This ammonium levels increased total nitrogen and finally the reduction that achieved in experiment with perlite column was 95.5% in contrast to other experiment that reduction was more than 99.7%. Finally, phosphate levels in first experiment were increased with many periods that the increase of phosphate levels was more than 15 mg/l. In second experiment with perlite, the reduction was noticeable from first days and average levels along the experiment were 1.5 mg/l with reduction more than 85%.

In N-60-L column, pH levels increased in both experiments. The increase was higher in the experiment with perlite and the highest average pH levels observed (pH=7.97). Conductivity levels were lower in the experiment with perlite. It is noticed that during experiment, average levels reduced from initial solution about 30 μS/cm (242.5 μS/cm). In first experiment there was increase of conductivity levels about 96 μS/cm (496.9 μS/cm). Redox potential increased at both experiment with the same way (2 mV). The lowest levels observed in experiment without perlite columns (148.5 mV). The same happened with TOC levels. There was an increase from initial solutions in both experiments. Again TOC levels in experiment without perlite columns were 3 time higher than experiment with perlite (22.26 ppm). Nitrogen levels were really Low Flow with Total Nitrogen reduction in both experiments more than 99.6%. It is noticed that nitrogen levels in experiment without perlite column came from the entire nitrogen compounds and the majority of them from nitrite levels. In the second experiment, nitrogen levels came

from ammonium levels. Finally, phosphate levels in first experiment increased during experiment more than 3 mg/l in average levels and in second experiment, the reduction is visible and observed levels more than 95%.

In N-40-H column, flow rate was higher and the retention time in column was smaller. With higher flow rate the system was expected to react with different way. The pH levels again in that column increased in both experiments. The highest levels observed in experiment with perlite column with average pH=7.89. Conductivity levels increased in both experiments with the same way (50 μS/cm). The highest levels observed in experiment without perlite column (451.6 μS/cm). It was about 125 μS/cm higher than experiment with perlite. Redox potential remained almost the same like initial solution with small increase (1 mV) only in first experiment. The TOC levels in two experiments followed different approach. In first experiment, TOC levels were higher than second experiment but there was reduction from the initial solution (5 ppm) receiving average TOC levels along the experiment 15.94 ppm. In second experiment there was an increase of TOC levels for 1ppm and the average levels along the experiment were 7.68 ppm. The most important part was nitrogen compounds. In first experiment, the reduction achieved levels more 97%. In contrast to Low Flow rate columns there was a difference. Nitrate levels were higher in Low Flow rate columns. In second experiment, there was an important increase in nitrate levels. From the start of experiment till the end, the reduction in nitrate levels was not as high as expected with reduction only 60% (194.82 μmol/l). Except from high nitrate levels, ammonium levels were noticeable high in contrast to experiment without perlite. Finally, phosphate levels in experiment without perlite increased as it was noticed in all other columns with average levels 10.89 mg/l. In experiment with perlite column the reduction was more than 80% with average levels 1.98 mg/l.

Table 9.3.7: Tea columns average characteristics, experiments with GW (all results received after (N=3 replicates) triplicate analysis)

	T-40-L				T-60-L				T-40-H			
Results	**GW perlite**		**GW**		**GW perlite**		**GW**		**GW perlite**		**GW**	
SD												
pH	7.87	*0.19*	7.70	*0.16*	7.41	*0.19*	7.64	*0.23*	8.05	*0.26*	7.58	*0.11*
Conductivity (μS/cm)	273.87	*36.04*	638.81	*20.09*	250.35	*53.93*	483.51	*16.89*	247.24	*50.90*	439.42	*34.91*
Redox Potential (mV)	+169.40	*34.18*	+145.40	*21.76*	+171.8	*36.92*	+141.5	*19.80*	+177.9	*27.21*	+147.1	*19.42*
TOC (ppm)	8.92	*5.07*	77.22	*25.27*	11.84	*5.45*	44.26	*9.10*	8.22	*4.71*	21.53	*2.99*
K (mg/l)	29.13	*2.21*	27.82	*1.10*	30.10	*2.68*	27.69	*1.75*	29.57	*2.28*	30.63	*3.85*
PO4 (mg/l)	0.31	*0.55*	11.57	*12.16*	0.16	*0.22*	12.89	*17.85*	0.96	*0.98*	12.77	*15.85*
NO3 (μmol/l)	0.28	*0.33*	0.00	*0.00*	0.93	*2.16*	0.15	*0.29*	1.63	*5.89*	1.65	*3.00*
NO2 (μmol/l)	0.08	*2.99*	0.00	*0.00*	0.02	*0.02*	1.55	*1.33*	0.05	*1.44*	2.49	*7.20*
NH4 (μmol/l)	6.43	*8.93*	0.23	*0.74*	9.11	*12.12*	10.17	*19.70*	12.03	*20.67*	1.37	*2.33*
TN (μmol/l)	6.98	*9.08*	0.23	*0.74*	10.28	*12.41*	11.86	*19.30*	13.75	*24.68*	5.51	*9.36*

The second substrate material was tea waste materials. As it is noticed from previous part, it is the substrate material that the best results were expected. The detailed results are in Table 9.3.7.

In T-40-L column, pH levels increased in both experiments. The highest levels observed again in experiment with perlite column and average pH was 7.87. Conductivity levels in two experiments followed different approach. In experiment without perlite column there was an increase of conductivity more than 235 μS/cm. The average levels of conductivity were the highest than all columns (638.8 μS/cm). In contrast, in experiment with perlite column there was a small reduction in conductivity levels than initial solution (273.9 μS/cm). Redox potential reduced in both experiments with the same level (1 mV) and the highest levels observed in experiment with perlite column (169.4 mV). TOC levels increased in both experiments. It is noticed at experiment without perlite column the increase of TOC levels was the highest that noticed with average levels 77.22 ppm. In experiment with perlite the increase was smaller and only 2 ppm on average levels. TOC levels were 10 time higher in first experiment without perlite column. The reduction of total nitrogen was more than 98% at both experiments. The nitrogen compound observed to be most significant levels in both experiment was ammonium. The best reduction at all experiments was noticed in experiment with groundwater without perlite column, achieving levels more than 99.95%. Finally, phosphate levels in experiment without perlite increased with average levels 11.57 mg/l. In experiment with perlite columns the reduction of phosphate levels was more than 95%.

In T-60-L column, pH levels were the lowest than any other column. The trend of results for the two experiments was different. In experiment without perlite column there was an increase in pH levels in contrast to initial solution with average pH=7.64. In experiment with perlite, pH levels reduced and average levels were 7.41. It was the lowest average pH than any column. Conductivity levels follow the same trend like pH. In first experiment there was increase in conductivity and in second experiment with perlite there was reduction. Conductivity levels were lower in experiment with perlite column with average levels 250.35 μS/cm which was lower from first experiment about 230 μS/cm. Redox potential, in both experiments also reacted with different way. In first experiment there was reduction from initial groundwater solution (-5 mV). Redox potential in first experiment was lower than experiment with perlite for 30 mV. In experiment with perlite there was a small increase in redox potential (1 mV) with average levels 171.8 mV. TOC levels as it was noticed in previous column, increased. More specifically there was a significant increase in the first experiment that the organic carbon levels were duplicated in contrast to the initial solution with average levels 44.26 ppm. TOC levels in first part were four time higher than second experiment with perlite. In second experiment there was increase also in TOC levels with average levels 11.84 ppm. In nitrogen levels there was reduction at both experiments more than 97.7%. In details, the reduction was more than 99.5% in nitrate

compounds at both experiments. The main nitrogen compound that was noticed in both experiments was ammonium with similar levels in both experiments (10 μmol/l). In Total Nitrogen, the experiment with perlite column observed slightly higher reduction (98%) than the first experiment (97.7%). Finally, phosphate levels in first experiment increased with average levels 12.89 mg/l. At second experiment with perlite the reduction was more than 98%.

In T-40-H column, pH levels increased in both experiments. The increase in experiment with perlite column was higher than experiment without perlite column. The average levels in experiment with perlite were basic with pH 8.05. The average pH levels in first experiment were 7.58. Conductivity levels in experiments reacted with different way. In first experiment there was an increase from initial solution about 40 μS/cm achieving average levels 439.42 μS/cm. In second experiment with perlite column there was decrease in conductivity. The reduction was more than 25 μS/cm, with average levels 247.24 μS/cm which was much lower than first experiment about 190 μS/cm. Redox potential levels were increased in both experiments. The highest levels observed in experiment with perlite column which was higher than initial solution more than 7 mV and higher than first experiment for 30 mV. The average redox potential was 177.9 mV. TOC levels increased at both experiments. Due to the fact that the flow rate was higher TOC levels were lower than other columns. TOC levels in first experiment were almost 3 times higher than second experiment with average TOC levels 21.53 ppm. The increase at both experiments was 2 ppm. Nitrate levels reduced more than 99.6% at both experiments. Nitrate levels were higher than other Tea columns. Nitrite levels were higher in first experiment in contrast to second experiment. Ammonium levels were 10 time higher in second experiment than first experiment, but the levels were really Low Flow. Total Nitrogen levels, were lower in first experiment (5.51 μmol/l) with reduction levels more than 98.9%. In second experiment with perlite column total nitrogen levels were 13.75 μmol/l and the reduction is 97.4%. Finally, phosphate levels were following the same trend as other Tea columns. There was increase in average levels (12.77 mg/l) in first experiment. In contrast, in second experiment with the perlite column reduction achieved levels more than 90%.

9.4 Summary

The environmental impact of the use of the new substrate materials and the results that receives was important to main hypothesis that described in Chapter 1. The selected materials can enhance nitrogen compounds removal and in combination can also remove the P compounds. The detailed approach of two last experiments Chapter 8 and 9 can describe accurately the

approach that was followed. All the conditions were in agreement with other studies (Lee et al., 2004; Saeed and Sun, 2011; Delay et al., 2013) that investigate denitrification process for the microbial kinetics and the microbial activity that exist in the columns. The combination of batch tests and columns experiments in short and long term, ensured that the removal of N and P compounds can be achieved by the proposed design and the proposed research. The system can work in preferable and non-preferable environmental conditions providing the reductions that are expected. The correct organic material that can be the organic source for the system is crucial to provide the best results in combination with the correct HRT and the liquid solution. In the end of the columns experiment samples from all columns analysed to find out the denitrifying bacteria. It was the proof of the results that received along the experiments from the microbial activity that supports the existing conclusions.

According to the results that provided from the q-PCR analysis and for denitrification process the main issue was to focus on the nirK and nirS reductases. The nirK and nirS genes were useful targets for PCR primers to detect communities of denitrifying bacteria in samples from batch test flasks. 20ml of each sample was filter and countered the communities of denitrifying bacteria. The results are providing in Table 9.3.8.

Table 9.3.8: nirK and nirS genes in 20ml filtered sample Columns

log[(nirK/S) /ml]	**nirK**	**nirS**
NO_3 solution	3.77	2.46
Sand L	1.07	1.99
Sand H	0.67	1.51
T-40-H	1.22	3.06
T-40-L	0.90	1.19
T-60-L	1.39	2.68
N-40-H	1.48	2.58
N-40-L	2.55	2.43
N-60-L	3.55	4.20

The results of q-PCR support the hypothesis that denitrifying bacteria exist in the columns and the nitrogen compounds removal is successful. The denitrifier colony exists and the microbial activity that noticed in the results of Ion Chromatography can ensure by the microbial activity.

CHAPTER 10

CONCLUSION AND RECOMMENDATIONS

10.1 Restatement of the objectives

The major aim of the research was to investigate high nitrogen levels mainly high phosphate levels in water in the Samos Island area in Greece and evaluate methods that use waste substrate materials available from agricultural activity to reduce this contamination. The simulation of the field conditions, laboratory scale artificial constructed wetland and columns studies to simulated groundwater flow in the area. Tap water and groundwater from two different agricultural areas around UK (Scotland and Northern Ireland) were used. Three novel substrate materials used, that are connected with the area of investigation, tea waste materials, hazelnut husk and perlite, were studied and the results showed improve the efficiency over published results by others. Finally, there is a proposed solution to the problem of high nitrogen and phosphate levels in the field.

10.2 Major conclusions

The reduction of nitrate levels is noticed at all experiments with all substrate materials. The best reduction was achieved with the new substrate materials, tea waste materials and hazelnut husk wastes. The reduction in those materials achieved 99% in short and long term experiments.

In all water samples the denitrification process is likely connected with the carbon source and the substrate material used. The corrected combination of the substrate material is very important for the best results. The best combination is inert material in 40-60% and waste materials that contain high carbon levels to promote the denitrification process. That approach is the best because there is a combination of inert material and substrate material and can provide to the design system the suitable environmental conditions to enhance denitrification process.

The carbon source of the experiment is connected with the degradation rates and high the carbons levels are, higher carbon, faster the degradation rates. The degradation rates are following specific route at all experiments. An initial adaptation lag phase that the reduction of nitrogen compounds was received, and the stable phase that is following the zero order kinetics.

The phosphate levels were reduced in almost all experiments except from the experiment with tea waste materials and hazelnut husk wastes. In contrast in that experiment there was an increase of phosphate levels more than expected. For that reason, perlite was added in the experiments resulting in the removal of phosphate by more than 80% at all combinations.

The microbial activity in all experiments is active, according to the results and the reduction of Nitrogen compounds for all substrate material under all conditions, batch experiment and columns studies. For the batch tests and at the last column experiments (Chapters 7, 8 and 9) denitrifier bacteria were identified by q-PCR (Results in Chapter 7 and 9).

Perlite without organic substrate was not successful for nitrate removal, 40%, but in combination with tea wastes and hazelnut husk wastes the removal increased to 99%. The property of perlite to absorb phosphate was the best combination in an ultimate full-scale engineering design for the best results.

In the Table 10.1 there are the main outcomes of all experiments about denitrification process and nitrate removal.

Table 10.1: Total experimental characteristics

	Substrate materials	**HRT**	**Solution**	**NO_3-N removal**	**Degradation rates**	**λ value ($Days^{-1}$)**	**$T_{½}$ (Hours)**
Chapter 5	Sand Wheat straw	7 hours	GW pre-treated	55-80%	Adaptation Lag Phase cannot be described by one kinetic law Stable phase is described by zero order kinetics	4.410-5.330	3.05-5.00
		50 hours		40-88%		0.244-1.500	12.07-67.10
Chapter 6	Sand Mulch Perlite	16 hours	GW pre-treated	27-56%		0.522-1.409	14.38-33.62
Chapter 8	Tea Nut Sand	3.25 d 6.10 d	TW	27-99.99%		0.043-1.152	4.08-518.78
			GW	24-100%		0.039-1.150	2.30-217.50
Chapter 9	Tea Nut Sand Perlite	3.25 d 6.10 d	GW	24-99.94%		0.042-1.663	11.64-426.74

10.3 Secondary conclusions

The ideal conditions in pH, conductivity levels, redox potential and significant TOC levels are connected with denitrification process. The ideal conditions were observed at all experiments and the reduction of nitrate levels was noticed at all experiment.

The water source used for experiments was varied to see if the source of water was important. Interestingly, tap water working better in the experiments, but natural groundwater also showed good activity. At all cases the rates of reactions determined using groundwater is preferred

because is simulation of the natural water. The results are promising with reduction at batch experiment for tea waste materials between 90-99% that is also met in column experiments short and long term without reduction of removal rates. In hazelnut husk waste materials, the removal rates were lower between 50-99% in batch experiment. In column experiment there is a concern for the high flow rate experiment because in contrast to all other nut columns with removal rate 90-99%, the removal rate reduced to 60%. The correct combination of substrate material and the correct flow rate can provide the best results in denitrification process and the phosphate removal.

In batch experiment the ideal conditions are not the same with tap and groundwater solution. The critical point in tap water was 198 hours but in contrast in groundwater, more time need to achieve the critical point. This is very interesting as this suggest the microbial populations already exist in the waste material and are able to thrive in tap water (pure) rather than compete with natural microorganisms found in groundwater. Further research is needed to study this; however microbial biochemistry was no part of this thesis.

10.4 Recommendation for future work

- Study of specific pesticides and microbial contamination and measurement along with detailed study of the microbial activity.
- Measurement of dissolved oxygen at all the duration of experiments to find out the interaction that exist between oxygen levels and microbial activity.
- Use sensors that can measure all the detailed about anions, cations, pH, DO, ORP and conductivity at all the duration of experiment.
- Use groundwater from the area that is under investigation (Greece) and use more substrate material from that area.
- Monitoring groundwater that is used during all the year from specific area to find out also the connections with the pollution of the study area.
- More detailed approach on the duration of experiments, with at least 90 days experimental period, to find out the life-time of the system in long term experiments.
- Application of results to specific cultivations like a hydroponic greenhouse that there is a main control for all activities.
- Application of results in the field scale environment to test other factors that can affect the research including weather, the soil, the water, and the cultivations.

References

Addiscott, T.M., Whitmore, A.P., Powlson, D.S. (1991). Farming, Fertilizers and the Nitrate Problem. Wallingford: CAB International, 170pp.

Aikpokpodian, P.E., Ipinmoroti, R.R., Omotoso, S.M. (2010).Bisorption of Nickel (II) from aqueous solution using waste tea (Camellia Sinensis) materials. American-Eurasian Journal of Toxicological Sciences. 2(2), 72-82.

Ajmal, M.A., Hussain, S., Ahmad, A. (1998). Role of sawdust in the removal of copper (II) from industrial wastes. Wat. Res., 32, 3085-3091.

Akar, E., Altinis¸ A., Seki, Y. (2013). Using of activated carbon produced from spent tea leaves for the removal of malachite green from aqueous solution. Ecol. Eng., 52, 19–27.

Albuquerque, A., Oliveira, J., Semitela, S., Amaral, L. (2009). Influence of bed media characteristics on ammonia and nitrate removal in shallow horizontal subsurface flow constructed wetlands. Bioresource Technology.,100, 6269–6277.

Alcala, M., Jones, K., Ren, J., Andreassen, T. (2009). Compost product optimization for surface water nitrate treatment in biofiltration applications. Bioresource Technology., 100, 3991–3996.

Amarasinghe, B., Williams, A.R. (2007). Tea waste as a low cost adsorbent for the removal of Cu and Pb from wastewater. Chem. Eng. J., 132, 299-309.

APHA/AWWA. (1998). Standard Methods for the examination of water and waste- water. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA

APHA. (1992). Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association, Washington, DC.

APHA. (1999). Standard Methods for the Examination of Water and Wastewater, 20th Edition. APHA, AWWA & WWF, Washington, D.C.

Appelo, C.A.J., Postma, D. (2005). Geochemistry,GroundWater And Pollution" Second Edition, AA Bakema Publishers, Lediden, The Netherlands, 649pp.

Arvanitoyannis, I.S., Varzakas T.H. (2008). Vegetable waste treatment: comparison and critical presentation of methodologies. Crit. Rev. Food Sci. Nutr., 48, 205-247.

Aslan, S. (2005). Combined removal of pesticides and nitrates in drinking waters using biodenitrification and sand filter system. Process Biochemistry., 40, 417–424.

Aslan, S., Turkman, A. (2005). Combined biological removal of nitrate and pesticides using wheat straw as substrates. Process Biochem., 40, 935–943.

Ayyasamy, P., Rajakumar, S., Sathishkumar, M., Swaminathan, K., Shanthi, K., Lakshmanaperumalsamy, P. (2009). Nitrate removal from synthetic medium and groundwater with aquatic macrophytes. Desalination., 242, 286–296.

Bacteria Growth Curve. http://www.blog.naturaleza.eldietista.es/2013/09/el-ser-humano-en-peligro-de-extincion.html, (Accessed 10/11/2015)

Bange, H.W. (2000).Global change. It's not a gas. Nature, 16, 301–302.

Bell, S., McGillivray, D. (2008). Environmental Law. 7th Edition. Oxford: Oxford University Press, 860pp.

Bernard, R., Mortazavi, B., Kleinhuizen, A. (2015). Dissimilatory nitrate reduction to ammonium (DNRA) seasonally dominates NO3 reduction pathways in an anthropogenically impacted sub-tropical coastal lagoon. Biogeochemistry., 125, 47–64.

Blok, J. (1994). Classification of biodegradability by growth kinetic parameters. Ecotoxicol. Environ. Saf., 27,, 294–305.

Bockris, M., Kim, J. (1997). Electrochemical treatment of low-level nuclear wastes. Appl. Electrochem., 27, 623-634.

Boogerd, F.C., Van Verseveld, H.W., Stouthamer, A.H. (1981). Respiration- driven proton translocation with nitrite and nitrous oxide in Paracoccus denitrificans. Biochim. Biophys. Acta, 638, 181-191.

Boubaker, K., Colantoni, A., Allegrini, E., Longo, L., Giacinto, S., Monarca, D., Cecchini, M. (2014). Assessment of Energetic Potential by Hazelnuts Pruning in Black Sea Region in Turkey in Reference to the Achieved Viterbo Area Sustainability of Agricultural Systems Projects in Italy. American Journal of Biomass and Bioenergy, 2 (3), 113-127.

Bouraoui, F., Grizzetti, B., Aloe, A. (2009). JRC EUR 24002 EN. (2009) 72 http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/8130/1/eur_fatescenario_baseline.pdf

Boyer, J., Groffman, P. (1996). Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. Soil Biology & Biochemistry, 28, 783-790.

Bradberry, S. (2007). Methaemoglobinaemia. Medicine, 35, 552-553.

Braker, G., Ayala-del-Rio, H., Devol, A., Fesefeldt, A., Tiedje, J. (2001). Community structure of denitrifiers, Bacteria, and Archaea along redox gradients in Pacific Northwest marine sediments by terminal restriction fragment length polymorphism analysis of amplified nitrite reductase (nirS) and 16S rRNA genes. Applied and Environmental Microbiology, 67, 1893-1901.

Braker, G. Fesefeldt, A., Witzel, K.P. (1998). Development of PCR primer systems for amplification of nitrite reductase genes (nirK and nirS) to detect denitrifying bacteria in environmental samples. Applied and Environmental Microbiology, 64, 3769-3775.

Bratieres, K., Fletcher, T., Deletic, A., Zinger, Y. (2008). Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. Water Research., 42, 3930-3940.

Bryan, N.S. (2006). Nitrite in nitric oxide biology: Cause or consequence? A systems-based review Free Rad. Biol. Med., 41, 691-701.

Calderer, M., Marti, V., de Pablo, J., Guivernau, M., Prenafeta-Boldú, F.X., Viñas, M. (2014). Effects of enhanced denitrification on hydrodynamics and microbial community structure in a soil column system. Chemosphere, 111, 112–119.

Çalişkan, T. (1995). Fındık Çeşit Katalogu. Tarım ve Köy isleri Bakanlığı Tarımsal Üretim ve Geliştirme Genel Müdürlüğü.

Cameron, S., Schipper, L. (2010). Nitrate removal and hydraulic performance of carbon substrates for potential use in denitrification beds. Ecol. Eng., 36, 1588–1595.

Cameron, S., Schipper, L. (2011). Evaluation of passive solar heating and alternative flow regimes on nitrate removal in denitrification beds. Ecological Engineering., 37, 1195–1204.

Canter, L.W. (1997). Nitrates in Groundwater. CRC, Lewis Publishers, Boca Raton, FLL.

Cantor, K.P. (1997). Drinking water and cancer. Cancer Cause Control, 8, 292-308.

Castaldi, S., Riondino, M., Baronti, S., Esposito, F., Marzaioli, R., Rutigliano, F., Vaccari, F., Miglietta, F. (2011). Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. Chemosphere., 85, 1464–1471.

Cavdar, D.A., Kalaycioglu, H., Mengeloglu, F. (2011). Tea mill waste fibers filled thermoplastic composites: the effects of plastic type and fiber loading. Journal of Reinforced Plastics and Composites, 30(10), 833–844.

Centi, G., Perathoner, S. (2003). Catalysis and sustainable (green) chemistry. Applied Catalysis B-Environmental, 41, 15-29.

Chabani, M., Amrane, A., Bensmaili, A. (2007). Kinetics of nitrates adsorption on Amberlite IRA 400 resin. Desalination., 206, 560–567.

Chen, Y., Wen, Y., Cheng, J., Xue, C., Yang, D., Zhou, Q. (2011). Effects of dissolved oxygen on extracellular enzymes activities and transformation of carbon sources from plant biomass: implications for denitrification in constructed wetlands. Bioresource Technology, 102, 2433–2440.

Cheyns, K., Mertens, J., Diels, J., Smolders, E., Springael, D. (2010). Monod kinetics rather than a first-order degradation model explains atrazine fate in soil mini-columns: implications for pesticide fate modelling. Environmental Pollution., 158, 1405–1411.

Christianson, L.E. (2011). Pilot-scale evaluation of denitrification drainage bioreactors: reactor geometry and performance. Journal of Environmental Engineering, 137 (4), 213-220.

Chu, L., Wang, J. (2013). Denitrification performance and biofilm characteristics using biodegradable polymers PCL as carriers and carbon source. Chemosphere., 91, 1310–1316.

Colenutt, BA., Trenchard, P.J. (1985). Ion chromatography and its application to environmental analysis: a review. Environmental pollution, 10, 77-96.

Connolly, R., Zhao, Y., Sun, G., Allen, S. (2009). Removal of ammoniacal-nitrogen from an artificial landfill leachate in downflow reed beds. Process Biochemistry, 39, 1971–1976.

Costa, C., Efstathiou, A. (2007). Mechanistic Aspects of the H2-SCR of NO on a Novel Pt/MgO−CeO2 Catalyst. J. Phys. Chem., 111 (7), 3010–3020.

Costello, D., Lamberti, G. (2009). Biological and physical effects of non-native earthworms on nitrogen cycling in riparian soils. Soil Biology & Biochemistry., 41, 2230–2235.

Cox, C.D., Payne, W.J. (1973). Separation of soluble denitrifying enzymes and cytochromes from Pseudomonas perfectomarinus. Can. J. Microbiol., 19, 861-872.

Cuello, C., Correa, P., Haenszel, W., Gordillo, G., Brown, C., Archer, M., Tannenbaum, S. (1976). Gastric cancer in Colombia. I. Cancer risk and suspect environmental agents. J Natl Cancer Inst., 57(5), 1015–1020.

Curry, S. (1982). Methemoglobinemia.Ann. Emerg. Med., 11, 214-221.

Dandie, C.E., Miller, M.N., Burton, D.L., Zebarth, B.J., Trevors, J.T., Goyer, C. (2007). Nitric oxide reductase-targeted real-time PCR quantification of denitrifier populations in soil. Appl. Environ. Microbiol., 73, 4250–4258.

Daskalaki, P., Voudouris, K. (2006). The impacts of the Irrational Water Resources Management on Groundwater Quality of Greece. 2nd International Conference, Water Science and Technology.

Daskalaki, P., Voudouris, K. (2008). Groundwater quality of porous aquifers in Greece: a synoptic review. Environ. Geol., 54, 505–513.

Davidson, E.A., Swank, W.T. (1986). Environmental parameters regulating gaseous nitrogen losses form two forested ecosystems via nitrification and denitrification. Appl. Environ. Microbiol., 52, 1287-1292.

DeCatanzaro, J., Beauchamp E., Drury, C. (1987). Denitrification VS Dissimulatory nitrate reduction in soil with alfalfa, straw and glucose and sulphide treatments. Soil Bioil. Biochem., 19 (5), 583-587.

Dede, O., Dede, G., Ozdemir, S. (2012). The use of hazelnut husk and biosolid in substrate preparation for ornamental plants. African Journal of Agricultural Research, 7(43), 5837-5841.

DEFRA. (2008). Water Framework Directives – Daughter Directives [online] available fromhttp://www.defra.gov.uk/environment/water/wfd/daughter-dirs.htm

Delay, F., Porel, G., Chatelier, M. (2013). A dual flowing continuum approach to model denitrification experiments in porous media colonized by biofilms. Journal of Contaminant Hydrology., 150, 12–24.

Della Rocca, C., Belgiorno, V., Meriç, S. (2006). An heterotrophic/autotrophic denitrification (HAD) approach for nitrate removal from drinking water. Process Biochemistry, 41, 1022–1028.

Della Rocca, C., Belgiorno, V., Meric, S. (2005). Cotton-supported heterotrophic denitrification of nitrate-rich drinking water with a sand filtration post-treatment. Water SA, 31, 229–236.

Della Rocca, C., Belgiorno, V., Meriç, S. (2007). Overview of in-situ applicable nitrate removal processes. Desalination, 204, 46–62.

Delwiche, C.C. (1970). The nitrogen cycle. Sci. Am., 223, 137-146.

Dem D., Tarjan P.P., Englehardt J.D. (2004). Kinetic Studies of the Electrochemical Treatment of Nitrate and Nitrite Ions on Iridium-Modified Carbon Fiber Electrodes.Chem. Eng. Technol., 7, 56-64.

Demir, T. (2014). Molecular characterization of Turkish Hazelnut cultivars and accessions. J. Anim. Plant Sci., 24(3), 820-828.

Demir, T., Beyhan, N. (2000). A research on the selection of hazelnuts grown in Samsun (Samsun ilinde yetiştirilen fındıkların seleksiyonu üzerine bir araştırma). Turk J. Agric For., 24, 173-183.

Dimitriou, E., Moussoulis, E. (2010). Hydrological and nitrogen distributed catchment modeling to assess the impact of future climate change at Trichonis Lake, western Greece. Hydrogeology Journal, 18, 441–454.

Donmez, A., Kalaycioglu, H., Mengeloglu, F. (2011). Tea mill waste fibers filled thermoplastic composites: the effects of plastic type and fiber loading. Journal of Reinforced Plastics and Composites., 30(10), 833–844.

Drever, J.I. (1997). The Geochemistry of Natural Waters, Surface and Groundwater Environments, 3rd edition, Prentice-Hall Publishers, 440 pp.

Dusdieker, L.B., Stumbo, P.J., Kross, B.C., Dungy, C.I. (1996). Does Increased Nitrate Ingestion Elevate Nitrate Levels in Human Milk. Arch. Pediatr. Adolesc. Med., 150, 311-314.

Dyer, A.,Tangkawanit, S., Rangsriwatananon, K. (2004). Exchange diffusion of Cu^{2+},Ni^{2+},Pb^{2+} and Zn^{2+} into analcime synthesized from perlite. Microporous and Mesoporous Materials., 75, 273–279.

Eastman, J. A. and Ferguson, J. F. (1981). Solubilization of Particulate Organic Carbon During the Acid Phase of Anaerobic Digestion. Journal of the Water Pollution Control Federation, 53, 352-366.

EEA, Nutrients in European ecosystems. Topic Report 4. (1999). 156pp.

EEC. (1980). Council directive on the quality of water for human consumption. (No– 80/778) off), J. EEC., 229 (11), 1980

EOS Samos. The Union of Vinicultural Cooperatives of Samos. (http://www.samoswine.gr/) (Accesses 10-8-2014)

EPA. http://water.epa.gov/type/rsl/monitoring/vms52.cfm accessed (22/8/2014)

EPA. http://water.epa.gov/type/rsl/monitoring/vms59.cfm accessed (22/8/2014)

Erdogan, V., Koksal, A., Aygun, A. (2010). Assessment of Genetic Relationships among Turkish Hazelnut (Corylus avellana L.) Cultivars by RAPD Markers. Romanian Biotechnological Letters., 15 (5), 5591-5601.

Essandoh, H.M.K., Tizaoui, C., Mohamed, M.H. (2013). Removal of dissolved organic carbon and nitrogen during simulated soil aquifer treatment. Water research., 47, 3559-3572.

European Union Directive. (1991). European Union Nitrates Directive (91/676/EC). (http://eur-lex.europa.eu/)

European Union Directive. (2000). European Union Water Framework Directive (2000/60/EEC). (http://eur-lex.europa.eu/)

European Union Directive. (2006). European Union Groundwater Directive (2006/118/EC). (http://eur-lex.europa.eu/)

Fan, A.E., Steinberg, V.E. (1996). Health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity. Regul. Toxicol. Pharmacol., 23, 35–43.

Fanning, J.C. (2000). The chemical reduction of nitrate in aqueous solution. Coordin. Chem. Rev., 199, 159–179.

FAO. (2013). Current world fertilizers trend and outlook 2013. ftp://ftp.fao.org/agl/agll/docs/cwfto13.pdf (Accessed 05-02-2015)

FAO. (2012). FAOSTAT Database. FAO, Rome. Retrieved from http://faostat.fao.org/faostat/ cited on 2012. (Accessed 08-01-2015)

Feng, L.J., Zhu, L., Yang, Q., Yang, G.F., Xu, J., Xu, X.Y. (2013). Simultaneous enhancement of organics and nitrogen removal in drinking water biofilm pretreatment system with reed addition. Bioresource Technology, 129, 274–280.

Fotiadis, G., Siasios, A. (2007). Ιστορικό Δασικών Πυρκαγιών Ν. Σάμου (Περίοδος 1993 - 2007), Περιφέρεια Βορείου Αιγαίου - Διεύθυνση Δασών Σάμου.

García, J., Aguirre, P., Mujeriego, R., Huang, Y., Ortiz, L., Bayona, J.M. (2004). Initial contaminant removal performance factors in horizontal flow reed beds used for treating urban wastewater. Water Research, 38, 1669–1678.

Ge, S., Peng, Y., Wang, S., Lu, C., Cao, X., Zhu, Y. (2012). Nitrite accumulation under constant temperature in anoxic denitrification process: The effects of carbon sources and COD/NO_3-N. Bioresource Technology, 114, 137–143.

Ghiglieri, G., Barbieri, G., Vernier, A., Carletti, A., Demurtas, N., Pinna, R., Pittalis, D. (2009). Potential risks of nitrate pollution in aquifers from agricultural practices in the Nurra region , northwestern Sardinia , Italy. Journal of Hydrology, 379, 339–350.

Gibert, O., Pomierny, S., Rowe, I., Kalin, R.M. (2008). Selection of organic substrates as potential reactive materials for use in a denitrification permeable reactive barrier (PRB). Bioresour. Technol., 99, 7587–7596.

Gikas, G., Tsihrintzis, V., Akratos, C., Haralambidis G. (2009). Water quality trends in Polyphytos reservoir, Aliakmon River, Greece. Environ. Monit. Assess., 149, 163–181.

Gikas, G., Tsihrintzis V., Akratos C. (2011). Performance and modeling of a vertical flow constructed wetland–maturation pond system. Journal of Environmental Science and Health Part A, 46, 692–708.

Gironas, J., Adriasola, J., Fernadez, B. (2008). Experimental analysis and modelling of a stormwater perlite filter. Water environment Research., 80 (6), 524-539.

Gonzaga, R., Martinez-Landa, P., Perez, A., Villanueva, P. (2009). Mechanical properties dependency of the pearlite content of ductile irons, J. of Achievement in Materials and Manufacturing Engineering, 33 (2), 150-158.

Google maps, Ireland, Scotland, Samos, https://www.google.co.uk/maps., (Accessed 15/5/2013)

Grady, Jr., C.P.L., Daigger, G.T. (1998). Biological Wastewater Treatment Theory and Applications. Marcel Dekker, Inc., New York, NY.

Greenan, C.M., Moorman, T.B., Kaspar, T.C., Parkin, T.B., Jaynes, D.B. (2006). Comparing carbon substrates for denitrification of subsurface drainage water. J. Environ. Qual., 35, 824–829.

Groffman, P.M., Altabet, M.A., Bohlke, J.K., Butterbach-Bahl, K., David, M.B., Firestone, M.K., Giblin, A.E., Kana, T.M., Nielsen, L.P., Voytek, M.A. (2006). Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications, 16, 2091-2122.

Groffman, P.M., Axelrod, E.A., Lemunyon, J.L., Sullivan, W.M. (1991). Denitrification in grass and forest vegetated filter strips. Journal of Environmental Quality, 20, 671-674.

Guo, Q., Blowes, D.W. (2009). Biogeochemistry of two types of permeable reactive barriers, organic carbon and iron-bearing organic carbon for mine drainage treatment: Column experiments. Journal of Contaminant Hydrology, 107, 128–139

Gustafsson, J., Renman, A., Renman, G., Poll, K. (2008). Phosphate removal by mineral-based sorbents used in filters for small-scale wastewater treatment. Water Research, 42, 189-197.

Hameed, B.H. (2009). Spent tea leaves: a new non-conventional and low-cost adsorbent for removal of basic dye from aqueous solutions. J. Hazard. Mater., 161, 753–759.

Hammersley, M., Howes, B. (2002). Control of denitrification in a septage-treating artificial wetland: the dual role of particulate organic carbon. Water Res., 36 (17), 4415–4427.

Hanter, W.J., Shaner, D. L., (2010). Biological remediation of groundwater containing both nitrate and atrazine. Curr Microbiol., 60, 42–46

Hashim, M., Mukhopadhyay, S., Sahu, J.N., Sengupta, B. (2011). Remediation technologies for heavy metal contaminated groundwater. Journal of Environmental Management., 92, 2355-2388.

Hekmatzadeh, A.A.,Karimi-Jashani, A., Talebbeydokhti, N., Kløve, B. (2012). Modeling of nitrate removal for ion exchange resin in batch and fixed bed experiments. Desalination., 284, 22- 31.

Henry, S., Bru, D., Stres, B., Hallet, S., Philippot, L. (2006). Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Appl. Environ. Microbiol., 72, 5181–5189.

Henry, S., Baudoin, E., Lopez-Gutierrez, J.C., Matin-Laurent, F., Brauman, A., Philippot, L. (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. Journal of Microbiological Methods., 59, 327-335.

Henze, M., Harremoes, H. (1977). Biological denitrification of Sewage: A literature review. Prog. Wat. Tech., 8, 509-555.

Henze, M., van Loosdrecht, M.C.M., Ekama, G.A. (2008). Biological Wastewater Treatment: Principles, Modeling, and Design. Intl Water Assn Publishing, 528 pp.

Hill, A. (1996). Nitrate removal in stream riparian zones. Journal of Environmental Quality, 25, 743-755.

Hill, A., Devito, K., Campagnolo, S., Sanmugadas, K. (2000). Subsurface denitrificationin a forest riparian zone: Interactions between hydrology and supplies of nitrate and organic carbon. Biogeochemistry, 51, 193-223.

Hill, M.J. (1999). Nitrate toxicity: myth or reality. J. Brit, Nutr., 81, 343-344.

Ho, Y.S., Chiang, T.H., Hsueh, Y.M. (2005). Removal of basic dye from aqueous solution using tree fern as a biosorbent. Process Biochem., 40(1), 119-124.

Hochstein, L.I. Betlach, M., Kritikos ,G. (1984). The effect of oxygen on denitrification during steady-state growth of Paracoccus denitrificans. Arch. Microbiol., 137, 74-78.

Horold, S., Tacke, T., Vorlop, K.D. (1993). Catalytic removal of Nitrate and Nitrite from drinking water: Screening for hydrogenation catalysts and influence of reaction conditions on activity and selectivity. Environ. Technol., 14, 931-939.

Horvátha, L., Groszb, B., Machonc, A., Tubac, Z., Nagyc, Z., Czóbelc, S., Baloghd, B., Pélid, E., Fótid, S., Weidingere, T., Pintérc, K., Führer, E. (2010). Estimation of nitrous oxide emission from Hungarian semi-arid sandy and loess grasslands; effect of soil parameters, grazing, irrigation and use of fertilizer. Agriculture, Ecosystems and Environment, 139, 255–263.

Huang, B., Chi, G., Chen, X., Shi, Y. (2011). Removal of highly elevated nitrate from drinking water by pH-heterogenized heterotrophic denitrification facilitated with ferrous sulfide-based autotrophic denitrification. Bioresource Technology., 102, 10154–10157.

Huang, G., Fallowfield, H.,Guan, H., Liu, F. (2012). Remediation of Nitrate-Nitrogen Contaminated Groundwater by a Heterotrophic-Autotrophic Denitrification Approach in an Aerobic Environment. Water Air Soil Pollut., 223, 4029–4038.

Huett, D.O., Morris, S.G., Smith, G., Hunt, N. (2005). Nitrogen and phosphorus removal from plant nursery runoff in vegetated and unvegetated subsurface flow wetlands. Water Research., 39, 3259–3272.

Jackson, P.E., Chassaniol, K., (2002). Advances in the determination of inorganic ions in potable waters by ion chromatography. Journal of environmental monitoring., 4, 10-15.

Jamei, M., Guiras, H., Chtourou, Y., Kallel, A., Romero, E., Georgopoulos, I. (2011). Water retention properties of perlite as a material with crushable soft particles. Engineering Geology., 122, 261–271.

Jing, L., Wang, H., Jiang, T., Li, D., Feng, H., Chen, M. (2010). Denitrification of Groundwater Using Walnut Shell as Solid Carbon Source. Conference on Environmental Pollution and Public Health., 1059-1063.

Johir, M.H., George, J., Vigneswaran, S., Kandasamy, J., Grasmick, A. (2011). Removal and recovery of nutrients by ion exchange from high rate membrane bio-reactor (MBR) effluent. Desalination., 275, 197–202.

Johnson, D., Keller, E., Rockwell, T. (1990). Dynamic pedogenesis: new views on some key soil concepts, and a model for interpreting Quaternary soils. Quaternary Research., 33, 306-319.

Ilstedt, U., Singh, S., Nordgren, A. (2007). Using perlite as a substrate carrier for measuring microbial available phosphorus by respiration kinetics in soils. Biol. Fertil. Soils., 43, 503–510.

International Fertilizer Industry Association – IFA webpage: http://www.fertilizer.org (Accessed 24/1/2015)

Iwasaki, H., Shidara, S., Suzuki, H., Mori, T. (1963). Studies on denitrification. VII. Further purification and properties of denitrifying enzyme. J. Biochem., 53, 299-303.

Kaelin, D., Manser, R., Rieger, L., Eugster, J., Rottermann, K., Siegrist, H. (2009). Extension of ASM3 for two-step nitrification and denitrification and its calibration and validation with batch tests and pilot scale data. Water Research., 43, 1680-1692.

Kammas, P. (1998). Hydrogeological study of island Samos, IGME.

Kanbur, G., Arslan, D., Özcan, M. (2013). Some compositional and physical characteristics of some Turkish hazelnut (Corylus avellana L.) variety fruits and their corresponding oils. IFRJ, 20(5), 2161-2165.

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., Philippot, L. (2006). Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. Appl. Environ. Microbiol., 72, 5957–5962.

Kapoor, A., Viraraghavan, T. (1997). Nitrate Removal from Drinking Water-Review J. Environ. Eng., 123 (4), 371-380.

Karanasios, K.A., Vasiliadou, I.A., Pavlou, S., Vayenas, D.V. (2010). Hydrogenotrophic denitrification of potable water: a review. Journal of Hazardous Materials, 180, 20–37.

Karavoltsos, S., Sakellari, A., Mihopoulos, N., Dassenakis, M., Scoullos, M. (2008). Evaluation of the quality of drinking water in regions of Greece. Desalination., 224, 317–329.

Kasirajam, S., Ngouajio, M. (2012). Polyethylene and biodegradable mulches for agricultural applications: a review, Agron. Sustain. Dev., 32, 501-529.

Kebbekus, B.B., Mintra, S. (1998). Environmental Chemical Analysis. London: Blackie. 330pp.

Kiely, G. (1997). Environmental Engineering, McGraw Hill Publishers. 979pp.

King, S.E. (2005). Riparian buffer effectiveness in removing groundwater nitrate as influenced by vegetative type. 184pp.

Kim, H., Seagren, E.A., Davis, A.P. (2003). Engineered bioretention for removal of nitrate from stormwater runoff. Water Environ. Res., 75, 355–367.

Knapp, C., Graham, D. (2007). Nitrite-oxidizing bacteria guild ecology associated with nitrification failure in a continuous-flow reactor. FEMS microbiology ecology., 62, 195-201.

Knies, S.V. (2009). Riparian buffer effectiveness at removal of NO b3 s-N from groundwater in the middle Coastal Plain of North Carolina. 118pp.

Knowles, R. (1982). Denitrification. Microbiol. Rev., 46, 43-70.

Koch, A.L. (1997). The Monod model and its alternatives. In: Koch,A.L.,Robinson,J., Milliken, G.A. (Eds.), Mathematical Modeling in Microbial Ecology. Chapman and Hall, New York. 62–93.

Koh, D.C., Mayer, B., Lee, K,S., Ko, K.S. (2010). Land-use controls on sources and fate of nitrate in shallow groundwater of an agricultural area revealed by multiple environmental tracers. Journal of Contaminant Hydrology, 118, 62–78.

Köksal, A.İ., Artik, N., Şimşek, A., Güneş, N. (2006). Nutrient composition of hazelnut (Corylus avellana L.) varieties cultivated in Turkey. Food Chemistry, 99, 509-515.

Konstantinou, I.K., Hela D.G., Albanis, T. (2006). The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence levels. Environmental Pollution, 141, 555-570.

Kovarova-Kovar, K., Egli, T. (1998). Growth kinetics of suspended microbial cells: From single-substrate-controlled growth to mixed-substrate kinetics. Microbiology and molecular biology reviews, 62(3), 646–666.

Kraft, B., Strous, M., Tegetmeyer, H. (2011), Microbial nitrate respiration - genes, enzymes and environmental distribution. J. Biotech., 155, 104-117.

Kross, B.C., Olson, M.L., Ayebo, A., Johnson, J.K. (1995). Humans. In: Soil Amendments: Impacts on Biotic Systems. JE Rechcigl (ed). Ann Arbor: CRC Press, Inc., 153-214.

Kuenen, J. (2008). Anammox bacteria: from discovery to application. Nat. Rev. Microbiol., 4, 320-326.

Kulkarni, P. (2013). Nitrophenol removal by simultaneous nitrification denitrification (SND) using T. pantotropha in sequencing batch reactors (SBR). Bioresource Technology, 128, 273–280.

Lacasa, E., Cañizares, P., Sáez, C., Fernández, F., Rodrigo, M. (2011). Removal of nitrates from groundwater by electrocoagulation. Chemical Engineering Journal., 171, 1012– 1017.

Lecloux, A.J. (1999). Chemical, biological and physical constrains in catalytic reduction processes for purification of drinking water. Catal. Today, 53, 23-34.

Lee, C., Fletcher, T.D., Sun, G. (2009). Nitrogen removal in constructed wetland systems. Engineering in Life Sciences, 9, 11–22.

Lee, K., Bong, Y., Lee, D., Kim, Y., Kim, K. (2008). Tracing the sources of nitrate in the Han River watershed in Korea, using δ^{15}N-NO_3 and δ^{18}O-NO_3 values. Science of the Total Environment., 395 (2), 117-124.

Lee, L. Ong, S., Ng, W. (2004). Denitrification of Nitrate Wastewater using Packed-Bed Columns. Water Environment Research, 76 (5), 388-393.

Leverenz, H. L., Haunschild, K., Hopes, G., Tchobanoglous, G., Darby, J.L. (2010). Anoxic treatment wetlands for denitrification. Ecological Engineering, 36, 1544–155.

Li, J., Chang, H., Ma, L., Hao, J., Yang, R.T. (2011). Low-temperature selective catalytic reduction of NOx with NH3 over metal oxide and zeolite catalysts. Catalysis Today.,175, 147–156.

Lin, T., Chen, W., Wang, L. (2010). Particle properties in granular activated carbon filter during drinking water treatment. Journal of Environmental Sciences., 22(5), 681–688.

Lin, Y., Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. Applied Microbiology and Biotechnology., 69, 627–642.

Liu, S., Zhao, Z., Li, J., Wang, J., Qi, Y. (2013). An anaerobic two-layer permeable reactive biobarrier for the remediation of nitrate-contaminated groundwater. Water Research., 47, 5977-5985.

Long, L.M., Schipper, L., Bruesewitz, D. (2011). Long-term nitrate removal in a denitrification wall. Agriculture, Ecosystems and Environment, 140, 514–520.

Lopez-Ruiz, B., (2000). Advances in the determination of inorganic anions by ion chromatography. Journal of Chromatography A., 881, 607–627.

Maag, M., Vinther, F. (1996). Nitrous oxide emission by nitrification and denitrification indifferent soil types and at different soil moisture contents and temperatures. AppliedSoil Ecology., 4, 5-14.

Madigan, M.T., Martinko, J.M., Parker, J. (1997). Biology of Microorganisms. 8th edition. Prentice-Hall, New Jersey. 986pp.

Mahavi, A.H., Naghipour, D., Vaezi, F., Nazmara, S. (2005). Tea waste as an adsorbent for heavy metal removal from industrial wastewaters. American J. App. Sci., 2(1), 372-375.

Maia, G., Day, G., Gates, R., Taraba, J., Coyne, M. (2012). Moisture effects on greenhouse gases generation in nitrifying gas-phase compost biofilters. Water Research., 46, 3023-3031.

Maier, R.M., Pepper, I.L., Gerba, C.P. (2009). Environmental microbiology. Amsterdam; London: Elsevier Academic Press. 589pp.

Marshall, W.E., Champagne, E.T. (1995). Agricultural by-products as adsorbents for metal ions in laboratory prepared solutions and in manufacturing waste water. J. Environment Sciences Health., 2, 241- 261.

Martin, D., Salminen, J.M., Niemi, R.M., Heiskanen, I.M., Valve, M.J., Hellsten, P.P., Nysten, T.H. (2009). Acetate and ethanol as potential enhancers of low temperature denitrification in soil contaminated by fur farms: a pilot-scale study. J. Hazard. Mater., 163, 1230–1238.

Martin, J. (2008). Scottish Nitrate Vulnerable Zones. (Accessed 10-9-2014) http://www.netregs.gov.uk/netregs/businesses/agriculture/61887.aspx

Martinez, A., Carrey, R., Torrento, C., Folch., A., Soler, A., Otero, N. (2015). Evaluation of two carbon sources for inducing denitrification: Batch and column experiments. Procedia Earth and Planetary Science, 13, 124 – 128.

Mattison, R.G., Taki, H., Harayama, S. (2002). The bacterivorous soil flagellate Heteromita globosa reduces bacterial clogging under denitrifying conditions in sand-filled aquifer columns. Appl. Environ. Microbiol., 68, 4539-4545.

Mayer, P. M., Reynolds, S.K., McCutchen, M.D., Canfield, T.J. (2009). Meta-analysis of nitrogen removal in riparian buffers. Journal of Environmental Quality., 36, 1172-1180.

McEwan, A.G., Greenfield, A.J., Wetzstein, H.G., Jackson, J.B., Ferguson, S.J. (1985). Nitrous oxide reduction by members of the family Rhodospirilaceae and the nitrous oxide reductase of Rhodopseudomonas capsulata. J. Bacteriol., 164, 823-830.

McLaughlan, R.G., Al-Mashaqbeh, O. (2009). Effect of media type and particle size on dissolved organic carbon release from woody filtration media. Bioresource Technology, 100, 1020–1023.

Metcalf and Eddy (2003). Wastewater Engineering: Treatment, Disposal, Reuse. Revised by G. Tchobanoglous, F. Burton. McGraw-Hill, Singapore. 1771 pp .

Microbial Kinetics., http://chemwiki.ucdavis.edu/Physical_Chemistry/Kinetics/Reaction_Rates, (Accessed 15/11/2015)

Milovanovic, M. (2007). Water quality assessment and determination of pollution sources along the Axios/Vardar River, Southeastern Europe. Desalination, 213, 159–173.

Ministry of Agriculture Greece., http://www.minagric.gr/ , Samos Land Use., (Accessed 15/10/2015)

Minet, E., Goodhue, R., Coxon, C.E., Kalin, R.M., Meier-Augenstein, W. (2011). Simplifying and improving the extraction of nitrate from freshwater for stable isotope analyses. J. Environ. Monit., 13, 2062–2066.

Misaelides, P. (2011). Application of natural zeolites in environmental remediation: A short review. Microporous and Mesoporous Materials., 144, 15–18.

Monod, J. (1949). The growth of bacterial cultures. Ann. Rev. Microbiol., 3, 371-394.

Moreno-Vivian, C., Blasco, M., Mart, N., Cabello P. (1999). Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. J. Bacteriol., 181, 6573-6584.

Morris, J.T., Lajtha, K. (1986). Decomposition and nutrient dynamics of litter from four species of freshwater emergent macrophytes. Hydrobiologia., 131, 215-223.

Mourtzios, P. (2008). Γεωμορφολογικά και υδρογραφικά χαρακτηριστικά που συνδέονται με την ανάπτυξη των χειμάρρων της νήσου Σάμου, Διατριβή. Ειδίκευσης, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη.

Moutsopoulos, K.N., Poultsidis, V.G., Papaspyros, J.N.E., Tsihrintzis, V. (2011). Simulation of hydrodynamics and nitrogen transformation processes in HSF constructed wetlands and porous media using the advection–dispersion-reaction equation with linear sink-source terms. Ecological Engineering., 37, 1407–1415.

Mulch, http://mulchandmore.webs.com/mulch.htm., (Accessed, 15/2/2014)

Muscutt, A.D., Harris, G.L., Bailey, S.W., Davies, D.B. (1993). Buffer zones to improve water quality: a review of their potential use in UK agriculture. Agriculture Ecosystems & Environment., 45, 59–77.

Myteletsi, S., Theodorou, F. (2000). Ειδική μελέτη κατασκευής αντιπλημμυρικών – αντιδιαβρωτικών έργων των καμένων εκτάσεων της νήσου Σάμου, Περιφέρεια Βορείου Αιγαίου, Διεύθυνση Δασών Σάμου.

Nakatani, A., Martines, A., Nogueira, M, Fagotti, D., Oliveira, A., Bini, D., Sousa, J., Cardoso, E. (2011). Changes in the genetic structure of Bacteria and microbial activity in an agricultural soil amended with tannery sludge. Soil Biology & Biochemistry., 43, 106-114.

Nandal, M., Hooda, R., Dhania, G. (2014). Tea Wastes as a Sorbent for Removal of Heavy Metals from wastewater. International Journal of Current Engineering and Technology., 4 (1), 243-247.

Narváez, L., Cunill, C., Cáceres, R., Marfà, O. (2011). Design and monitoring of horizontal subsurface-flow constructed wetlands for treating nursery leachates. Bioresource Technology., 102, 6414–6420.

Ni, S., Zhang Q. (2013). Anaerobic Ammonium Oxidation: From Laboratory to Full-Scale Application. BioMed. Research International., 1-10.

Nikolaidis, C., Mandalos, P., Vantarakis, A. (2008). Impact of intensive agricultural practices on drinking water quality in the EVROS Region (NE GREECE) by GIS analysis. Environ. Monit. Assess., 143, 43–50.

Nitrogen Cycle in the earth and the movement in the atmosphere. (Accessed 16-11-2014). http://californiaagriculture.ucanr.edu/landingpage.cfm?article=ca.E.v067n01p68&fulltext=yes

Ng, I.S., Wu, X., Yang, X., Xie, Y., Lu, Y., Chen, C. (2013). Synergistic effect of Trichoderma reesei cellulases on agricultural tea waste for adsorption of heavy metal Cr(VI). Bioresour. Technol., 145, 297–301.

Nordström, A. (2014). Denitrification in a Low Temperature Bioreactor System - Laboratory column studies. ISSN 1650-6553, 290pp.

Okpokwasili, G.C., Nweke C.O. (2005). Microbial growth and substrate utilization kinetics. African Journal of Biotechnology., 5(4), 305-317.

Ovez, B. (2006). Batch biological denitrification using Arundo donax, Glycyrrhiza glabra, and Gracilaria verrucosa as carbon source. Process Biochem., 41, 1289–1295.

Ozel, U., Akdemir, A., Ergun, O. (2012). Utilization of natural zeolite and perlite as landfill liners for in situ leachate treatment in landfills, Int. J. Environ. Res. Public Health., 9, 1581-1592.

Papanikolaou, D. (1979). Unités tectoniques et phases de déformation dans I' île de Samos, Mer Egée. Bull. Soc. Géol. France, 745-752.

Parslow, R.C., McKinney, P.A., Law, G.R., Staines, A., Williams, R., Bodansky, H.J. (1997). Incidence of childhood diabetes mellitus in Yorkshire, northern England, is associated with nitrate in drinking water: an ecological analysis. Diabetologia., 40(5), 551-561.

Payne, E., Fletcher, T., Russell, D., Grace, M., Cavagnaro, T., Evrard, V., Deletic, A., Hatt, B., Cook, P. (2014). Temporary storage or permanent removal? The division of nitrogen between biotic assimilation and denitrification in stormwater biofiltration systems. PLoS ONE 9(3): e90890.

Payne, W.J. (1981) Denitrification. John Wiley & sons, New York. 214 pp.

Pedrero, F., Kalavrouziotis, I., Alarcón, J., Koukoulakis, P., Asano, T. (2010). Use of treated municipal wastewater in irrigated agriculture—Review of some practices in Spain and Greece. Agricultural Water Management., 97, 1233–1241.

Peña-Haro, S., Llopis-Albert, C., Pulido-Velazquez, M., Pulido-Velazquez, D. (2010). Fertilizer standards for controlling groundwater nitrate pollution from agriculture: El Salobral-Los Llanos case study, Spain. Journal of Hydrology., 392, 174–187.

Pe-Piper, G., Tsolis-Katagas, P. (1991). K-rich mordenite from Late Miocene rhyolitic tuffs, island of Samos, Greece. Clays and Clay Minerals., 39(3), 239-247.

Perlite., http://www.succseed.com/sundries/potting-material-perlite-ca-6-litre.html ., (Accessed 12/2/2013)

Petersen, J., Thomsen, K., Mattsson, L., Hansen, M., Christensen, T. (2012). Estimating the crop response to fertilizer nitrogen residues in long-continued field experiments. Nutr.Cycl. Agroecosyst., 93, 1–12.

Philippot, L. (2002). Denitrifying genes in bacterial and Archaeal genomes. Biochimica Et Biophysica Acta-Gene Structure and Expression., 1577, 355-376.

Philippot, L., Hallin, S. (2005). Finding the missing link between diversity and activity using denitrifying bacteria as a model functional community. Current Opinions in Microbiology., 8, 234-239.

Philippot, L., Hallin, S., Schloter, M. (2007). Ecology of denitrifying prokaryotes in agricultural soil. In Advances in Agronomy., 96, 249-305.

Philippot, L., Piutti, S., Martin-Laurent, F., Hallet, S., Germon, J. (2002). Molecular analysis of the nitrate-reducing community from unplanted and maize-planted soils. Applied and Environmental Microbiology., 68, 6121-6128.

Pintar, A. (2003). Catalytic processes for the purification of drinking water and industrial effluents. Catalysis Today., 77, 451–465.

Pirt, S.J. (1975). Principles of microbe and cell cultivation, Wiley, New York. 274 pp.

Polatides C., Kyriacou, G. (2000). Electrochemical removal of nitrate ion from aqueous solution by pulsing potential electrolysis. Electrochi. Acta., 50, 5237-5241.

Powlson, D.S. (1993). Understanding the soil nitrogen cycle. Soil Use and Management., 9, 86–93.

Prasad, S., Singh, A., Joshi, H.C. (2007). Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resources Conservation and Recycling., 50, 1–39.

Puckett, L. (2004). Hydrogeologic controls on the transport and fate of nitrate in groundwater beneath riparian buffer zones: results from thirteen studies across the United States, 47-53.

Qambrani, N.A., Oh, S.E. (2013). Effect of dissolved oxygen tension and agitation rates on sulfur-utilizing autotrophic denitrification: batch tests. Appl. Biochem. Biotechnol., 169, 181–191.

Quevauviller, P. (2006). Agreement on New EU Groundwater Directive. Journal of Solids and Sediments., 6(4), 254-260.

Ray, A., Selvakumar, A., Tafuri, A. (2006). Removal of selected pollutants from aqueous media by hardwood mulch. Journal of Hazardous Materials., B136, 213–218.

Read-Daily, B., Tank, J., Nerenberg, R. (2011). Stimulating denitrification in a stream mesocosm with elemental sulfur as an electron donor. Ecol. Eng., 37, 580–588.

Reddy, K.R., DeLaune, R.D. (2008). Biogeochemistry of wetlands science and applications. 800pp.

Reed bed tank/ pre-treatment tank., https://www.specifiedby.com/building-regulations/approved-document-h-drainage-waste-disposal ., (Accessed 17/4/2013)

Riedl, H. (1989). Beitraege zur Landschaftsstruktur und Morphogenese von Samos und Ikaria (Ostaegaeische In- seln). Salzburger geographische Arbeiten, 143-243.

Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.W.N., Bemment, C.D. (2008). Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. Water Res., 42, 4215–4232.

Robertson, W.D. (2010). Nitrate removal rates in woodchip media of varying age. Ecological Engineering., 36, 1581–1587.

Robinson-Lora, M., Brennan, R. (2009). The use of crab-shell chitin for biological denitrification: batch and column tests. Bioresource Technology., 100, 534–541.

Rodríguez, D. C., Pino, N., Peñuela, G. (2011). Monitoring the removal of nitrogen by applying a nitrification-denitrification process in a Sequencing Batch Reactor (SBR). Bioresource Technology, 102, 2316–2321.

Sa, J., Montero, J., Duncan, E., Anderson, J.A. (2007). Bi modified Pd/SnO2 catalysts for water denitration. Applied Catalysis B-Environmental., 73, 98-105.

Saeed, T., Sun, G. (2012). A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. Journal of Environmental Management., 112, 429-448.

Saeed, T., Sun, G. (2011a). Enhanced denitrification and organics removal in hybrid wetland columns: comparative experiments. Bioresource Technology., 102, 967–974.

Saeed, T., Sun, G. (2011b). Kinetic modelling of nitrogen and organics removal in vertical and horizontal flow wetlands. Water Research., 45(10), 3137-3152.

Saleh-Lakha, S., Shannon, K.E., Goyer, C., Trevors, J.T., Zebarth, B.J., Burton, D.L. (2008). Nitric oxide reductase gene expression and nitrous oxide production in nitrate- grown Pseudomonas mandelii. Appl. Environ. Microbiol., 74, 6876–6879.

Saleem, M., Chakrabarti, M., Hasan, D. (2011). Electrochemical removal of nitrite in simulated aquaculture wastewater. African Journal of Biotechnology., 10(73), 16566-16576.

Saliling, W., Westerman, P., Losordo, T. (2007). Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. Aquacultural Engineering., 37, 222–233.

Samos Municipality. (http://www.vathi.gr/) (Accesses 10-3-2014)

Sawada, E., Satoh, T. (1980). Periplasmic location of dissimilatory nitrate and nitrite reductases in a denitrifying phototrophic bacterium, Rhodo-pseudomonas sphaeroides forma sp. denitrificans. Plant Cell Physiol., 21, 205-210.

Schipper, L., Barkle, G., Vojvodic-Vukovic, M. (2005). Maximum rates of nitrate removal in a denitrification wall. Journ. Envirom. Qual., 34(4), 1270-1276.

Schipper, L., Cameron, S.C., Warneke, S. (2010a). Nitrate removal from three different effluents using large-scale denitrification beds. Ecological Engineering., 36, 1552–1557.

Schipper, L., Robertson, W.D., Gold, A.J., Jaynes, D.B., Cameron, S.C. (2010b). Denitrifying bioreactors-An approach for reducing nitrate loads to receiving waters. Ecological Engineering., 36, 1532–1543.

Schmidt-Hieber, C, Jonas, P, Bischofberger, J. (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature., 429(6988), 184–187.

Schnobrich, M.R., Chaplin, B.P., Semmens, M.J., Novak, P.J. (2007). Stimulating hydrogenotrophic denitrification in simulated groundwater containing high dissolved oxygen and nitrate concentrations. Water Res., 41, 1869–1876.

Seki.T. (1980). Chromatographiczhonration of some biogenic amines on a weakly acidic ion-exchange resin. Journal of Chromatography A., 207(2), 286-287.

SEPA. (2005). The Future for Scotland's Water. (http://www.sepa.org.uk/) (Accessed 12/1/2015)

Sgouridis, F., Heppell, C., Wharton, G., Lansdown, K., Trimmer, M. (2011). Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in a temperate re-connected floodplain. Water Research., 45, 4909-4922.

Shaikh, M., Bhatti, I. (2011). Utilization of tea waste for the removal of arsenic (III) from aqueous solution. Sindh Uni. Res. Jour. (Sci. Ser.)., 43(1), 97-104.

Shimada, H., Orii, Y. (1975). The nitric oxide compounds of Pseudomonas aeruginosa nitrite reductase and their probable participation in the nitrite reduction. FEBS Lett., 54, 237-240.

Shoun, H., Kim, D., Uchiyama, H., Sugiyama, J. (1992). Denitrification by fungi. Fems Microbiology Letters, 94, 277-281.

Sicolo, M., Tringali, M., Orsi, F., Santagostino, A. (2009). Porphyrin pattern and methemoglobin levels in Columba livia applied to assess toxicological risk by air pollution in urban areas. Arch Environ Contam Toxicol., 57(4), 732-40.

Silber, A., Bar-Yosef, B., Levkovitch, I., Soryano, S. (2010). pH-Dependent surface properties of perlite: Effects of plant growth. Geoderma, 158, 275–281.

Skoulikidis, N., Amaxidis, Y., Bertahas, I., Laschou, S., Gritzalis, K. (2006). Analysis of factors driving stream water composition and synthesis of management tools- a case study on small/medium Greek catchments. Science of the Total Environment., 362, 205–241.

Small, H., Stevens, T.S., Bauman, W.C. (1975). Novel Ion Exchange Chromatographic Method Using Conductimetric Detection, Anal Chem., 47, 1801-1805.

Smith, R.E. (1988). Ion Chromatography Applications Boca Raton, USA: CRC Press. 451 pp.

Soares, M.I.M. (2000). Biological denitrification of groundwater. Water Air Soil Pollut., 123, 183–193.

Soares, M.I.M., Abeliovich, A. (1998). Wheat straw as substrate for water denitrification. Water Res., 32, 3790–3794.

Soares, M.I.M., Braester, C., Belkin, S., Abeliovich, A. (1991). Denitrification in laboratory sand columns – carbon regime, gas accumulation and hydraulic- properties. Water Res., 25, 325–332.

Stamatakis, M. (1989). A boron-bearing potassium feldspar in volcanic ash and tuffaceous rocks from Miocene lake deposits, Samos Island, Greece. American Mineralogist., 74, 230-235.

Stamatakos, D. (2010). Εφαρμογή του μοντέλου Mike She σε περιόδους κλιματικής αλλαγής. ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ.

Stamatis, G., Parpodis, K., Filintas, A., Zagana, E. (2011). Groundwater quality, nitrate pollution and irrigation environmental management in the Neogene sediments of an agricultural region in central Thessaly (Greece). Environ. Earth. Sci., 64, 1081–1105.

Stanescu, D., Chen-Charpentier, B. (2009). Random coefficient differential equation models for monod kinetics. Discrete and continuous dynamical systems Supplement 2009. AimScience., 719-728.

Starr, C., Gillham, R. (1993). Denitrification and organic-carbon availability in 2 aquifers. Ground Water., 31, 934-947.

Stavrianou, K. (2009). Αλλαγές στις χρήσεις Γης και στο φυσικό περιβάλον στη Σάμο κατά τον 20 αιώνα. Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης.

Strebel, O., Duynisved, W., Boettcher, J. (1989). Nitrate pollution of groundwater in Western Europe. Agric. Ecosyst. Environ., 26, 189-214.

Su, C., Puls, R.W. (2006). Removal of added nitrate in cotton burr compost, mulch compost, and peat: mechanisms and potential use for groundwater nitrate remediation. Chemosphere., 66, 91–98.

Su, C., Puls, R.W. (2007). Removal of added nitrate in the single, binary, and ternary systems of cotton burr compost, zerovalent iron, and sediment: Implications for groundwater nitrate remediation using permeable reactive barriers. Chemosphere, 67, 1653–1662

Talebnia, F., Karakashev, D., Angelidaki, I. (2010). Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. Bioresource Technology., 101, 4744–4753.

Tanaka, Y., Yatagai, A., Masujima, H., Waki, M., Yokoyama, H. (2007). Autotrophic denitrification and chemical phosphate removal of agro-industrial wastewater by filtration with granular medium, Bioresource Technology., 98, 787-798.

Tang, S., Yang, Q., Shang, H., Sun, T. (2010). Removal of nitrate by autosulfurotrophic denitrifying bacteria: optimization, kinetics and thermodynamics study. Fresenius Environmental Bulletin., 19(12), 3193-3198.

Throback, I.N., Enwall, K., Jarvis, A., Hallin, S. (2004). Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. Fems Microbiology Ecology., 49, 401-417.

Thullner, M., Zeyer, J., Kinzelbach, W. (2002). Influence of microbial growth on hydraulic properties of pore networks. Transport Porous Med., 49, 99–122.

Topuz, A., Dincer C.,Torun, M., Tontul, İ., Şahin-Nadeem, H., Haznedar, A.,Ozdemir, F. (2014). Physicochemical properties of Turkish green tea powder: effects of shooting period, shading, and clone. Turkish Journal of Agriculture and Forestry., 38, 233-241.

Torrentó, C., Urmeneta, J., Otero, N., Soler, A., Viñas, M., Cama, J. (2011). Enhanced denitrification in groundwater and sediments from a nitrate-contaminated aquifer after addition of pyrite. Chem. Geol., 287, 90–101.

Tran, T.H., Park, H.D., Jo, H.Y., Yun, S.T., Nguyen, T.M. (2011). Influence of different substrates in wetland soils on denitrification.Water Air Soil Pollut., 215, 549–560.

Trois, C., Pisano, G., Oxarango, L. (2010). Alternative solutions for the bio-denitrification of landfill leachates using pine bark and compost. Journal of Hazardous Materials., 178, 1100–1105.

Tunçsiper, B. (2009). Nitrogen removal in a combined vertical and horizontal subsurface-flow constructed wetland system. Desalination., 247, 466–475

Turner, J.C.R., Anderson, J.R., Boudart, M. (1981). Catalysis Science and Technology, vol. 1. Springer-Verlag, Berlin, Germany, 1981.

Tziritis, E., Kelepertzis, A., Stamatakis, M. (2008). Hydrogeochemical conditions and groundwater quality in the SE part of Samos Island, Greece. MINERAL WEALTH 149/2008.

Urata, K., Satoh, T. (1985). Mechanism of nitrite reduction to nitrous oxide in a photodenitrifier, Rhodopseudomonas sphaeroides forma sp. denitrificans. Biochim. Biophys. Acta., 841, 201-207.

U.S. Environmental Protection Agency. (1975). Process Design Manual for Nitrogen Control. Office of Technology Transfer, Washington, D.C. 311pp.

USGS. (http://water.usgs.gov/edu/dissolvedoxygen.html) accessed (22/8/2014)

USGS. (http://water.usgs.gov/ogw/karst/aquifers) (Accesses 15-10-2014)

Vandevivere, P., Baveye, P. (1992). Effect of bacterial extracellular polymers on the saturated hydraulic conductivity of sand columns. Appl. Environ. Microbiol., 58, 1690–1698.

van Haandel, A.C., Ekama, G.A., Marais, G. v. R. (1981). The activated sludge process - 3. Single sludge denitrification. Wat. Res., 15, 1135-1152.

Vanek, V. (1993). Transport of groundwater-borne phosphorus to lake Byson, south Sweden. Hydrobiologia., 251, 211–216.

van Loon, A.J.M., Botterweck, A.A.M, Goldbohma, R.A., Brants, H.A.M., van den Brandt, P.A. (1997). Nitrate intake and gastric cancer risk: results from the Netherlands cohort study. Cancer Lett., 114, 259-261.

Vasiliadou, I., Karanasios, K., Pavlou, S., Vayenas, D. (2009). Experimental and modelling study of drinkingwater hydrogenotrophic denitrification in packed-bed reactors. Journal of Hazardous Materials, 165, 812–824.

Vassilopoulos, A., Evelpidou, N., Tziritis, E., Boglis, A. (2008). Wetlands the example of Samos, University of Athens

Vavliankakis, E. (2002). Έρευνα παρακολούθησης των αλλαγών στις αποτεφρωμένες εκτάσεις της Σάμου και των απιπτώσεων αυτών στη διάβρωση των εδαφών, στην εκδήλωση πλημμυρών και στην υποβάθμιση των υδατικών πόρων, Ερευνητικό Πρόγραμμα Τομέα Φυσικής & Περιβαλλοντικής Γεωγραφίας ΑΠΘ, Φορέας Ανάθεσης: Νομαρχιακή Αυτοδιοίκηση Σάμου.

Venkateswari, R., Ganesh, R., Deenadayalan, M., Mahender, E., Ramachandran, B., Janakiraman, L. (2007). Transient Methemoglobinemia in an Infant, The Indian Journal of Pediatrics., 74(11), 1037-1038.

Vidal, S., Rocha, C., Galvão, H. (2002). A comparison of organic and inorganic carbon controls over biological denitrification in aquaria. Chemosphere., 48, 445-451.

Vignais, P.M., Henry, M., Sim, E., Kell D.B. (1981). The electron transport system and hydrogenase of Paracoccus denitrificans. Current Topics in Bioenergetics Academic Press, New York., 12, 115-196.

Volokita, M., Belkin, S., Abeliovich, A., Soares, M. (1996). Biological denitrification of drinking water using newspaper. Wat. Res., 30 (4), 965-971.

von Rohr, R., Hering, J.., Kohler, H., von Gunten, U. (2014). Column studies to assess the effects of climate variables on redox processes during riverbank filtration. Water Research., 61, 263-275.

Vought, L.B.M., Dahl, J., Pedersen, C.L., Lacoursiere, J.O. (1994). Nutrient retention in riparian ecotones. Ambio., 23, 342–348.

Voutsa, D.,Manoli, E., Samara, C., Sofoniou, M., Stratis, I. (2001). A study of surface water quality in Macedonia, Greece: speciation of nitrogen and phosphorus. Water, Air, and Soil Pollution., 129, 13–32.

Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. Science of the Total Environment., 380, 48–65.

Wallenstein, M. D., Myrold, D., Firestone, M., Voytek, M. (2006). Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications., 16, 2143-2152.

Walters, A.H. (1985). Clinical ecology in environmental health. Rev.Environ.Health., 5, 271-293.

Walker, R. (1990). Nitrate in drinking water and human health. Food Addit. Contam., 5, 717-768.

Ward, M.H., Mark, S.D., Cantor, K.P., Weisenburger, D.D., Correa-Villaseñor, A., Zahm, S.H. (1996). Drinking water nitrate and the risk of non-Hodgkin's lymphoma. Epidemiology., 7(5), 465–471

Warneke, S., Schipper, L., Bruesewitz, D., McDonald, I., Cameron, S. (2011). Rates, controls and potential adverse effects of nitrate removal in a denitrification bed. Ecological Engineering., 37, 511–522.

Wasewar, K.L., Atif, M., Prasad, B., Mishra, I.M. (2009). Batch adsorption of zinc on tea factory waste. Desalination., 244, 66–71.

Wasewar, K.L., Mohammad, A., Prasad, B., Mishra I.M. (2008). Adsorption of Zn using factory tea waste: kinetics, equilibrium and thermodynamics. Soil, Water, Air., 36(3), 320-329.

Weng, C., Lin, Y., Hong, D., Sharma, Y., Chen, S., Tripathi, K. (2014). Effective removal of copper ions from aqueous solution using base treated black tea waste. Ecological Engineering., 67, 127–133.

Weng, C.H., Lin, Y.T., Chen, Y.J., Sharma, Y.C. (2013). Spent green tea leaves for decolorization of raw textile industry wastewater. Color. Technol., 129, 298–304.

WHO. (1993). Guidelines for drinking water quality. Geneva, 1993.

Wicht, H. (1995). N2O-Emission durch den Betrieb biologischer Kläranlagen". Dissertation vom 01.12.1995 der Universität Hannover.

Willems, H.P.L., Rotelli, M.D., Berry, D.F., Smith, E.P., Reneau, R.B., Mostaghimi, S. (1997). Nitrate removal in riparian wetland soils: Effects of flow rate, temperature, nitrate concentration and soil depth. Water Research., 31, 841-849.

Wu, W., Yang, F., Yang, L. (2012). Biological denitrification with a novel biodegradable polymer as carbon source and biofilm carrier. Bioresource Technology., 118, 136–140.

Xing, X., Gao, B., Zhong, Q., Yue, Q., Li, Q. (2011). Sorption of nitrate onto amine-crosslinked wheat straw: characteristics, column sorption and desorption properties. Journal of Hazardous Materials., 186, 206–211.

Xu, X., Gao, B., Tan, X., Zhang, X., Yue, Q., Wang, Y., Li, Q. (2013). Nitrate adsorption by stratified wheat straw resin in lab-scale columns. Chemical Engineering Journal., 226, 1–6.

Yamashita, T., Yokoyama, H., Kanafusa, S., Ogino, A., Ishida, M., Osada, T., Tanaka, Y. (2011). Nitrate removal activity of a biofilm attached to a perlite carrier under continuous aeration conditions, Biosci. Biotechnol. Biochem., 75(3), 578-581.

Yang, Z., Zheng, S., Chen, J., Sun, M. (2008). Purification of nitrate-rich agricultural runoff by a hydroponic system. Bioresource Technology., 99, 8049–8053.

Yavuz, F., Birinci, A. Peker, K., Atsan T. (2005). Econometric Modeling of Turkey's Hazelnut Sector: Implications on Recent Policies. Turkish J. Agric. Forestry., 29, 1-7.

Yoshida, M., Ishii, S., Otsuka, S., Senoo, K. (2009). Temporal shifts in diversity and quantity of nirS and nirK in a rice paddy field soil. Soil Biology & Biochemistry., 41, 2044-2051.

Zhao, Y.Q., Zhao, X.H., Babatunde, O. (2009). Use of dewatered alum sludge as main substrate in treatment reed bed receiving agricultural wastewater: long-term trial. Bioresource Technology., 100, 644–648.

Zhong, X., Wu, Y., Xu, Z. (2013). Bioclogging in porous media under discontinuous flow condition. Water Air Soil Pollut., 224, 1543-1550.

Zhou, W., Sun, Y., Wu, B., Zhang, Y., Huang, M., Miyanaga, T., Zhang, Z. (2011). Autotrophic denitrification for nitrate and nitrite removal using sulfur-limestone. Journal of Environmental Sciences., 23(11), 1761–1769.

Zhao, Y., Feng, C., Wang, Q., Yang, Y., Zhang, Z., Sugiura, N. (2011). Nitrate removal from groundwater by cooperating heterotrophic with autotrophic denitrification in a biofilm-electrode reactor. Journal of Hazardous Materials, 192, 1033– 1039.

Zumft, W.G. (1997). Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews., 61, 533-541.

Zumft, W.G., Vega J.M. (1979). Reduction of nitrite to nitrous oxide by a cytoplasmic membrane fraction from the marine denitrifier Pseudomonas perfectomarinus. Biochim. Biophys. Acta., (54)8, 484-499.

Appendix I

Methods

I.1 pH Calibration

The pH calibration became with the same way in all the experiments. The pH and conductivity measured using Mettler Toledo-Seven Multi meter.

To obtain a pH value, the probe is dipped in a buffer solution of pH=4, a buffer solution of pH=7 and a buffer solution pH=10 to ensure the probe is correctly calibrated. Once the probe is calibrated it is dipped into the aqueous sample, allowed to equilibrate and the pH value obtained is recorded.

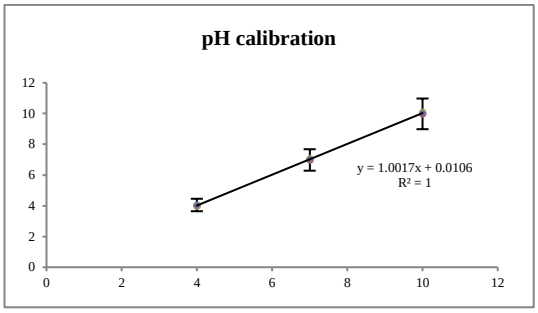


Figure I.1: pH calibration curve

The calibration curve is the curve that is visible in Figure (I.1) below and there is coefficient of determination R^2=100%. This shows that the instrument that used was very well calibrated and the error from the calibration was minor according to the results that received. Additionally the measurements were reliable and acceptable.

I.2 Oxidation reduction potential calibration

The measurement of ORP became with Mettler Toledo-Seven Multi meter used. To obtain an ORP value, the probe is dipped in a buffer solution of ORP 240 mV and a buffer solution ORP 470 mV to ensure the probe is correctly calibrated.

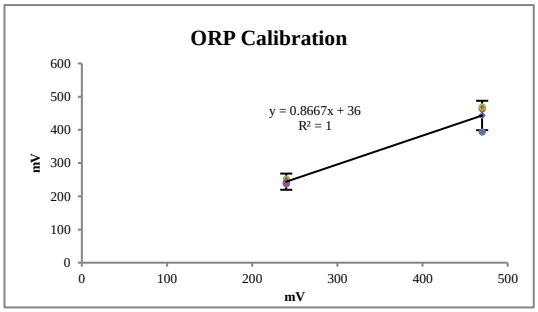


Figure I.2: Oxidation Reduction Potential calibration curve

The coefficient of determination for Redox potential was R^2=100%. The problem of redox calibration was that there are only two buffer solutions that used to calibrate the instrument. With that point the measurements were accurate but not as accurate as the measurements of other characteristics.

I.3 Conductivity calibration

The conductivity calibration became with the same way in all the experiments. The pH and conductivity measured using Mettler Toledo-Seven Multi meter.

The buffer solutions are 88 μS/cm, 1413 μS/cm and 12880 μS/cm. For every buffer solution triplicate results were received to calibrate the instrument.

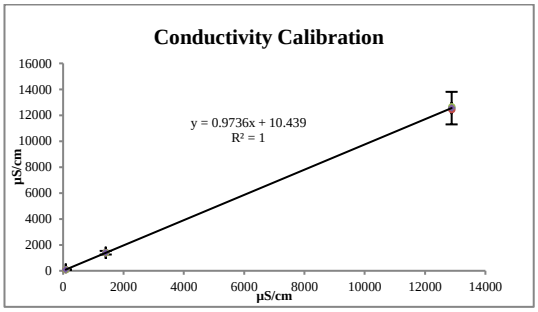


Figure I.3: Conductivity calibration curve

The calibration curve is the curve that is visible in Figure (I.3) below and there is coefficient of determination R^2=100%. That shows that the instrument was calibrated very well and the prices that received from the instrument were reliable.

I.4 Spectrophotometer

At the first experiment the methods that used to analysed the anions and cations was the method with microplates and spectrophotometer. The methods were according to ASTM. The instrument that was used to analyse was a spectrophotometer from BioTek™ Epoch™ Microplate Spectrophotometer as it is visible at Figure I.4. With Epoch spectrophotometer nitrite, ammonium and phosphate were analysed.

Figure I.4: BioTek™ Epoch™ Microplate Spectrophotometer

The analytical method for each of the anions and cations are the following:

I.4.1 Nitrite

The method that is used in to analysed according to Standard Methods. The method was 4500 B NO_2 Nitrogen (Nitrite) – Colorimetric method.

I.4.1.1 Solutions

The solutions that are used to create the working solutions were the following. For the stock solution preparation dry and dissolved $NaNO_2$ used. In 1000 ml water 0.1232gr $NaNO_2$ were added. For every 25 mg/l of the solution 1 ml of chloroform was added. The stock solution can be stored up to one month in refrigerator.

The second solution that used was NED solution. To create the NED solution 0.1gr of NED added in 100 ml of deionized water.

NED solution N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride). To 800 mL water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in refrigerator.

The third solution that used was sulfanilic acid. The preparation for the sulfanilic acid was the following. In 250 ml of water 47.5 ml of concentrated HCl added. 1gr of the solution added in 100 ml of the solution that prepared before (2M HCl).

The calibration of nitrite became in the microplates. For every standard triplicate of results received. The standard solution that analysed has range between 0-0.25 mg/l with specified solutions for 0 mg/l, 0.05 mg/l, 0.10mg/l, 0.15mg/l, 0.20mg/l and 0.25 mg/l.

I.4.1.2 Methodology for the samples

Every microplate has 96 wells and all of them used for the analysis of the samples and the calibration of the instrument. Firstly 250 μl of standard or sample added to the well. Then 25 μl of sulfanific acid solution added and the microplate was shaken for 20 minutes. After that addition of 25 μl of NED solution was made and the microplate was shaken for more than 1 hour. Finally, after that process the microplate was analysed in 535nm at BioTek™ Epoch™ Microplate Spectrophotometer

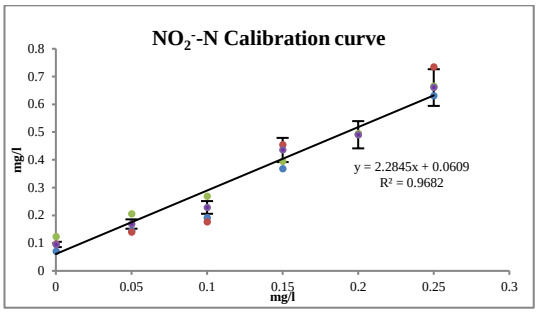


Figure I.5: Nitrite calibration Epoch spectrophotometer

The calibration curve is the curve that is visible in Figure (I.5) below and there is coefficient of determination R^2=96.82%. The calibration curve was acceptable with the calibration curve that received and the results of the measurement were reliable. At all the measurement were triplicate of the results to accurate as much as possible all the results. The only disadvantage of that method was the limitation of the measurement between 0 and 0.25 mg/l that force many times to dilute the samples and the increase the possibility of the errors.

That method used only at the first two experiments. The first one only in columns with sand and straw used as substrate materials and the second one with columns again and substrate materials sand, mulch and perlite.

I.4.2 Ammonium

The method that is used in to analysed according to Standard Methods. The method was Standard Method 4500 – NH_3 F Ammonia phenate method.

I.4.2.1 Solutions

For the preparation of the stock solution 0.3821gr of dry and dissolve NH_4Cl added in 1000ml of water. With that solution an initial solution with 100 mg/l NH_4^+-N was created and remained in the refrigerator till the time of use.

The second solution for the analysis was EDTA solution. To prepare the EDTA solution 6 gr of Na_2EDTA added in 100 ml of deionized water with pH=7.

The next solution that used for the analysis was phenol-nitroprusside solution. To prepare this solution 7 gr of phenol and 34 mg of sodium nitroprusside added in 100 ml of water. This solution stored in dark environment in the fridge till the time of usage.

The last solution that used was Buffered hypochlorite. For the preparation of the solution 1.48 gr of NaOH and 4.98 gr of Na_2HPO_4 and 20 ml of 5% NaOCl added to 100 ml of water creating a solution with pH between 11.4 and 12.2.

I.4.2.2 Methodology for the samples

Every microplate has 96 wells and all of them used for the analysis of the samples and the calibration of the instrument. Firstly 60 µl of sample or standard added to each microplate well. Then 15 µl of EDTA solution added and the samples stand for 5 minutes. Following 25 µl phenol nitroprusside added in the microplates wells and stand for 1 minute. After that 50 µl hypochlorite added and the microplates stand for 1 minute. Finally, 160 µl of deionized water was added in the microplate wells. The microplates shake for an hour in stable temperature of 37 ^{o}C. The microplates after one hour remain for 10 minutes to cool down and then added to the spectrophotometer and analysed in 636nm.

For the calibration 2.5 ml stock diluted in 100 ml matrix to give 2.5 mg/l working solution. The calibration of ammonium became in the microplates. For every standard triplicate of results received. The standard solution that analysed has range between 0-2.5 mg/l with specified solutions for 0 mg/l, 0.5 mg/l, 1.0mg/l, 1.5mg/l, 2.0mg/l and 2.5 mg/l.

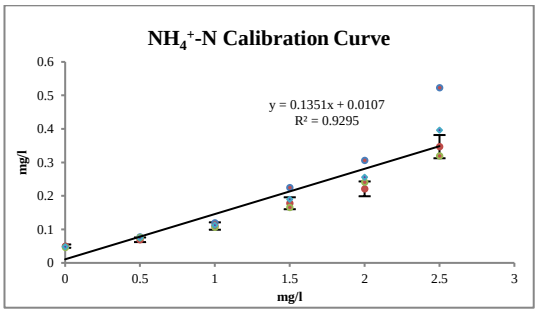


Figure I.6: Ammonium calibration Epoch spectrophotometer

The calibration curve is the curve that is visible in Figure (I.6) below and there is coefficient of determination R^2=92.95%. The determination coefficient is lower than the other measurement with microplates but again accurate more than 92%. Again with that percentage the measurements are reliable. As it is mention in the previous section the only problem with that method was the limitation in the levels of ammonium that can be measured. The dilution that became in the solutions was an issue for the accuracy of the results. The method used in the first two experiments.

I.4.3 Nitrate

The method that is used in to analysed according to Standard Methods. The method was 4500 B NO_3 Nitrogen (Nitrate) – Ultraviolet spectrophotometric screening method.

I.4.3.1 Solutions

The stock solution for the analysis of nitrates was created by adding 0.7218 gr dry and dissolved KNO_3 in 1000 ml of water to create a stock solution with 100mg/l NO_3-N.

The second solution that used was NED solution. To create the NED solution 0.1gr of NED added in 100 ml of deionized water.

The third solution that used was sulfanilic acid. The preparation for the sulfanilic acid was the following. In 250 ml of water 47.5 ml of concentrated HCl added. 1gr of the solution added in 100 ml of the solution that prepared before (2M HCl).

The next solution was sodium hydroxide solution. To create that solution 40 gr of sodium hydroxide added in 1 litre of water.

The next solution was copper and zinc catalyst. The preparation of the solution was the following. 35.4 mg $CuSO_4*5H_2O$ and 900 mg $ZnSO_4*7H_2O$ added in 1l water.

The last solution was the hydrazine sulphate solution. To prepare that solution 1.71 gr of hydrazine sulphate added in 1l of water.

The last three solutions used to create a catalyst mix. The preparation of the catalyst mix was made by adding 1 part of the above three solutions.

I.4.3.2 Methodology for the samples

Every microplate has 96 wells and all of them used for the analysis of the samples and the calibration of the instrument. Firstly 140 µl of standard or sample added to each microplate well. Then 60 µl of catalyst mix added and the microplates were shaken for 45 minutes. After that 75 µl sulfanilic acid added and each microplate was shaken for 20 minutes. Finally, 20 µl of NEDD solution added and each microplate was shaken for two hours. Finally, the microplate analysed in the BioTek™ Epoch™ Microplate Spectrophotometer with wavelength of 535 nm.

For the calibration 1ml of stock solution was added in 100 ml of water. The calibration of nitrate became in the microplates. For every standard triplicate of results received. The standard solution that analysed has range between 0-1mg/l with specified solutions for 0 mg/l, mg/l, 0.25 mg/l, 0.5 mg/l, 0.75 mg/l and 1.0 mg/l.

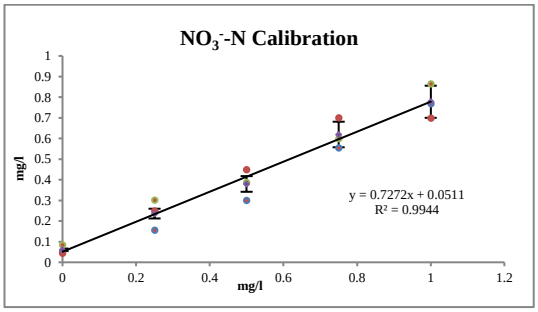


Figure I.7: Nitrate calibration Epoch spectrophotometer

The calibration curve is the curve that is visible in Figure (I.7) below and there is coefficient of determination R^2=99.44%. The accuracy of the method was really high. The results were very accurate but the problem was the range of the results that received in microplate analysis. For

that reason, in the time of the dilution was more than 4 the spectrophotometric method with Thermo Scientific UV-Vis Helios Zeta Spectrophotometer used to measure higher levels of nitrates.

The method used at the first experiment only due to the problem that exist with the dilution samples.

I.4.4 Orthophosphate

The method that is used in to analysed according to Standard Methods. The method was Standard Method 4500 PE – Ascorbic Acid Method.

I.4.4.1 Solutions

The preparation of stock solution is the following. 1.099 gr of KH_2PO_4 added in 250 ml of water. In that solution added 1.25 ml of concentrated HCl acid and a drop of toluene to create a stock solution with 1000 mg/l PO_4-P.

The second solution was sulphuric acid. To prepare sulphuric acid solution 8 ml of sulphuric acid added in 100 ml of water.

The next solution was ammonium molybdate solution 1.2%. To prepare that solution 12 gr ammonium molybdate and 0.3 gr antimony potassium tartartae added in 600 ml water. Then 150 ml of concentrated H_2SO_4 added and till 1l water was added. This solution remains in a cool place till the time that was used. Before the use of that solution 12.5 ml of that solution diluted in 100 ml of water.

The last solution that used was ascorbic acid solution. To prepare that solution 1.5 gr of ascorbic acid added in 100 ml of water. That solution prepared every single day of the analysis.

I.4.4.2 Methodology for the samples

Every microplate has 96 wells and all of them used for the analysis of the samples and the calibration of the instrument. Firstly 50 μl of sample or standard added in each of the microplate wells. Then 10 μl of sulphuric acid added to the microplates and it stood for 5 minutes. After that 200 μl of ammonium molybdate solution was added in the microplate wells. Finally, 50 μl of ascorbic acid added in the wells and the microplates were shaken for 30 minutes. Then the microplates analysed in spectrophotometer with wavelength 880nm.

For the calibration 1 ml stock solution diluted in 100 ml of water to received concentration 10 mg l/1 PO_4-P. The calibration of phosphate became in the microplates. For every standard triplicate of results received. The standard solution that analysed has range between 0-7 mg/l

with specified solutions for 0 mg/l, 1.0 mg/l, 2.0mg/l, 3.0mg/l, 4.0mg/l, 5.0 mg/l, 6.0 mg/l and 7.0 mg/l.

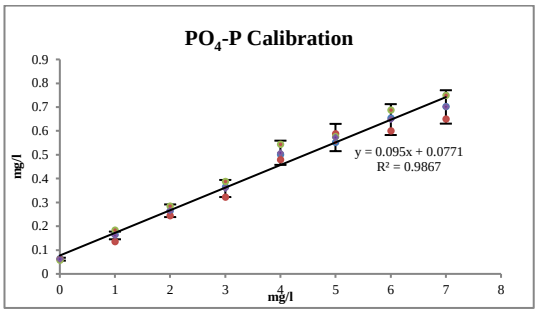


Figure I.8: Phosphate calibration Epoch spectrophotometer

The calibration curve is the curve that is visible in Figure (3.8) below and there is coefficient of determination R^2=98.67%. The accuracy of the calibration is high and the results that received are very accurate without any concerning point about the measurements. The limitation point of the method was the range of the results.

I.5 IC Calibration results

The calibration of anions and cations (NO_2-N, NO_3-N, NH_4-N and PO_4-P) were analyses with the same method as the first experiment with sand and straw.

Except from the calibration with the colorimetric methods there was the IC calibration for anions and cations that finally used as more accurate method for analysis. With IC there was calibration for NO_2, NO_3, NH_4, PO_4, SO_4, Cl, K, Li, Fl, Mg, Mn, Ca, Br and Na. The concentrations that used for calibration were 0.1 mg/l, 0.5 mg/l, 1 mg/l, 5 mg/l and 10 mg/l.

Below in Figure I.9 and I.10 there are working solution chromatograms for anions and cations.

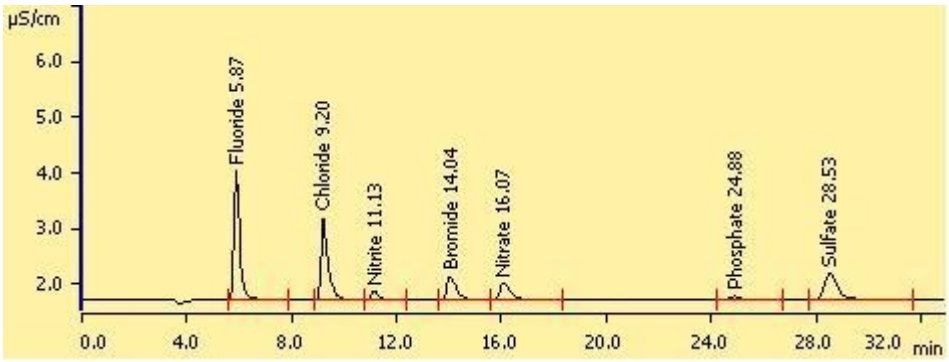


Figure I.9: 5mg/L anion working solution chromatogram

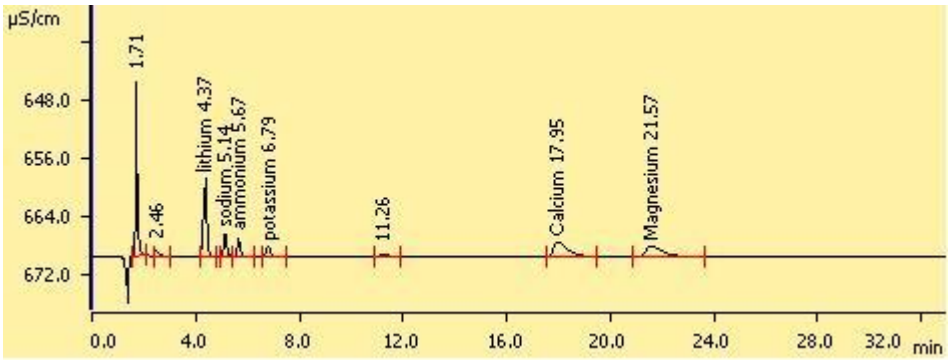


Figure I.10: 5mg/L cation working solution chromatogram

I.5.1 Nitrogen compounds and phosphate calibration

In the following section there is the calibration of IC for nitrite, nitrate, and ammonium that are the main measurements that I investigate.

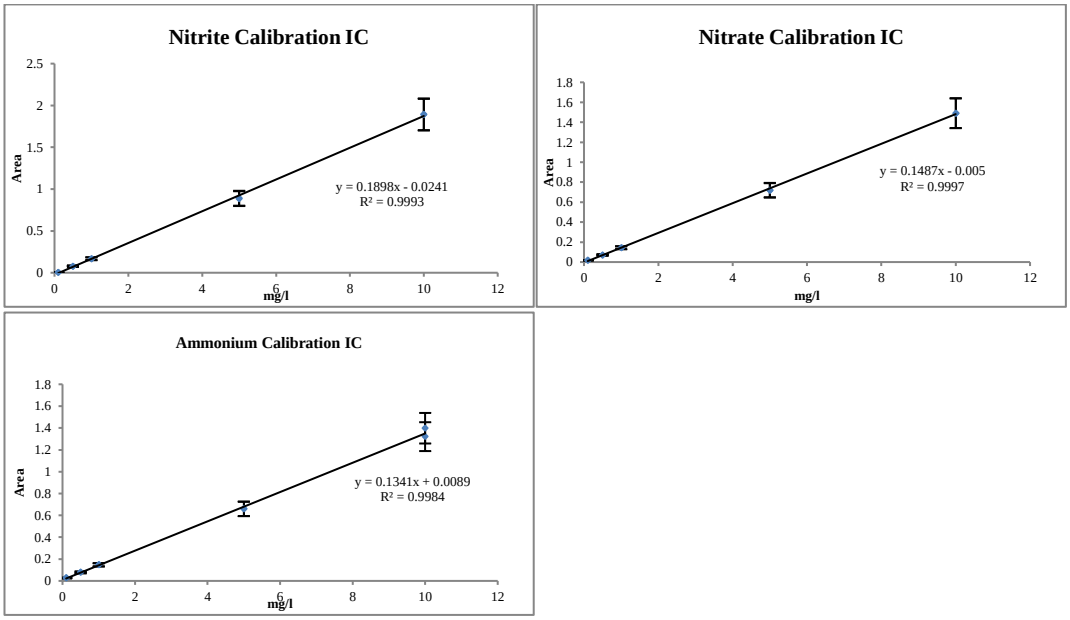


Figure I.11: Nitrite, nitrate and ammonium calibration curve IC

In contrast to colorimetric methods the coefficient of determination R^2 at all cases is more than 99% and the measurements with IC are more accurate. Additionally to this the results that can be received have wide range so no dilution is needed to receive the results. The measurements were very accurate and the results were very reliable.

The IC chromatography was used at all the experiments except from the initial one (columns with sand and straw substrate material).

Additionally to this with the same sample with have measurements for more anions and cations so the range of results is bigger. The anion that is more useful and I focused on my research was phosphate and the calibration curve is visible below.

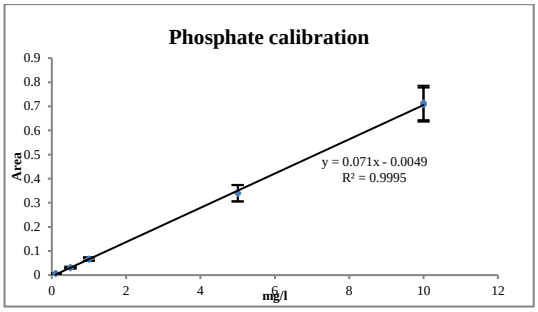


Figure I.12: Phosphate calibration curve IC

The coefficient of determination for phosphate was R^2=99.9%, following the accurate levels of nitrogen compounds.

I.6 TOC Calibration

Samples were analysed using a liquid TOC analyser Teledyne Tekmar Dohrmann Series Apollo 9000.

Standard solutions vials with concentration between 1-400 mg/L were analysed to receive the calibration curve. All samples were ran in triplicate (n=3) and the average peak area recorded and used to calculated the concentration of TOC present in the sample. The standard solution that analysed has range between 1-400 mg/l with specified solutions for 1mg/l, mg/l, 2 mg/l, 5 mg/l, 10 mg/l, 20 mg/l, 50 mg/l, 100 mg/l, 200 mg/l and 400 mg/l.

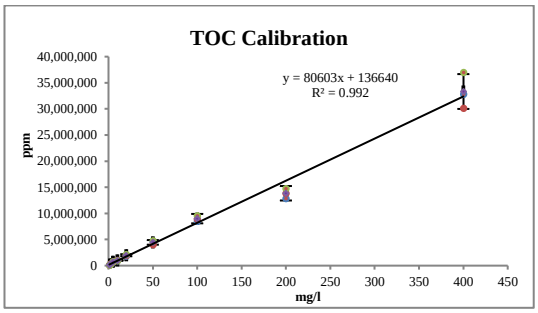


Figure I.13: TOC calibration curve

The calibration curve is the curve that is visible in the Figure (I.13) below and there is coefficient of determination R^2=99.2%. The accuracy levels are really high and the measurement really precise. The levels of TOC that measured at all the experiment were between 0 and 600ppm showing that only one dilution probably used in some samples.

The TOC levels measured in the second experiment with sand, mulch and perlite substrate materials, in batch experiments with tea waste materials and hazelnut husk wastes and finally in the last experiment in columns with the same substrate materials as the batch tests.

Appendix II

COLUMNS EXPERIMENT WITH SAND AND WHEAT STRAW AS SUBSTRATE MATERIALS

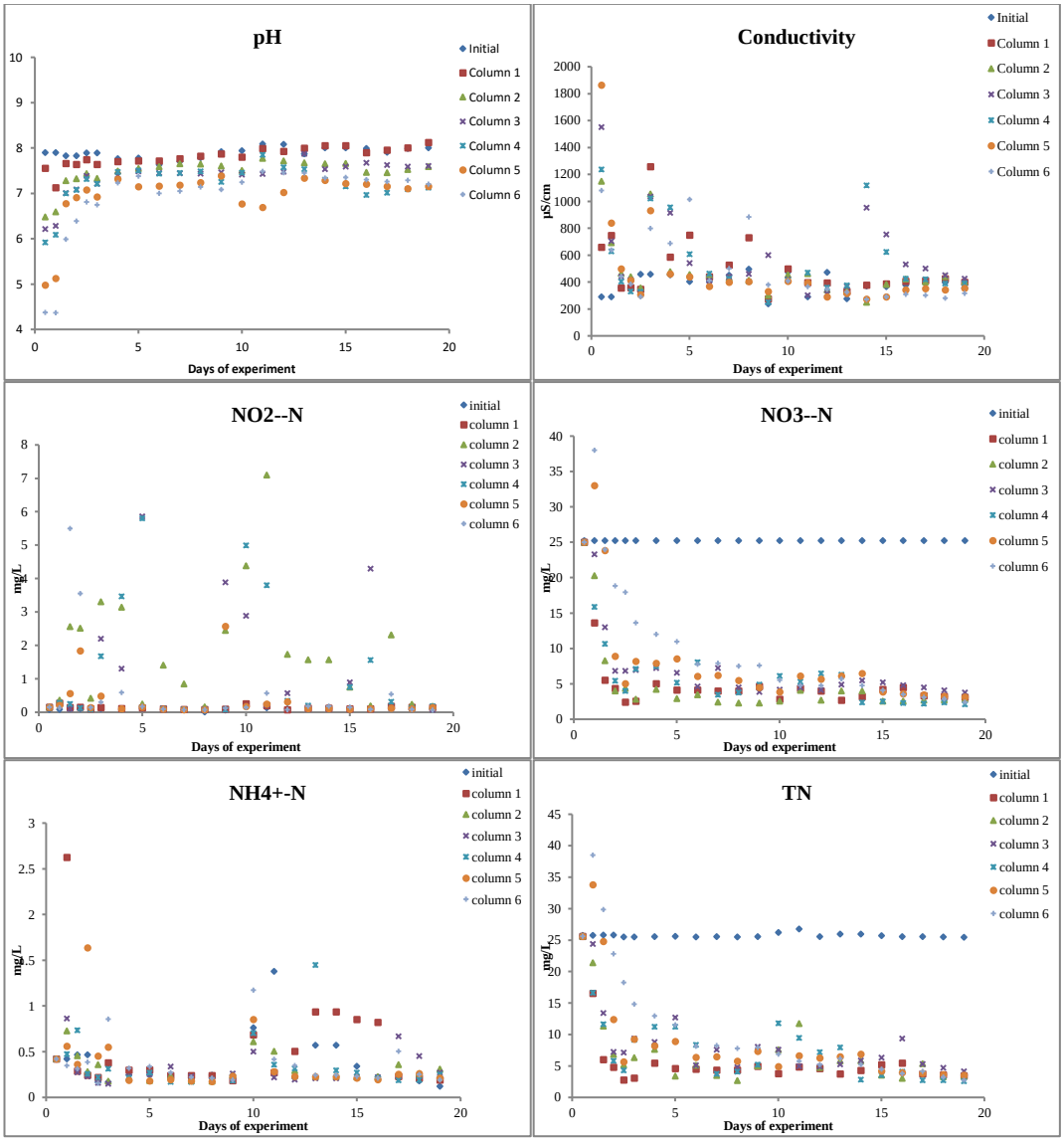


Figure II.1: Experiment 1 details

Table II.1: pH levels experiment 1

	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	7.90	7.56	6.48	6.21	5.92	4.97	4.37
	7.90	7.13	6.59	6.28	6.08	5.12	4.36
Day 2	7.83	7.66	7.28	7.01	7.00	6.77	5.99
	7.83	7.64	7.32	7.08	7.07	6.91	6.39
Day 3	7.89	7.74	7.43	7.39	7.31	7.08	6.81
	7.89	7.64	7.33	7.21	7.21	6.92	6.74
Day 4	7.77	7.70	7.49	7.40	7.47	7.31	7.23
Day 5	7.78	7.71	7.55	7.49	7.50	7.15	7.38
Day 6	7.63	7.71	7.59	7.45	7.43	7.16	7.00
Day 7	7.70	7.77	7.65	7.44	7.45	7.18	7.05
Day 8	7.78	7.82	7.64	7.43	7.48	7.24	7.14
Day 9	7.92	7.87	7.61	7.46	7.25	7.38	7.08
Day 10	7.94	7.80	7.51	7.41	7.45	6.77	7.25
Day 11	8.09	7.99	7.77	7.43	7.85	6.69	7.48
Day 12	8.08	7.93	7.71	7.48	7.57	7.02	7.46
Day 13	7.87	8.00	7.67	7.87	7.54	7.33	7.45
Day 14	8.00	8.05	7.65	7.53	7.30	7.28	7.34
Day 15	8.01	8.05	7.66	7.59	7.16	7.22	7.35
Day 16	7.99	7.90	7.46	7.67	6.97	7.20	7.30
Day 17	7.90	7.95	7.45	7.63	7.01	7.16	7.26
Day 18	7.99	8.01	7.52	7.59	7.09	7.10	7.29
Day 19	8.00	8.12	7.59	7.60	7.12	7.16	7.20

Table II.2: Conductivity levels experiment 1

µS/cm	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	290	659	1148	1551	1235	1862	1080
	290	746	692	704	627	838	638
Day 2	358	355	461	439	403	497	440
	358	357	437	414	329	413	384
Day 3	458	346	355	320	328	305	288
	458	1257	1054	1035	1020	930	799
Day 4	453	585	475	914	953	459	687
Day 5	403	749	455	541	607	438	1014
Day 6	402	438	449	409	461	368	410
Day 7	450	525	430	439	425	399	500
Day 8	495	729	412	459	403	403	884
Day 9	236	276	300	600	248	330	379
Day 10	474	498	453	419	411	405	407
Day 11	289	395	463	301	470	394	364
Day 12	471	394	340	313	357	289	345
Day 13	274	330	334	368	374	314	325
Day 14	369	377	250	951	1117	273	269
Day 15	365	385	382	752	622	290	289
Day 16	377	405	421	531	423	340	308
Day 17	389	409	401	500	420	350	302
Day 18	396	425	440	450	389	340	280
Day 19	355	399	400	425	388	352	315

Table II.3: Nitrite levels experiment 1

mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	0.09	0.15	0.15	0.15	0.15	0.15	0.15
	0.09	0.28	0.37	0.21	0.29	0.21	0.13
Day 2	0.11	0.14	2.57	0.11	0.25	0.57	5.50
	0.11	0.16	2.52	0.13	0.13	1.84	3.56
Day 3	0.11	0.11	0.42	0.12	0.09	0.14	0.14
	0.11	0.14	3.31	2.20	1.68	0.49	0.30
Day 4	0.09	0.12	3.15	1.30	3.47	0.10	0.59
Day 5	0.11	0.14	0.25	5.86	5.80	0.16	0.18
Day 6	0.07	0.11	1.41	0.11	0.11	0.10	0.10
Day 7	0.08	0.09	0.85	0.09	0.09	0.08	0.08
Day 8	0.01	0.08	0.16	0.08	0.08	0.07	0.08
Day 9	0.07	0.09	2.45	3.89	0.08	2.57	0.13
Day 10	0.21	0.26	4.39	2.89	4.99	0.17	0.15
Day 11	0.13	0.20	7.10	0.21	3.80	0.25	0.58
Day 12	0.10	0.08	1.74	0.57	0.34	0.31	0.07
Day 13	0.15	0.13	1.57	0.12	0.20	0.11	0.19
Day 14	0.15	0.13	1.57	0.12	0.13	0.11	0.19
Day 15	0.12	0.11	0.75	0.90	0.75	0.10	0.15
Day 16	0.11	0.11	0.19	4.30	1.57	0.11	0.09
Day 17	0.13	0.18	2.32	0.11	0.31	0.13	0.54
Day 18	0.09	0.15	0.24	0.11	0.12	0.11	0.09
Day 19	0.10	0.14	0.18	0.08	0.19	0.14	0.07

Table II.4: Nitrate levels experiment 1

mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	25.23	25.10	25.22	25.31	25.11	25.14	25.19
	25.33	13.65	20.30	23.30	15.87	33.08	38.01
Day 2	25.13	5.56	8.30	12.99	10.65	23.83	24.02
	25.29	4.38	3.99	6.86	5.43	8.90	18.87
Day 3	25.21	2.42	4.38	6.86	3.99	5.03	17.95
	25.31	2.55	2.82	6.99	7.12	8.20	13.65
Day 4	25.12	5.03	4.25	7.25	7.51	7.90	12.08
Day 5	25.18	4.12	2.95	6.58	5.16	8.56	11.01
Day 6	25.09	4.12	3.47	4.64	8.04	6.08	7.77
Day 7	25.01	3.99	2.42	7.25	3.47	6.21	7.90
Day 8	25.15	4.01	2.30	4.50	3.80	5.50	7.50
Day 9	25.27	4.64	2.29	3.86	4.92	4.51	7.60
Day 10	25.13	2.82	2.55	4.20	6.10	3.86	5.56
Day 11	25.26	4.38	4.10	4.50	5.31	6.10	4.77
Day 12	25.11	3.99	2.68	4.12	6.50	5.69	4.77
Day 13	25.17	2.68	3.99	4.90	6.31	6.10	5.70
Day 14	25.12	3.21	3.99	5.50	2.40	6.50	4.77
Day 15	25.22	4.25	2.55	5.20	2.50	3.86	4.10
Day 16	25.23	4.50	2.58	4.80	2.31	3.60	3.50
Day 17	25.01	3.34	2.68	4.50	2.22	3.47	3.10
Day 18	25.09	3.22	2.75	4.10	2.40	3.25	2.90
Day 19	25.16	3.11	2.85	3.80	2.11	3.16	2.45

Table II.5: Ammonium levels experiment 1

mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	0.42	0.42	0.42	0.42	0.42	0.42	0.42
	0.42	2.63	0.73	0.86	0.47	0.56	0.35
Day 2	0.47	0.29	0.46	0.27	0.73	0.36	0.31
	0.47	0.24	0.28	0.23	0.26	1.64	0.39
Day 3	0.17	0.22	0.36	0.16	0.22	0.45	0.16
	0.17	0.38	0.18	0.15	0.31	0.55	0.86
Day 4	0.24	0.30	0.21	0.23	0.21	0.19	0.32
Day 5	0.25	0.30	0.18	0.25	0.27	0.18	0.34
Day 6	0.20	0.24	0.23	0.34	0.17	0.19	0.27
Day 7	0.21	0.24	0.18	0.23	0.17	0.18	0.22
Day 8	0.24	0.24	0.17	0.19	0.19	0.17	0.21
Day 9	0.24	0.18	0.24	0.26	0.18	0.23	0.19
Day 10	0.76	0.68	0.61	0.50	0.70	0.85	1.17
Day 11	1.38	0.27	0.50	0.22	0.36	0.28	0.42
Day 12	0.21	0.50	0.29	0.19	0.32	0.23	0.35
Day 13	0.57	0.93	0.22	0.21	1.45	0.22	0.24
Day 14	0.57	0.93	0.22	0.21	0.30	0.22	0.24
Day 15	0.34	0.85	0.22	0.22	0.27	0.21	0.23
Day 16	0.22	0.82	0.22	0.22	0.21	0.19	0.21
Day 17	0.21	0.23	0.36	0.67	0.19	0.25	0.50
Day 18	0.18	0.21	0.26	0.45	0.19	0.26	0.25
Day 19	0.12	0.19	0.31	0.27	0.25	0.21	0.19

Table II.6: TN levels experiment 1

mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	25.74	25.87	25.75	25.87	25.46	25.54	25.71
	25.74	16.55	21.40	24.38	16.63	33.77	38.48
Day 2	25.80	5.98	11.32	13.38	11.62	24.75	29.81
	25.80	4.78	6.79	7.22	5.81	12.38	22.81
Day 3	25.50	2.75	5.16	7.14	4.29	5.63	18.25
	25.50	3.08	6.30	9.34	9.11	9.24	14.81
Day 4	25.56	5.45	7.61	8.78	11.19	8.19	12.91
Day 5	25.59	4.56	3.38	12.69	11.23	8.89	11.51
Day 6	25.50	4.47	5.11	5.09	8.31	6.37	8.14
Day 7	25.52	4.32	3.45	7.57	3.73	6.47	8.20
Day 8	25.48	4.33	2.64	4.77	4.07	5.75	7.79
Day 9	25.54	4.92	4.99	8.01	5.17	7.32	7.92
Day 10	26.19	3.76	7.55	7.59	11.79	4.88	6.87
Day 11	26.74	4.85	11.70	4.93	9.46	6.62	5.77
Day 12	25.54	4.57	4.71	4.89	7.16	6.23	5.19
Day 13	25.94	3.74	5.78	5.23	7.94	6.44	6.13
Day 14	25.94	4.27	5.78	5.83	2.82	6.84	5.20
Day 15	25.69	5.21	3.53	6.32	3.52	4.17	4.48
Day 16	25.56	5.43	2.99	9.32	4.08	3.90	3.80
Day 17	25.56	3.75	5.36	5.28	2.70	3.85	4.14
Day 18	25.50	3.58	3.25	4.66	2.71	3.62	3.24
Day 19	25.44	3.44	3.34	4.15	2.55	3.51	2.71

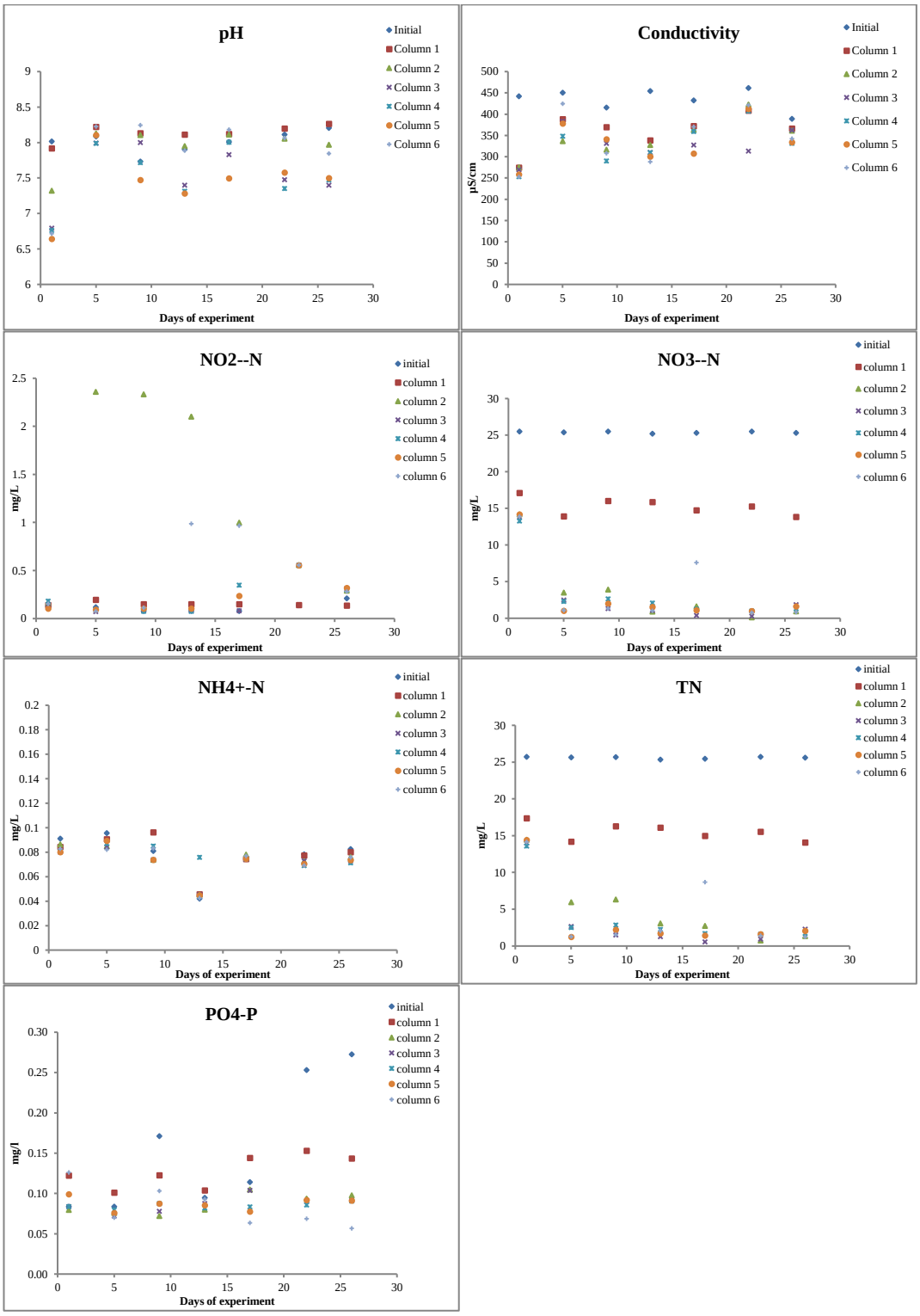


Figure II.2: Experiment 2 details

Table II.7: pH levels experiment 2

	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	8.01	7.92	7.32	6.79	6.76	6.64	6.71
Day 5	8.09	8.22	8.13	7.99	7.99	8.11	8.22
Day 9	7.73	8.13	8.11	8.00	7.71	7.47	8.24
Day 13	7.90	8.11	7.95	7.40	7.31	7.28	7.88
Day 17	8.01	8.12	8.11	7.83	8.00	7.49	8.18
Day 22	8.11	8.20	8.05	7.47	7.35	7.58	8.06
Day 26	8.21	8.26	7.97	7.40	7.47	7.50	7.84

Table II.8: Conductivity levels experiment 2

µS/cm	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	442	274	275	269	253	258	252
Day 5	450	388	336	383	348	378	424
Day 9	415	369	316	331	290	341	308
Day 13	454	338	327	310	309	300	288
Day 17	432	372	363	327	359	307	370
Day 22	461	408	422	313	407	412	421
Day 26	389	366	361	362	331	334	342

Table II.9: Nitrite levels experiment 2

mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	0.12	0.14	0.16	0.11	0.18	0.11	0.15
Day 5	0.12	0.20	2.36	0.07	0.09	0.09	0.08
Day 9	0.08	0.15	2.33	0.08	0.08	0.10	0.11
Day 13	0.08	0.15	2.10	0.08	0.08	0.10	0.99
Day 17	0.08	0.15	1.00	0.08	0.35	0.24	0.97
Day 22	0.14	0.14	0.56	0.56	0.56	0.56	0.56
Day 26	0.21	0.14	0.29	0.31	0.30	0.32	0.28

Table II.10: Nitrate levels experiment 2

mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	25.51	17.12	14.24	13.82	13.30	14.29	13.91
Day 5	25.40	13.91	3.50	2.50	2.30	1.03	1.10
Day 9	25.51	16.01	3.91	1.32	2.66	2.01	1.31
Day 13	25.20	15.89	0.90	1.12	2.08	1.57	1.02
Day 17	25.32	14.75	1.64	0.41	1.25	1.09	7.62
Day 22	25.50	15.29	0.12	0.29	0.98	0.98	0.75
Day 26	25.38	13.86	0.96	1.87	1.32	1.64	0.86

Table II.11: Ammonium levels experiment 2

mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	0.091	0.084	0.086	0.080	0.081	0.080	0.083
Day 5	0.096	0.090	0.085	0.084	0.087	0.089	0.082
Day 9	0.081	0.096	0.074	0.074	0.085	0.074	0.083
Day 13	0.042	0.046	0.045	0.045	0.076	0.045	0.042
Day 17	0.074	0.074	0.078	0.074	0.074	0.075	0.077
Day 22	0.078	0.077	0.073	0.073	0.069	0.070	0.069
Day 26	0.082	0.080	0.076	0.073	0.071	0.074	0.076

Table II.12: TN levels experiment 2

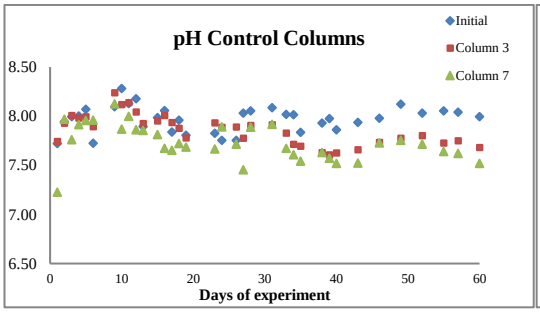
mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	25.72	17.34	14.45	13.99	13.56	14.39	14.14
Day 5	25.62	14.19	5.94	2.66	2.48	1.21	1.25
Day 9	25.66	16.26	6.32	1.48	2.82	2.19	1.51
Day 13	25.32	16.09	3.05	1.24	2.23	1.72	2.05
Day 17	25.45	14.97	2.72	0.56	1.68	1.40	8.66
Day 22	25.72	15.51	0.75	0.92	1.60	1.60	1.38
Day 26	25.59	14.08	1.33	2.25	1.69	2.03	1.21

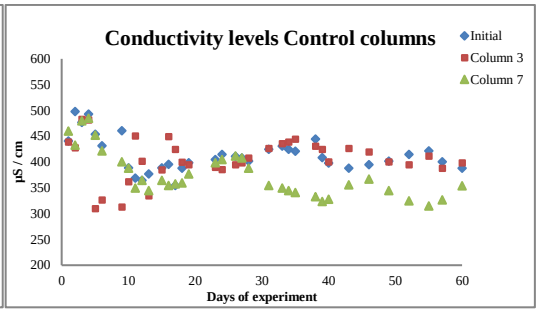
Table II.13: Phosphate levels experiment 2

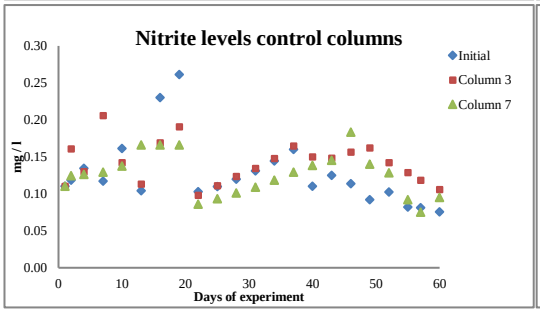
mg/l	Initial	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 1	0.084	0.122	0.080	0.084	0.084	0.099	0.126
Day 5	0.084	0.101	0.076	0.072	0.080	0.076	0.070
Day 9	0.171	0.123	0.072	0.078	0.088	0.088	0.103
Day 13	0.095	0.104	0.080	0.087	0.082	0.085	0.093
Day 17	0.114	0.144	0.105	0.104	0.083	0.078	0.063
Day 22	0.253	0.153	0.094	0.090	0.086	0.092	0.069
Day 26	0.273	0.143	0.098	0.091	0.090	0.091	0.057

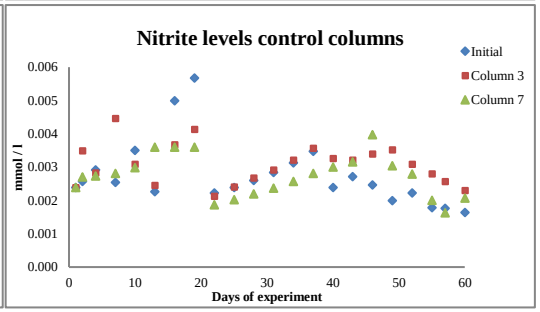
Appendix III

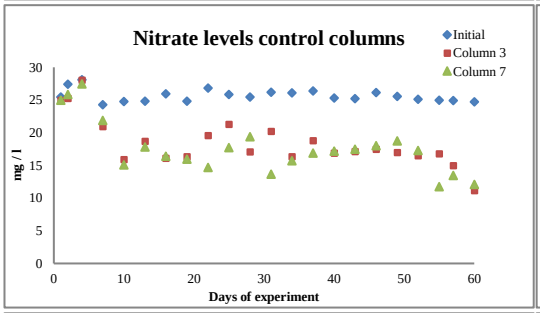
COLUMNS EXPERIMENT WITH SAND, MULCH AND PERLTE AS SUBSTRATE MATERIALS

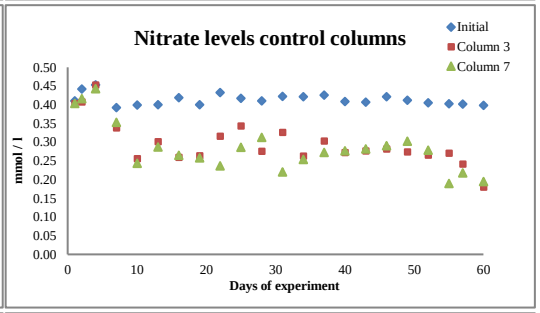


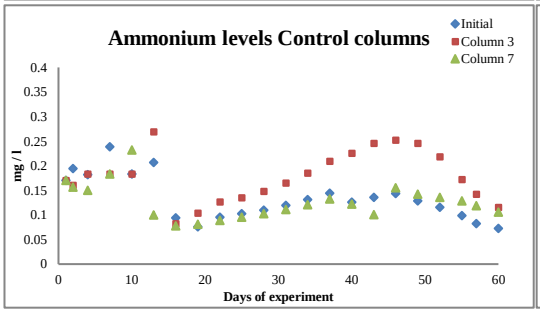


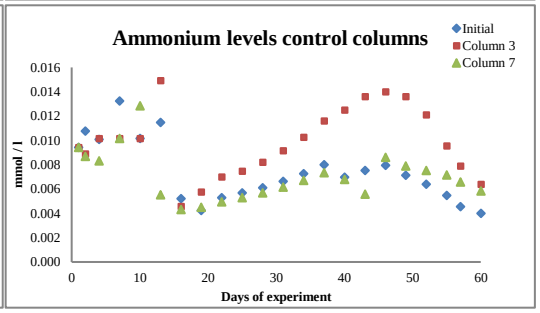












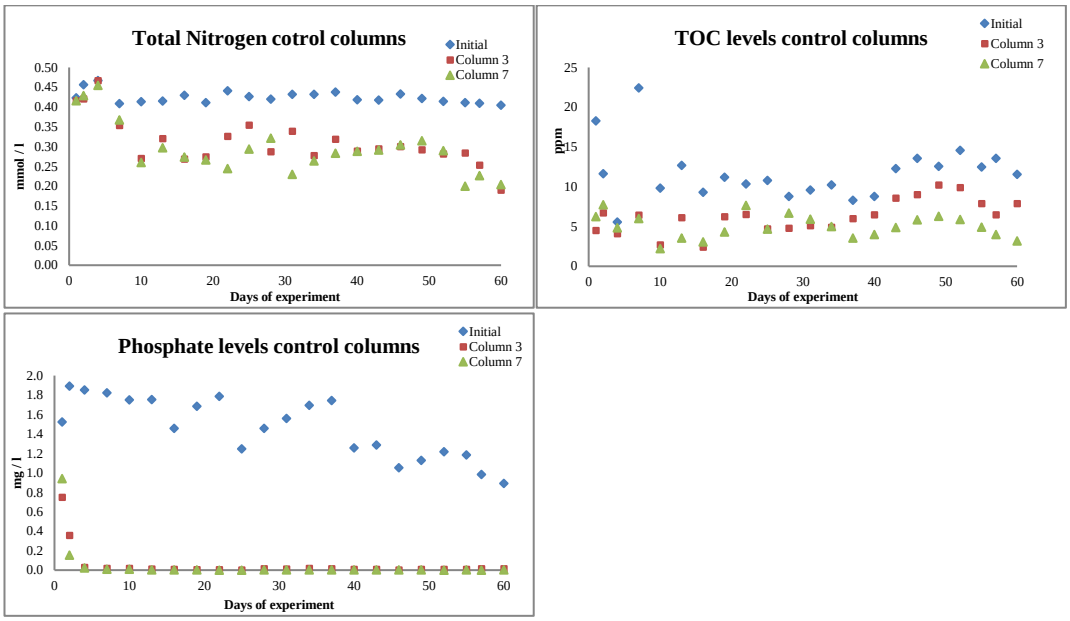


Figure III.1: Experiment Control Columns

Table III.1: pH and conductivity levels experiment Control Columns

pH	Initial	Column 3 (50% sand, 50% perlite)	Column 7 (100% perlite)	Conductivity	Initial	Column 3 (50% sand, 50% perlite)	Column 7 (100% perlite)
Day 1	7.72	7.75	7.23	**Day 1**	441	439	460
Day 2	7.94	7.93	7.97	**Day 2**	498	428	433
Day 3	8.00	8.01	7.76	**Day 3**	477	483	480
Day 4	8.00	7.98	7.92	**Day 4**	493	482	485
Day 5	8.07	8.00	7.96	**Day 5**	454	310	452
Day 6	7.73	7.90	7.96	**Day 6**	432	327	422
Day 9	8.10	8.24	8.13	**Day 9**	461	313	401
Day 10	8.28	8.12	7.87	**Day 10**	389	362	388
Day 11	8.13	8.14	8.00	**Day 11**	369	451	350
Day 12	8.18	8.05	7.86	**Day 12**	365	402	365
Day 13	7.90	7.93	7.86	**Day 13**	377	335	345
Day 15	7.99	7.95	7.81	**Day 15**	389	385	365
Day 16	8.06	8.01	7.67	**Day 16**	396	450	355
Day 17	7.84	7.94	7.66	**Day 17**	355	425	358
Day 18	7.96	7.88	7.72	**Day 18**	388	400	360
Day 19	7.81	7.78	7.69	**Day 19**	399	395	377
Day 23	7.83	7.94	7.67	**Day 23**	405	390	399
Day 24	7.76	7.89	7.89	**Day 24**	415	386	405
Day 26	7.76	7.89	7.72	**Day 26**	411	395	412
Day 27	8.03	7.78	7.45	**Day 27**	408	399	408
Day 28	8.06	7.91	7.89	**Day 28**	402	408	388
Day 31	8.09	7.92	7.92	**Day 31**	425	427	355
Day 33	8.02	7.83	7.68	**Day 33**	431	436	350
Day 34	8.02	7.72	7.61	**Day 34**	425	439	345
Day 35	7.84	7.70	7.54	**Day 35**	421	445	341
Day 38	7.93	7.63	7.63	**Day 38**	445	431	333
Day 39	7.98	7.61	7.57	**Day 39**	409	425	324
Day 40	7.86	7.63	7.52	**Day 40**	398	401	328
Day 43	7.94	7.66	7.52	**Day 43**	388	427	356
Day 46	7.98	7.74	7.73	**Day 46**	395	420	367
Day 49	8.13	7.78	7.76	**Day 49**	402	401	345
Day 52	8.03	7.80	7.72	**Day 52**	415	395	325
Day 55	8.06	7.73	7.64	**Day 55**	422	412	315
Day 57	8.04	7.75	7.62	**Day 57**	401	388	327
Day 60	8.00	7.69	7.52	**Day 60**	388	399	354

Table III.2: Nitrite and nitrate levels experiment Control Columns

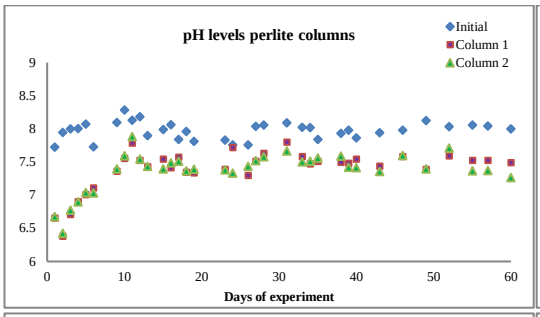
Nitrite	Initial		Column 3		Column 7		Nitrate	Initial		Column 3		Column 7	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l		mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
Day 1	0.110	0.002	0.110	0.002	0.110	0.002	**Day 1**	25.452	0.411	25.000	0.403	25.000	0.403
Day 2	0.118	0.003	0.161	0.003	0.124	0.003	**Day 2**	27.413	0.442	25.294	0.408	25.844	0.417
Day 4	0.134	0.003	0.130	0.003	0.126	0.003	**Day 4**	28.115	0.454	28.090	0.453	27.480	0.443
Day 7	0.117	0.003	0.206	0.004	0.129	0.003	**Day 7**	24.314	0.392	20.991	0.339	21.920	0.354
Day 10	0.161	0.003	0.142	0.003	0.137	0.003	**Day 10**	24.763	0.400	15.918	0.257	15.115	0.244
Day 13	0.104	0.002	0.113	0.002	0.166	0.004	**Day 13**	24.839	0.401	18.732	0.302	17.831	0.288
Day 16	0.230	0.005	0.169	0.004	0.166	0.004	**Day 16**	25.980	0.419	16.134	0.260	16.435	0.265
Day 19	0.261	0.006	0.191	0.004	0.166	0.004	**Day 19**	24.825	0.401	16.383	0.264	15.995	0.258
Day 22	0.103	0.002	0.098	0.002	0.086	0.002	**Day 22**	26.851	0.433	19.604	0.316	14.691	0.237
Day 25	0.110	0.002	0.111	0.002	0.093	0.002	**Day 25**	25.885	0.418	21.330	0.344	17.741	0.286
Day 28	0.120	0.003	0.123	0.003	0.101	0.002	**Day 28**	25.459	0.411	17.115	0.276	19.407	0.313
Day 31	0.131	0.003	0.134	0.003	0.109	0.002	**Day 31**	26.184	0.422	20.229	0.326	13.693	0.221
Day 34	0.144	0.003	0.148	0.003	0.118	0.003	**Day 34**	26.119	0.421	16.342	0.264	15.734	0.254
Day 37	0.160	0.003	0.165	0.004	0.129	0.003	**Day 37**	26.402	0.426	18.809	0.303	16.897	0.273
Day 40	0.110	0.002	0.150	0.003	0.139	0.003	**Day 40**	25.319	0.409	16.907	0.273	17.198	0.277
Day 43	0.125	0.003	0.148	0.003	0.145	0.003	**Day 43**	25.238	0.407	17.166	0.277	17.500	0.282
Day 46	0.114	0.002	0.156	0.003	0.183	0.004	**Day 46**	26.140	0.422	17.502	0.282	18.044	0.291
Day 49	0.092	0.002	0.162	0.004	0.140	0.003	**Day 49**	25.555	0.412	17.002	0.274	18.782	0.303
Day 52	0.102	0.002	0.142	0.003	0.129	0.003	**Day 52**	25.125	0.405	16.503	0.266	17.305	0.279
Day 55	0.082	0.002	0.129	0.003	0.092	0.002	**Day 55**	24.986	0.403	16.823	0.271	11.772	0.190
Day 57	0.081	0.002	0.118	0.003	0.075	0.002	**Day 57**	24.943	0.402	15.001	0.242	13.501	0.218
Day 60	0.076	0.002	0.106	0.002	0.095	0.002	**Day 60**	24.722	0.399	11.201	0.181	12.102	0.195

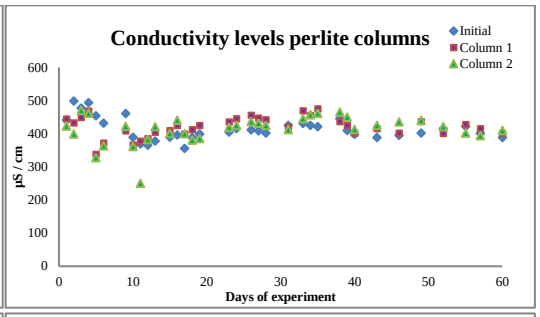
Table III.3: Ammonium and TN levels experiment Control Columns

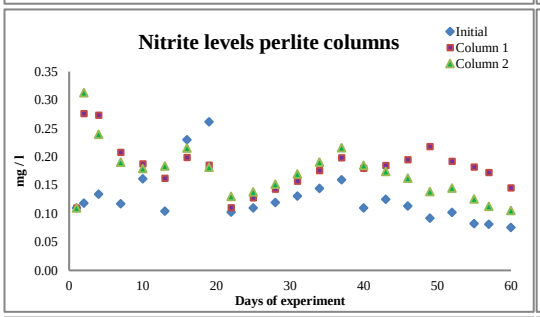
Ammonium	Initial		Column 3 (50% sand, 50% perlite)		Column 7 (100% perlite)		TN	Initial	Column 3	Column 7
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	**mmol/l**			
Day 1	0.170	0.009	0.170	0.009	0.170	0.009	**Day 1**	0.422	0.415	0.415
Day 2	0.194	0.011	0.161	0.009	0.157	0.009	**Day 2**	0.456	0.421	0.428
Day 4	0.182	0.010	0.183	0.010	0.150	0.008	**Day 4**	0.467	0.466	0.454
Day 7	0.239	0.013	0.183	0.010	0.183	0.010	**Day 7**	0.408	0.353	0.367
Day 10	0.183	0.010	0.183	0.010	0.232	0.013	**Day 10**	0.413	0.270	0.260
Day 13	0.207	0.011	0.269	0.015	0.100	0.006	**Day 13**	0.415	0.320	0.297
Day 16	0.094	0.005	0.082	0.005	0.078	0.004	**Day 16**	0.429	0.269	0.273
Day 19	0.076	0.004	0.104	0.006	0.081	0.005	**Day 19**	0.410	0.274	0.266
Day 22	0.096	0.005	0.126	0.007	0.089	0.005	**Day 22**	0.441	0.325	0.244
Day 25	0.103	0.006	0.135	0.007	0.095	0.005	**Day 25**	0.426	0.354	0.294
Day 28	0.110	0.006	0.148	0.008	0.103	0.006	**Day 28**	0.419	0.287	0.321
Day 31	0.120	0.007	0.165	0.009	0.111	0.006	**Day 31**	0.432	0.338	0.229
Day 34	0.131	0.007	0.185	0.010	0.121	0.007	**Day 34**	0.432	0.277	0.263
Day 37	0.144	0.008	0.209	0.012	0.132	0.007	**Day 37**	0.437	0.319	0.283
Day 40	0.126	0.007	0.225	0.012	0.122	0.007	**Day 40**	0.418	0.289	0.287
Day 43	0.136	0.008	0.246	0.014	0.101	0.006	**Day 43**	0.417	0.294	0.291
Day 46	0.143	0.008	0.252	0.014	0.155	0.009	**Day 46**	0.432	0.300	0.304
Day 49	0.129	0.007	0.246	0.014	0.142	0.008	**Day 49**	0.421	0.291	0.314
Day 52	0.116	0.006	0.219	0.012	0.136	0.008	**Day 52**	0.414	0.281	0.290
Day 55	0.099	0.005	0.172	0.010	0.129	0.007	**Day 55**	0.410	0.283	0.199
Day 57	0.082	0.005	0.142	0.008	0.119	0.007	**Day 57**	0.409	0.252	0.226
Day 60	0.072	0.004	0.116	0.006	0.106	0.006	**Day 60**	0.405	0.189	0.203

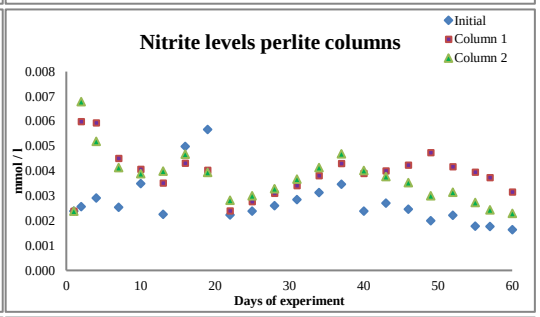
Table III.4: TOC and phosphate levels experiment Control Columns

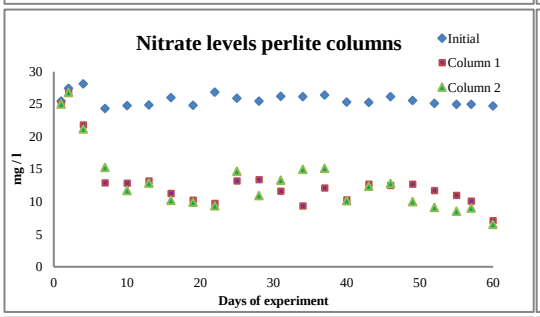
TOC	Initial	Column 3	Column 7	Phosphate	Initial	Column 3	Column 7
	ppm	ppm	ppm		mg/l	mg/l	mg/l
Day 1	18.246	4.481	6.235	**Day 1**	1.522	0.750	0.941
Day 2	11.603	6.703	7.731	**Day 2**	1.892	0.359	0.154
Day 4	5.556	4.107	4.830	**Day 4**	1.852	0.028	0.021
Day 7	22.428	6.412	5.989	**Day 7**	1.823	0.019	0.010
Day 10	9.795	2.702	2.196	**Day 10**	1.752	0.018	0.009
Day 13	12.655	6.121	3.518	**Day 13**	1.752	0.014	0.004
Day 16	9.277	2.405	3.047	**Day 16**	1.456	0.009	0.002
Day 19	11.187	6.221	4.294	**Day 19**	1.685	0.005	0.003
Day 22	10.320	6.509	7.627	**Day 22**	1.785	0.007	0.001
Day 25	10.776	4.705	4.673	**Day 25**	1.248	0.004	0.001
Day 28	8.752	4.763	6.656	**Day 28**	1.456	0.015	0.002
Day 31	9.562	5.112	5.891	**Day 31**	1.559	0.012	0.002
Day 34	10.232	4.895	4.989	**Day 34**	1.694	0.019	0.003
Day 37	8.266	5.993	3.545	**Day 37**	1.744	0.017	0.004
Day 40	8.774	6.455	3.963	**Day 40**	1.258	0.011	0.003
Day 43	12.253	8.566	4.856	**Day 43**	1.285	0.009	0.003
Day 46	13.555	8.990	5.830	**Day 46**	1.052	0.004	0.002
Day 49	12.556	10.220	6.255	**Day 49**	1.129	0.008	0.003
Day 52	14.562	9.885	5.880	**Day 52**	1.218	0.009	0.001
Day 55	12.465	7.895	4.895	**Day 55**	1.186	0.011	0.002
Day 57	13.547	6.452	3.987	**Day 57**	0.985	0.016	0.001
Day 60	11.552	7.897	3.152	**Day 60**	0.891	0.017	0.002

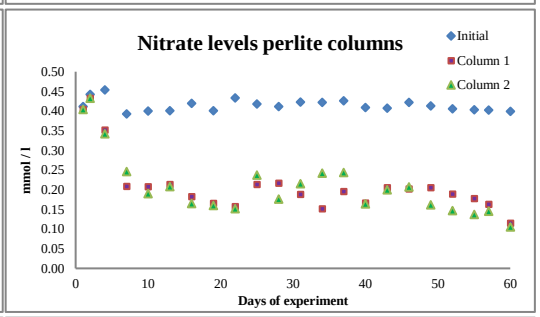

pH levels perlite columns
Initial
Column 1
Column 2
Days of experiment

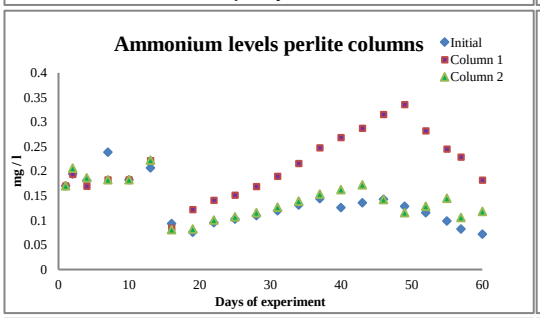

Conductivity levels perlite columns
Initial
Column 1
Column 2
µS / cm
Days of experiment

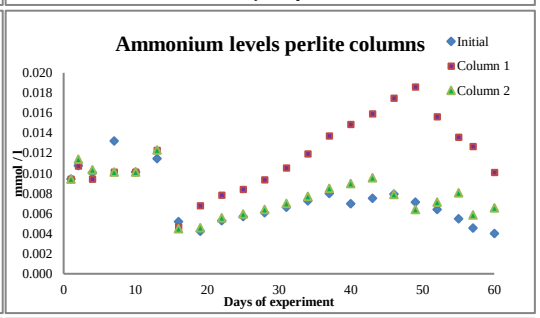

Nitrite levels perlite columns
Initial
Column 1
Column 2
mg / l
Days of experiment

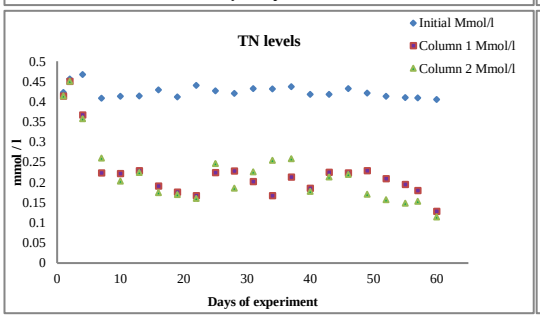

Nitrite levels perlite columns
Initial
Column 1
Column 2
mmol / l
Days of experiment

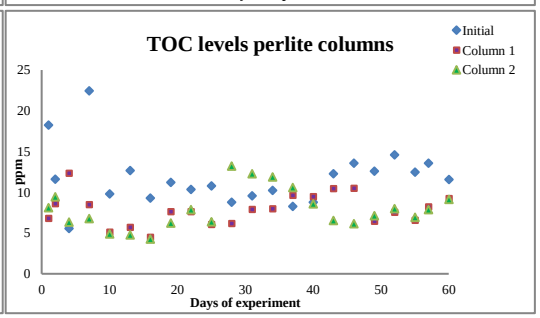

Nitrate levels perlite columns
Initial
Column 1
Column 2
mg / l
Days of experiment


Nitrate levels perlite columns
Initial
Column 1
Column 2
mmol / l
Days of experiment


Ammonium levels perlite columns
Initial
Column 1
Column 2
mg / l
Days of experiment


Ammonium levels perlite columns
Initial
Column 1
Column 2
mmol / l
Days of experiment


TN levels
Initial Mmol/l
Column 1 Mmol/l
Column 2 Mmol/l
mmol / l
Days of experiment


TOC levels perlite columns
Initial
Column 1
Column 2
ppm
Days of experiment

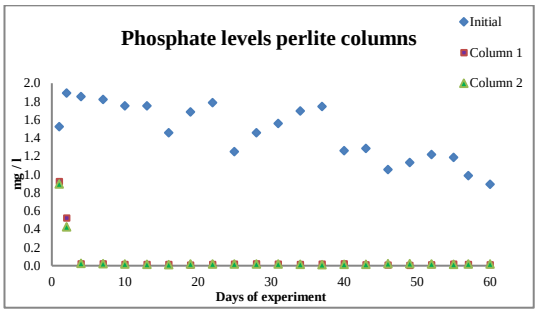


Figure III.2: Experiment Perlite Columns

Table III.5: pH and conductivity levels experiment Perlite Columns

pH	Initial	Column 1	Column 2	µS/cm	Initial	Column 1	Column 2
Day 1	7.72	6.65	6.68	**Day 1**	441	444	423
Day 2	7.94	6.38	6.43	**Day 2**	498	433	399
Day 3	8.00	6.71	6.77	**Day 3**	477	449	470
Day 4	8.00	6.90	6.90	**Day 4**	493	468	462
Day 5	8.07	7.01	7.04	**Day 5**	454	338	327
Day 6	7.73	7.11	7.03	**Day 6**	432	372	363
Day 9	8.10	7.36	7.40	**Day 9**	461	408	422
Day 10	8.28	7.55	7.59	**Day 10**	389	366	361
Day 11	8.13	7.78	7.88	**Day 11**	369	377	250
Day 12	8.18	7.53	7.55	**Day 12**	365	385	382
Day 13	7.90	7.43	7.43	**Day 13**	377	405	421
Day 15	7.99	7.54	7.40	**Day 15**	389	409	401
Day 16	8.06	7.41	7.49	**Day 16**	396	425	440
Day 17	7.84	7.57	7.51	**Day 17**	355	399	400
Day 18	7.96	7.35	7.36	**Day 18**	388	412	380
Day 19	7.81	7.34	7.39	**Day 19**	399	425	385
Day 23	7.83	7.39	7.38	**Day 23**	405	435	419
Day 24	7.76	7.72	7.33	**Day 24**	415	445	425
Day 26	7.76	7.30	7.44	**Day 26**	411	456	436
Day 27	8.03	7.51	7.52	**Day 27**	408	447	432
Day 28	8.06	7.63	7.58	**Day 28**	402	442	425
Day 31	8.09	7.80	7.66	**Day 31**	425	421	412
Day 33	8.02	7.58	7.50	**Day 33**	431	469	445
Day 34	8.02	7.47	7.51	**Day 34**	425	455	458
Day 35	7.84	7.51	7.56	**Day 35**	421	475	462
Day 38	7.93	7.50	7.59	**Day 38**	445	436	465
Day 39	7.98	7.48	7.42	**Day 39**	409	425	450
Day 40	7.86	7.54	7.41	**Day 40**	398	402	412
Day 43	7.94	7.44	7.36	**Day 43**	388	415	425
Day 46	7.98	7.58	7.60	**Day 46**	395	402	435
Day 49	8.13	7.39	7.39	**Day 49**	402	436	440
Day 52	8.03	7.59	7.71	**Day 52**	415	401	421
Day 55	8.06	7.52	7.36	**Day 55**	422	428	402
Day 57	8.04	7.53	7.37	**Day 57**	401	415	394
Day 60	8.00	7.49	7.27	**Day 60**	388	401	409

Table III.6: Nitrite and nitrate levels experiment Perlite Columns

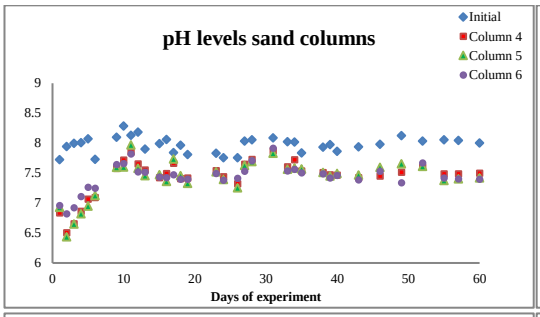
Nitrite	Initial		Column 1		Column 2		Nitrate	Initial		Column 1		Column 2	
	Mg/l	Mmol/l	Mg/l	Mmol/l	Mg/l	Mmol/l		Mg/l	Mmol/l	Mg/l	Mmol/l	Mg/l	Mmol/l
Day 1	0.110	0.002	0.110	0.002	0.110	0.002	**Day 1**	25.45	0.411	25.00	0.403	25.00	0.403
Day 2	0.118	0.003	0.276	0.006	0.313	0.007	**Day 2**	27.41	0.442	26.81	0.433	26.80	0.432
Day 4	0.134	0.003	0.274	0.006	0.240	0.005	**Day 4**	28.12	0.454	21.80	0.352	21.18	0.342
Day 7	0.117	0.003	0.208	0.005	0.191	0.004	**Day 7**	24.31	0.392	12.91	0.208	15.26	0.246
Day 10	0.161	0.003	0.188	0.004	0.179	0.004	**Day 10**	24.76	0.400	12.86	0.208	11.73	0.189
Day 13	0.104	0.002	0.162	0.004	0.184	0.004	**Day 13**	24.84	0.401	13.21	0.213	12.87	0.208
Day 16	0.230	0.005	0.199	0.004	0.215	0.005	**Day 16**	25.98	0.419	11.28	0.182	10.21	0.165
Day 19	0.261	0.006	0.186	0.004	0.182	0.004	**Day 19**	24.82	0.401	10.23	0.165	9.90	0.160
Day 22	0.103	0.002	0.111	0.002	0.130	0.003	**Day 22**	26.85	0.433	9.76	0.157	9.37	0.151
Day 25	0.110	0.002	0.128	0.003	0.138	0.003	**Day 25**	25.89	0.418	13.20	0.213	14.69	0.237
Day 28	0.120	0.003	0.143	0.003	0.151	0.003	**Day 28**	25.46	0.411	13.41	0.216	10.92	0.176
Day 31	0.131	0.003	0.158	0.003	0.169	0.004	**Day 31**	26.18	0.422	11.63	0.188	13.29	0.215
Day 34	0.144	0.003	0.176	0.004	0.190	0.004	**Day 34**	26.12	0.421	9.37	0.151	14.98	0.242
Day 37	0.160	0.003	0.199	0.004	0.216	0.005	**Day 37**	26.40	0.426	12.10	0.195	15.09	0.244
Day 40	0.110	0.002	0.180	0.004	0.185	0.004	**Day 40**	25.32	0.409	10.29	0.166	10.15	0.164
Day 43	0.125	0.003	0.185	0.004	0.174	0.004	**Day 43**	25.24	0.407	12.71	0.205	12.36	0.199
Day 46	0.114	0.002	0.195	0.004	0.163	0.004	**Day 46**	26.14	0.422	12.50	0.202	12.82	0.207
Day 49	0.092	0.002	0.219	0.005	0.139	0.003	**Day 49**	25.56	0.412	12.70	0.205	10.00	0.161
Day 52	0.102	0.002	0.192	0.004	0.145	0.003	**Day 52**	25.13	0.405	11.70	0.189	9.10	0.147
Day 55	0.082	0.002	0.182	0.004	0.126	0.003	**Day 55**	24.99	0.403	11.00	0.177	8.50	0.137
Day 57	0.081	0.002	0.172	0.004	0.113	0.002	**Day 57**	24.94	0.402	10.10	0.163	9.00	0.145
Day 60	0.076	0.002	0.146	0.003	0.106	0.002	**Day 60**	24.72	0.399	7.10	0.115	6.50	0.105

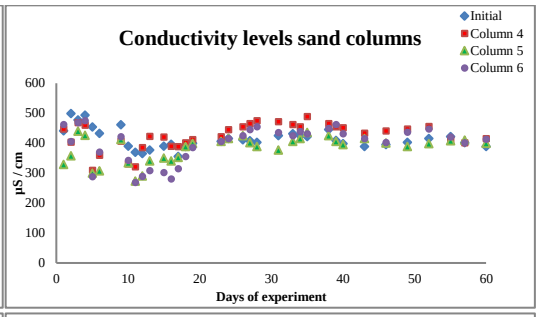
Table III.7: Ammonium and TN levels experiment Perlite Columns

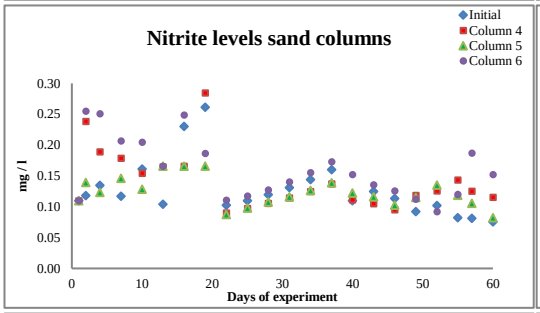
Ammonium	Initial		Column 1		Column 2		TN	Initial	Column 1	Column 2
	Mg/l	Mmol/l	Mg/l	Mmol/l	Mg/l	Mmol/l		Mmol/l	Mmol/l	Mmol/l
Day 1	0.170	0.009	0.170	0.009	0.170	0.009	**Day 1**	0.422	0.414	0.414
Day 2	0.194	0.011	0.194	0.011	0.206	0.011	**Day 2**	0.456	0.45	0.45
Day 4	0.182	0.010	0.170	0.009	0.187	0.010	**Day 4**	0.467	0.367	0.357
Day 7	0.239	0.013	0.183	0.010	0.183	0.010	**Day 7**	0.408	0.223	0.26
Day 10	0.183	0.010	0.183	0.010	0.183	0.010	**Day 10**	0.413	0.222	0.203
Day 13	0.207	0.011	0.222	0.012	0.222	0.012	**Day 13**	0.414	0.229	0.224
Day 16	0.094	0.005	0.084	0.005	0.081	0.004	**Day 16**	0.429	0.191	0.174
Day 19	0.076	0.004	0.122	0.007	0.083	0.005	**Day 19**	0.411	0.176	0.169
Day 22	0.096	0.005	0.141	0.008	0.101	0.006	**Day 22**	0.44	0.167	0.16
Day 25	0.103	0.006	0.151	0.008	0.107	0.006	**Day 25**	0.426	0.224	0.246
Day 28	0.110	0.006	0.169	0.009	0.116	0.006	**Day 28**	0.42	0.228	0.185
Day 31	0.120	0.007	0.190	0.011	0.127	0.007	**Day 31**	0.432	0.202	0.226
Day 34	0.131	0.007	0.216	0.012	0.139	0.008	**Day 34**	0.431	0.167	0.254
Day 37	0.144	0.008	0.248	0.014	0.154	0.009	**Day 37**	0.437	0.213	0.258
Day 40	0.126	0.007	0.269	0.015	0.162	0.009	**Day 40**	0.418	0.185	0.177
Day 43	0.136	0.008	0.287	0.016	0.172	0.010	**Day 43**	0.418	0.225	0.213
Day 46	0.143	0.008	0.315	0.017	0.142	0.008	**Day 46**	0.432	0.223	0.219
Day 49	0.129	0.007	0.336	0.019	0.116	0.006	**Day 49**	0.421	0.229	0.17
Day 52	0.116	0.006	0.282	0.016	0.129	0.007	**Day 52**	0.413	0.209	0.157
Day 55	0.099	0.005	0.245	0.014	0.145	0.008	**Day 55**	0.41	0.195	0.148
Day 57	0.082	0.005	0.229	0.013	0.106	0.006	**Day 57**	0.409	0.18	0.153
Day 60	0.072	0.004	0.182	0.010	0.119	0.007	**Day 60**	0.405	0.128	0.114

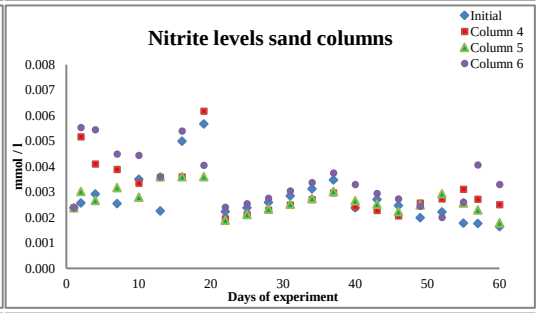
Table III.8: TOC and phosphate levels experiment Perlite Columns

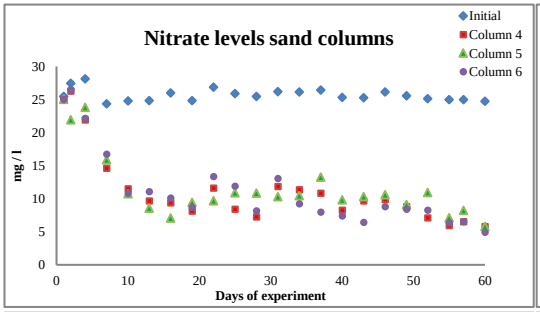
TOC	Initial	Column 1	Column 2	Phosphate	Initial	Column 1	Column 2
	ppm	ppm	ppm		mg/l	mg/l	mg/l
Day 1	18.25	6.79	8.11	**Day 1**	1.522	0.924	0.895
Day 2	11.60	8.60	9.42	**Day 2**	1.892	0.521	0.423
Day 4	5.56	12.34	6.32	**Day 4**	1.852	0.022	0.023
Day 7	22.43	8.47	6.75	**Day 7**	1.823	0.020	0.020
Day 10	9.79	5.09	4.85	**Day 10**	1.752	0.015	0.019
Day 13	12.65	5.69	4.74	**Day 13**	1.752	0.014	0.014
Day 16	9.28	4.47	4.24	**Day 16**	1.456	0.013	0.011
Day 19	11.19	7.61	6.19	**Day 19**	1.685	0.008	0.018
Day 22	10.32	7.61	7.85	**Day 22**	1.785	0.015	0.019
Day 25	10.78	6.04	6.38	**Day 25**	1.248	0.017	0.017
Day 28	8.75	6.15	13.19	**Day 28**	1.456	0.021	0.018
Day 31	9.56	7.90	12.26	**Day 31**	1.559	0.017	0.019
Day 34	10.23	7.98	11.85	**Day 34**	1.694	0.012	0.012
Day 37	8.27	9.63	10.55	**Day 37**	1.744	0.019	0.011
Day 40	8.77	9.45	8.55	**Day 40**	1.258	0.020	0.014
Day 43	12.25	10.45	6.52	**Day 43**	1.285	0.009	0.015
Day 46	13.56	10.48	6.15	**Day 46**	1.052	0.008	0.020
Day 49	12.56	6.46	7.13	**Day 49**	1.129	0.004	0.022
Day 52	14.56	7.55	7.97	**Day 52**	1.218	0.011	0.019
Day 55	12.47	6.55	6.92	**Day 55**	1.186	0.018	0.015
Day 57	13.55	8.22	7.86	**Day 57**	0.985	0.012	0.018
Day 60	11.55	9.22	9.12	**Day 60**	0.891	0.011	0.017

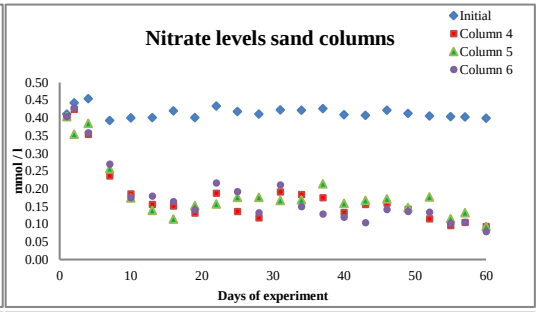

pH levels sand columns
Initial
Column 4
Column 5
Column 6
Days of experiment

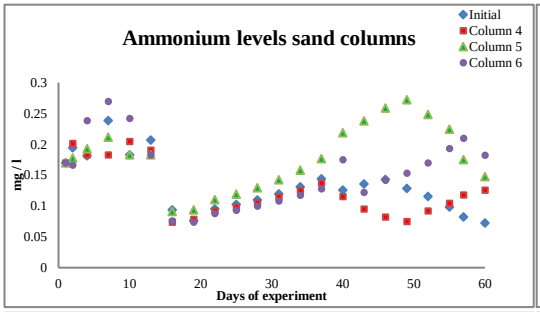

Conductivity levels sand columns
Initial
Column 4
Column 5
Column 6
µS / cm
Days of experiment

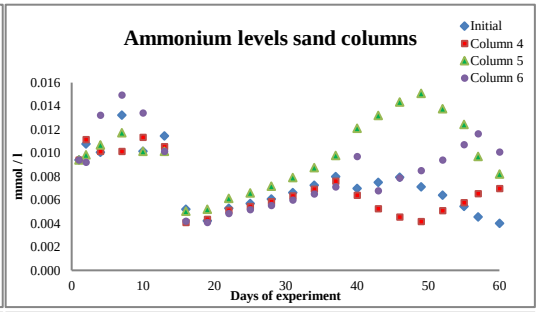

Nitrite levels sand columns
Initial
Column 4
Column 5
Column 6
mg / l
Days of experiment

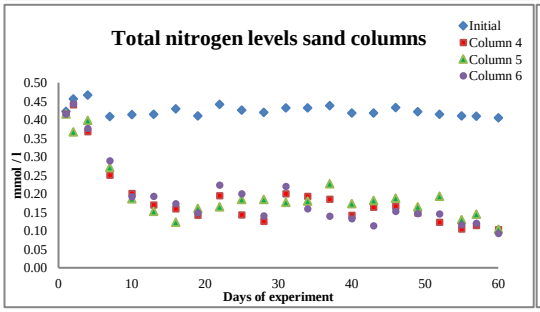

Nitrite levels sand columns
Initial
Column 4
Column 5
Column 6
mmol / l
Days of experiment

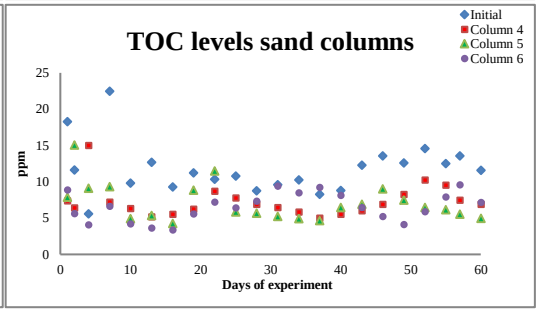

Nitrate levels sand columns
Initial
Column 4
Column 5
Column 6
mg / l
Days of experiment


Nitrate levels sand columns
Initial
Column 4
Column 5
Column 6
mmol / l
Days of experiment


Ammonium levels sand columns
Initial
Column 4
Column 5
Column 6
mg / l
Days of experiment


Ammonium levels sand columns
Initial
Column 4
Column 5
Column 6
mmol / l
Days of experiment


Total nitrogen levels sand columns
Initial
Column 4
Column 5
Column 6
mmol / l
Days of experiment


TOC levels sand columns
Initial
Column 4
Column 5
Column 6
ppm
Days of experiment

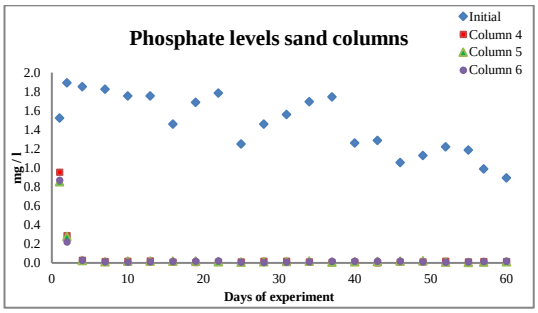


Figure III.3: Experiment Sand Columns

Table III.9: pH and conductivity levels experiment Sand Columns

pH	Initial	Column 4	Column 5	Column 6	µS/cm	Initial	Column 4	Column 5	Column 6
Day 1	7.72	6.84	6.93	6.96	**Day 1**	441	447	328	462
Day 2	7.94	6.51	6.43	6.82	**Day 2**	498	403	357	405
Day 3	8.00	6.65	6.65	6.92	**Day 3**	477	468	440	468
Day 4	8.00	6.86	6.82	7.11	**Day 4**	493	460	426	475
Day 5	8.07	7.06	6.95	7.27	**Day 5**	454	309	300	288
Day 6	7.73	7.09	7.12	7.25	**Day 6**	432	359	307	370
Day 9	8.10	7.58	7.60	7.64	**Day 9**	461	407	412	421
Day 10	8.28	7.72	7.60	7.66	**Day 10**	389	331	334	342
Day 11	8.13	7.85	7.97	7.82	**Day 11**	369	321	273	269
Day 12	8.18	7.65	7.58	7.52	**Day 12**	365	385	290	289
Day 13	7.90	7.55	7.46	7.51	**Day 13**	377	423	340	308
Day 15	7.99	7.42	7.47	7.43	**Day 15**	389	420	350	302
Day 16	8.06	7.49	7.36	7.43	**Day 16**	396	389	340	280
Day 17	7.84	7.67	7.74	7.47	**Day 17**	355	388	352	315
Day 18	7.96	7.41	7.46	7.40	**Day 18**	388	401	388	355
Day 19	7.81	7.42	7.33	7.40	**Day 19**	399	412	399	385
Day 23	7.83	7.54	7.52	7.49	**Day 23**	405	422	405	405
Day 24	7.76	7.44	7.39	7.38	**Day 24**	415	445	415	415
Day 26	7.76	7.32	7.25	7.41	**Day 26**	411	455	425	425
Day 27	8.03	7.65	7.63	7.53	**Day 27**	408	465	401	445
Day 28	8.06	7.73	7.69	7.71	**Day 28**	402	475	388	455
Day 31	8.09	7.88	7.83	7.91	**Day 31**	425	472	377	435
Day 33	8.02	7.60	7.56	7.54	**Day 33**	431	462	405	425
Day 34	8.02	7.72	7.59	7.56	**Day 34**	425	455	415	439
Day 35	7.84	7.54	7.57	7.50	**Day 35**	421	489	435	431
Day 38	7.93	7.51	7.51	7.49	**Day 38**	445	465	425	449
Day 39	7.98	7.47	7.45	7.42	**Day 39**	409	455	405	462
Day 40	7.86	7.47	7.50	7.46	**Day 40**	398	451	395	431
Day 43	7.94	7.42	7.47	7.38	**Day 43**	388	433	415	415
Day 46	7.98	7.45	7.59	7.53	**Day 46**	395	441	400	402
Day 49	8.13	7.51	7.65	7.34	**Day 49**	402	447	388	436
Day 52	8.03	7.60	7.61	7.67	**Day 52**	415	456	398	447
Day 55	8.06	7.49	7.38	7.42	**Day 55**	422	412	408	420
Day 57	8.04	7.49	7.40	7.40	**Day 57**	401	401	410	401
Day 60	8.00	7.50	7.42	7.40	**Day 60**	388	415	399	413

Table III.10: Nitrite and nitrate levels experiment Sand Columns

Nitrite	Initial		Column 4		Column 5		Column 6		Nitrate	Initial		Column 4		Column 5		Column 6	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l		mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
Day 1	0.110	0.002	0.110	0.002	0.110	0.002	0.110	0.002	**Day 1**	25.452	0.411	25.000	0.403	25.000	0.403	25.000	0.403
Day 2	0.118	0.003	0.238	0.005	0.139	0.003	0.255	0.006	**Day 2**	27.413	0.442	26.255	0.424	21.914	0.354	26.508	0.428
Day 4	0.134	0.003	0.189	0.004	0.123	0.003	0.251	0.005	**Day 4**	28.115	0.454	21.904	0.353	23.811	0.384	22.167	0.358
Day 7	0.117	0.003	0.179	0.004	0.146	0.003	0.207	0.004	**Day 7**	24.314	0.392	14.602	0.236	15.837	0.256	16.716	0.270
Day 10	0.161	0.003	0.154	0.003	0.129	0.003	0.205	0.004	**Day 10**	24.763	0.400	11.510	0.186	10.747	0.173	10.815	0.174
Day 13	0.104	0.002	0.165	0.004	0.166	0.004	0.166	0.004	**Day 13**	24.839	0.401	9.649	0.156	8.569	0.138	11.084	0.179
Day 16	0.230	0.005	0.166	0.004	0.166	0.004	0.249	0.005	**Day 16**	25.980	0.419	9.382	0.151	7.057	0.114	10.118	0.163
Day 19	0.261	0.006	0.285	0.006	0.166	0.004	0.187	0.004	**Day 19**	24.825	0.401	8.124	0.131	9.399	0.152	8.679	0.140
Day 22	0.103	0.002	0.090	0.002	0.087	0.002	0.111	0.002	**Day 22**	26.851	0.433	11.616	0.187	9.677	0.156	13.367	0.216
Day 25	0.110	0.002	0.098	0.002	0.098	0.002	0.117	0.003	**Day 25**	25.885	0.418	8.405	0.136	10.861	0.175	11.888	0.192
Day 28	0.120	0.003	0.106	0.002	0.107	0.002	0.127	0.003	**Day 28**	25.459	0.411	7.267	0.117	10.849	0.175	8.172	0.132
Day 31	0.131	0.003	0.115	0.002	0.116	0.003	0.140	0.003	**Day 31**	26.184	0.422	11.848	0.191	10.298	0.166	13.070	0.211
Day 34	0.144	0.003	0.125	0.003	0.127	0.003	0.155	0.003	**Day 34**	26.119	0.421	11.379	0.184	10.442	0.168	9.238	0.149
Day 37	0.160	0.003	0.137	0.003	0.139	0.003	0.173	0.004	**Day 37**	26.402	0.426	10.827	0.175	13.236	0.214	7.970	0.129
Day 40	0.110	0.002	0.112	0.002	0.122	0.003	0.152	0.003	**Day 40**	25.319	0.409	8.236	0.133	9.824	0.159	7.401	0.119
Day 43	0.125	0.003	0.105	0.002	0.116	0.003	0.136	0.003	**Day 43**	25.238	0.407	9.651	0.156	10.304	0.166	6.439	0.104
Day 46	0.114	0.002	0.095	0.002	0.103	0.002	0.126	0.003	**Day 46**	26.140	0.422	9.866	0.159	10.591	0.171	8.772	0.142
Day 49	0.092	0.002	0.119	0.003	0.115	0.003	0.113	0.002	**Day 49**	25.555	0.412	8.774	0.142	9.102	0.147	8.407	0.136
Day 52	0.102	0.002	0.126	0.003	0.135	0.003	0.092	0.002	**Day 52**	25.125	0.405	7.112	0.115	10.903	0.176	8.277	0.134
Day 55	0.082	0.002	0.143	0.003	0.119	0.003	0.120	0.003	**Day 55**	24.986	0.403	5.938	0.096	7.107	0.115	6.360	0.103
Day 57	0.081	0.002	0.126	0.003	0.106	0.002	0.187	0.004	**Day 57**	24.943	0.402	6.502	0.105	8.201	0.132	6.501	0.105
Day 60	0.076	0.002	0.116	0.003	0.082	0.002	0.152	0.003	**Day 60**	24.722	0.399	5.801	0.094	5.802	0.094	4.902	0.079

Table III.11: Ammonium and TN levels experiment Sand Columns

Ammonium	Initial		Column 4		Column 5		Column 6		TN	Initial	Column 4	Column 5	Column 6
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l		mmol/l	mmol/l	mmol/l	mmol/l
Day 1	0.170	0.009	0.170	0.009	0.170	0.009	0.170	0.009	**Day 1**	0.422	0.415	0.415	0.415
Day 2	0.194	0.011	0.201	0.011	0.178	0.010	0.166	0.009	**Day 2**	0.456	0.440	0.366	0.442
Day 4	0.182	0.010	0.183	0.010	0.193	0.011	0.239	0.013	**Day 4**	0.467	0.368	0.398	0.376
Day 7	0.239	0.013	0.183	0.010	0.212	0.012	0.270	0.015	**Day 7**	0.408	0.250	0.270	0.289
Day 10	0.183	0.010	0.205	0.011	0.183	0.010	0.242	0.013	**Day 10**	0.413	0.200	0.186	0.192
Day 13	0.207	0.011	0.191	0.011	0.183	0.010	0.183	0.010	**Day 13**	0.415	0.170	0.152	0.193
Day 16	0.094	0.005	0.074	0.004	0.091	0.005	0.076	0.004	**Day 16**	0.429	0.159	0.123	0.173
Day 19	0.076	0.004	0.079	0.004	0.094	0.005	0.074	0.004	**Day 19**	0.410	0.142	0.160	0.148
Day 22	0.096	0.005	0.093	0.005	0.111	0.006	0.088	0.005	**Day 22**	0.441	0.195	0.164	0.223
Day 25	0.103	0.006	0.098	0.005	0.119	0.007	0.093	0.005	**Day 25**	0.426	0.143	0.184	0.200
Day 28	0.110	0.006	0.106	0.006	0.129	0.007	0.100	0.006	**Day 28**	0.419	0.125	0.185	0.140
Day 31	0.120	0.007	0.115	0.006	0.143	0.008	0.108	0.006	**Day 31**	0.432	0.200	0.177	0.220
Day 34	0.131	0.007	0.125	0.007	0.158	0.009	0.117	0.007	**Day 34**	0.432	0.193	0.180	0.159
Day 37	0.144	0.008	0.137	0.008	0.177	0.010	0.128	0.007	**Day 37**	0.437	0.185	0.226	0.139
Day 40	0.126	0.007	0.116	0.006	0.219	0.012	0.175	0.010	**Day 40**	0.418	0.142	0.173	0.132
Day 43	0.136	0.008	0.095	0.005	0.238	0.013	0.122	0.007	**Day 43**	0.417	0.163	0.182	0.114
Day 46	0.143	0.008	0.082	0.005	0.259	0.014	0.142	0.008	**Day 46**	0.432	0.166	0.187	0.152
Day 49	0.129	0.007	0.075	0.004	0.272	0.015	0.153	0.008	**Day 49**	0.421	0.148	0.164	0.147
Day 52	0.116	0.006	0.092	0.005	0.249	0.014	0.170	0.009	**Day 52**	0.414	0.123	0.193	0.145
Day 55	0.099	0.005	0.105	0.006	0.225	0.012	0.193	0.011	**Day 55**	0.410	0.105	0.130	0.116
Day 57	0.082	0.005	0.118	0.007	0.175	0.010	0.210	0.012	**Day 57**	0.409	0.114	0.144	0.121
Day 60	0.072	0.004	0.126	0.007	0.148	0.008	0.182	0.010	**Day 60**	0.405	0.103	0.104	0.092

Table III.12: TOC and phosphate levels experiment Sand Columns

TOC	Initial	Column 4	Column 5	Column 6	Phosphate	Initial	Column 4	Column 5	Column 6
	ppm	ppm	ppm	ppm		mg/l	mg/l	mg/l	mg/l
Day 1	18.25	7.35	7.85	8.87	**Day 1**	1.522	0.954	0.852	0.870
Day 2	11.60	6.40	15.06	5.61	**Day 2**	1.892	0.286	0.278	0.219
Day 4	5.56	14.99	9.08	4.06	**Day 4**	1.852	0.028	0.024	0.029
Day 7	22.43	7.18	9.32	6.61	**Day 7**	1.823	0.014	0.012	0.011
Day 10	9.79	6.30	4.90	4.19	**Day 10**	1.752	0.019	0.019	0.009
Day 13	12.65	5.18	5.33	3.63	**Day 13**	1.752	0.020	0.017	0.011
Day 16	9.28	5.50	4.23	3.37	**Day 16**	1.456	0.009	0.018	0.016
Day 19	11.19	6.22	8.81	5.56	**Day 19**	1.685	0.004	0.019	0.017
Day 22	10.32	8.70	11.47	7.20	**Day 22**	1.785	0.008	0.012	0.020
Day 25	10.78	7.77	5.82	6.41	**Day 25**	1.248	0.011	0.009	0.009
Day 28	8.75	6.89	5.67	7.34	**Day 28**	1.456	0.018	0.011	0.004
Day 31	9.56	6.45	5.22	9.42	**Day 31**	1.559	0.019	0.016	0.008
Day 34	10.23	5.82	4.89	8.46	**Day 34**	1.694	0.011	0.017	0.011
Day 37	8.27	4.99	4.62	9.21	**Day 37**	1.744	0.005	0.009	0.019
Day 40	8.77	5.53	6.45	8.13	**Day 40**	1.258	0.007	0.011	0.017
Day 43	12.25	6.00	6.90	6.45	**Day 43**	1.285	0.002	0.016	0.018
Day 46	13.56	6.90	9.00	5.23	**Day 46**	1.052	0.009	0.017	0.019
Day 49	12.56	8.26	7.45	4.13	**Day 49**	1.129	0.012	0.020	0.012
Day 52	14.56	10.25	6.45	5.90	**Day 52**	1.218	0.018	0.009	0.009
Day 55	12.47	9.55	6.12	7.90	**Day 55**	1.186	0.012	0.004	0.011
Day 57	13.55	7.45	5.52	9.56	**Day 57**	0.985	0.014	0.008	0.016
Day 60	11.55	6.89	4.96	7.16	**Day 60**	0.891	0.013	0.011	0.017

Appendix IV

BATCH EXPERIMENTS WITH HAZELNUT HUSK WASTES AND TEA WASTE MATERIALS

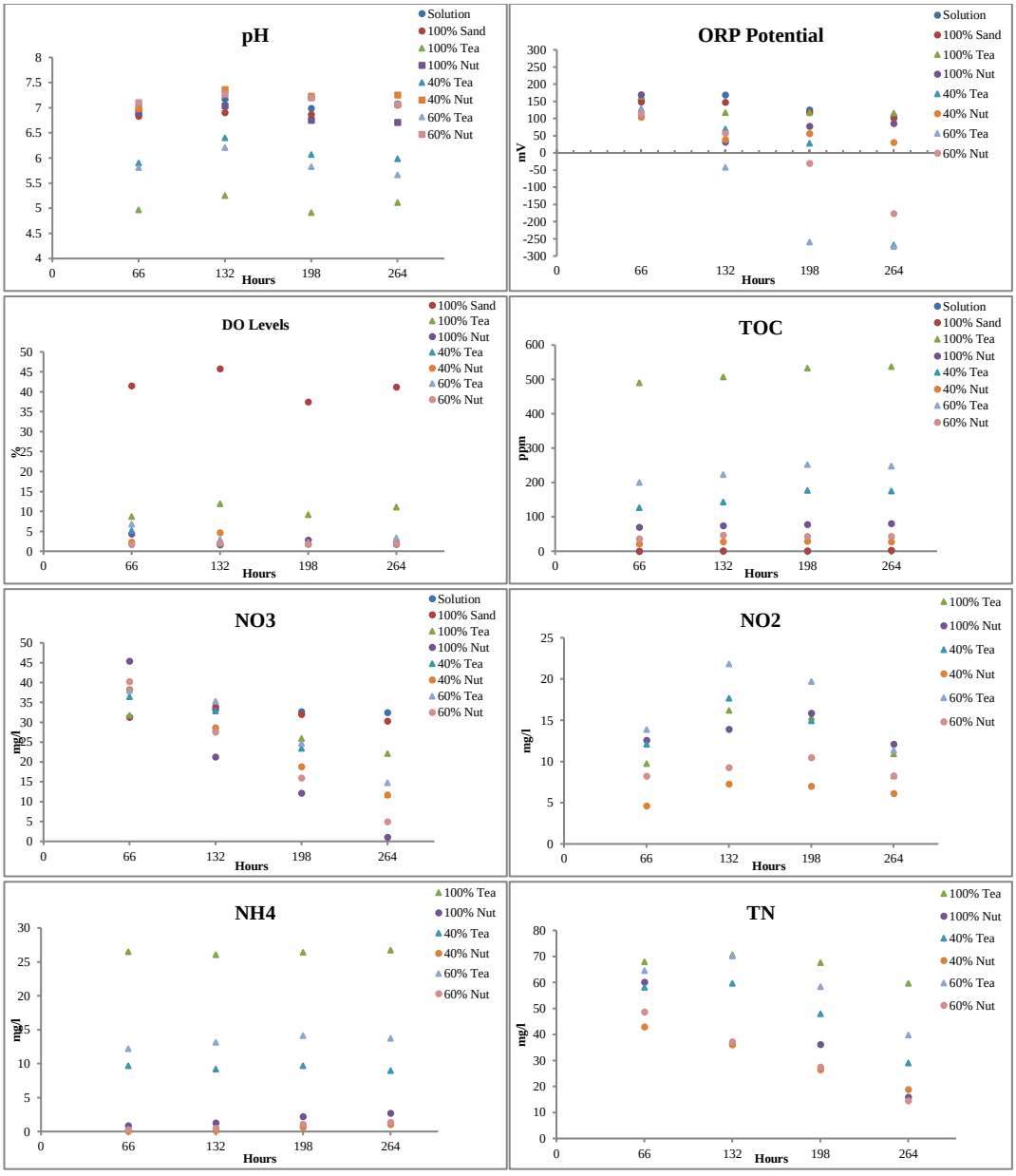


Figure IV.1: Batch experiment details

Table IV.1: pH and ORP levels batch experiment

	66 hours	132 hours	198 hours	264 hours	mV	66 hours	132 hours	198 hours	264 hours
Solution	6.86	7.17	6.99	7.08	**Solution**	155.6	168.4	125.5	106.7
100% Sand	6.83	6.90	6.87	7.05	**100% Sand**	148.2	147.6	116.9	101.1
100% Tea	4.96	5.25	4.91	5.11	**100% Tea**	164.4	117.1	117.3	115.4
100% Nut	6.90	7.04	6.75	6.71	**100% Nut**	169.3	31.1	77.6	85.1
40% Tea	5.90	6.40	6.07	5.98	**40% Tea**	128.2	69.6	28.0	-268.0
40% Nut	6.98	7.36	7.23	7.25	**40% Nut**	103.8	38.3	56.2	30.4
60% Tea	5.81	6.20	5.83	5.66	**60% Tea**	124.9	-42.4	-259.7	-272.2
60% Nut	7.10	7.27	7.20	7.06	**60% Nut**	112.3	56.9	-31.1	-176.4

Table IV.2: DO and TOC levels batch experiment

%	66 hours	132 hours	198 hours	264 hours	ppm	66 hours	132 hours	198 hours	264 hours
Solution					**Solution**	0.15	0.96	1.06	2.48
100% Sand	41.5	45.75	37.5	41.2	**100% Sand**	0.37	1.37	1.42	2.64
100% Tea	8.7	11.9	9.2	11.1	**100% Tea**	490.58	507.66	532.53	537.67
100% Nut	4.4	1.65	2.8	1.8	**100% Nut**	70.13	74.66	78.19	81.04
40% Tea	5.2	2.85	2.1	2.8	**40% Tea**	126.66	143.66	176.93	175.23
40% Nut	2.3	4.7	1.8	2.4	**40% Nut**	21.36	27.76	29.47	27.69
60% Tea	6.8	2.7	2.2	3.4	**60% Tea**	200.51	222.89	252.05	247.59
60% Nut	1.7	2	1.8	1.9	**60% Nut**	36.15	46.95	43.53	43.65

Table IV.3: Nitrogen species batch experiment with TW

Nitrate	66 hours		132 hours		198 hours		264 hours	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
Solution	31.24	0.504	33.18	0.535	32.67	0.527	32.40	0.523
100% Sand	31.30	0.505	33.89	0.547	31.98	0.516	30.26	0.488
100% Tea	31.73	0.512	28.43	0.459	25.90	0.418	22.11	0.357
100% Nut	45.39	0.732	21.23	0.343	12.10	0.195	1.06	0.017
40% Tea	36.37	0.587	32.81	0.529	23.39	0.377	11.75	0.190
40% Nut	38.27	0.617	28.61	0.462	18.79	0.303	11.68	0.188
60% Tea	37.93	0.612	35.22	0.568	24.65	0.398	14.72	0.238
60% Nut	40.26	0.650	27.49	0.444	15.98	0.258	4.93	0.080

Nitrite	66 hours		132 hours		198 hours		264 hours	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
Solution								
100% Sand								
100% Tea	9.71	0.211	16.17	0.351	15.34	0.333	10.91	0.237
100% Nut	12.56	0.273	13.90	0.302	15.85	0.344	12.09	0.262
40% Tea	12.09	0.262	17.66	0.383	14.91	0.324	8.26	0.179
40% Nut	4.60	0.100	7.27	0.158	6.98	0.151	6.12	0.133
60% Tea	13.87	0.301	21.82	0.474	19.68	0.427	11.33	0.246
60% Nut	8.20	0.178	9.26	0.201	10.45	0.227	8.26	0.179

Ammonium	66 hours		132 hours		198 hours		264 hours	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
Solution								
100% Sand								
100% Tea	26.52	1.470	26.04	1.443	26.39	1.463	26.71	1.481
100% Nut	0.87	0.048	1.28	0.071	2.20	0.122	2.73	0.151
40% Tea	9.71	0.538	9.19	0.509	9.68	0.537	8.97	0.497
40% Nut	0.06	0.003	0.15	0.008	0.67	0.037	1.04	0.058
60% Tea	12.22	0.677	13.13	0.728	14.15	0.784	13.73	0.761
60% Nut	0.27	0.015	0.50	0.028	1.09	0.060	1.38	0.076

TN	66 hours	132 hours	198 hours	264 hours
	mmol/l	mmol/l	mmol/l	mmol/l
Solution	0.504	0.535	0.527	0.523
100% Sand	0.505	0.547	0.516	0.488
100% Tea	2.193	2.253	2.214	2.074
100% Nut	1.053	0.715	0.661	0.431
40% Tea	1.387	1.422	1.238	0.866
40% Nut	0.721	0.628	0.492	0.379
60% Tea	1.590	1.770	1.609	1.244
60% Nut	0.842	0.672	0.545	0.335

Table IV.4: Nitrogen species batch experiment TW and GW

Nitrate	Solution		100% Sand		100% Tea		100% Nut		40% Tea		40% Nut		60% Tea		60% Nut	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
NO3 TW	33.68	0.543	31.26	0.504	1.57	0.025	0.18	0.003	1.26	0.020	14.92	0.241	8.50	0.137	1.91	0.031
NO3 GW	33.02	0.533	36.08	0.582	0.03	0.00	6.76	0.109	0.00	0.000	16.07	0.259	0.02	0.000	14.54	0.235

Nitrite	Solution		100% Sand		100% Tea		100% Nut		40% Tea		40% Nut		60% Tea		60% Nut	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
NO2 TW	0.01	0.000	1.33	0.029	18.96	0.412	4.86	0.105	0.03	0.001	6.58	0.143	7.17	0.156	4.01	0.087
NO2 GW	0.06	0.001	0.33	0.007	0.03	0.001	0.83	0.018	0.05	0.001	1.57	0.034	2.65	0.058	1.19	0.026

Ammonium	Solution		100% Sand		100% Tea		100% Nut		40% Tea		40% Nut		60% Tea		60% Nut	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
NH4 TW	48.34	2.680	46.72	2.590	0.31	0.017	0.23	0.013	2.79	0.155	1.05	0.058	1.20	0.067	1.25	0.069
NH4 GW	48.92	2.712	51.45	2.852	41.84	2.319	34.27	1.900	46.85	2.597	39.19	2.172	45.22	2.507	37.27	2.066

TN	Solution		100% Sand		100% Tea		100% Nut		40% Tea		40% Nut		60% Tea		60% Nut	
	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l	mg/l	mmol/l
TN TW	82.01	3.223	79.31	3.123	20.84	0.454	5.27	0.121	4.06	0.176	22.56	0.442	17.77	0.359	7.70	0.187
TN GW	87.95	3.246	82.08	3.441	41.92	2.320	41.24	2.027	46.98	2.598	56.92	2.466	47.11	2.564	53.10	2.326

Appendix V
COLUMNS EXPERIMENT WITH TEA WASTE MATERIALS AND HAZELNUT HUSK WASTES

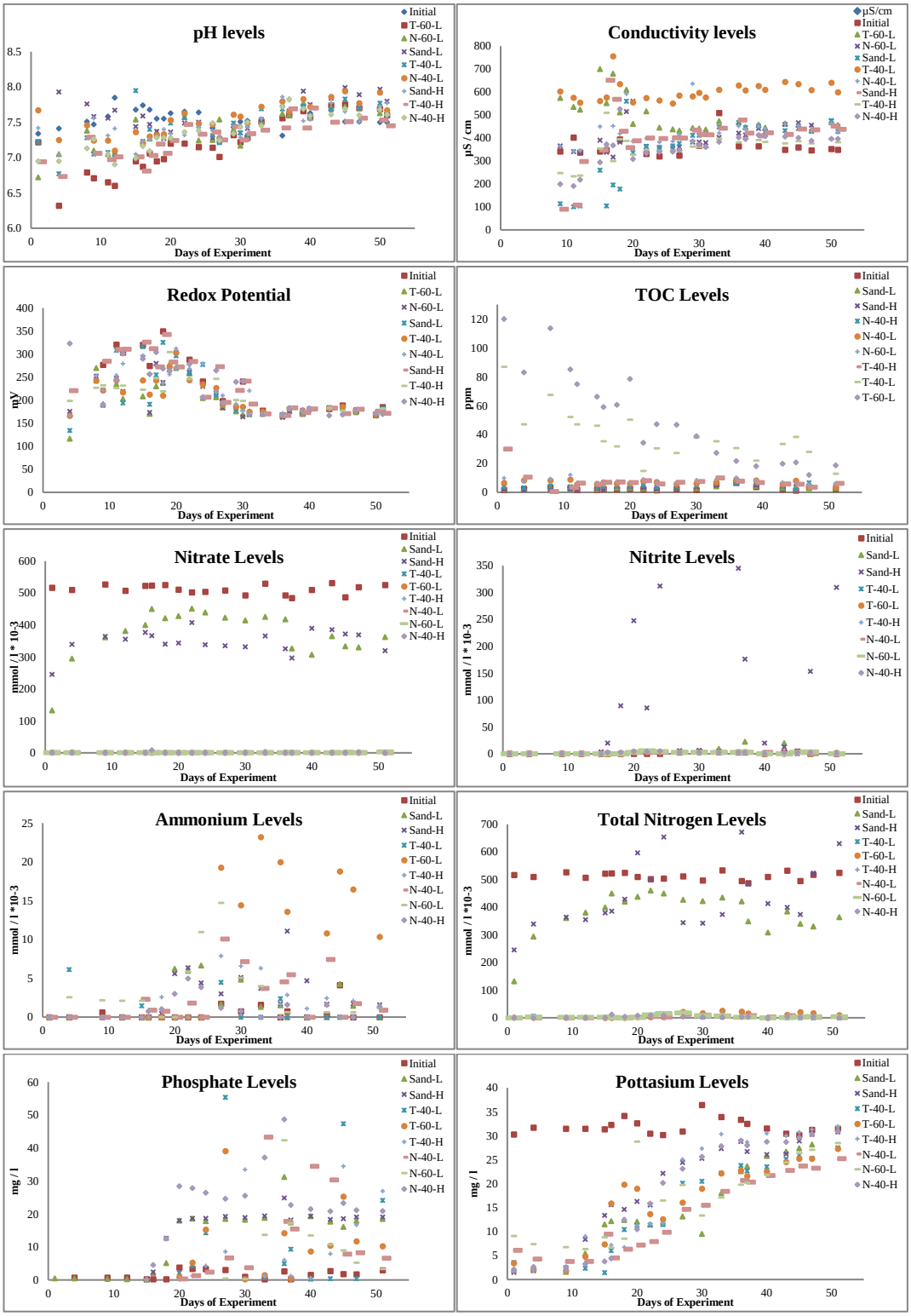


Figure V.1: Columns experiment with tea, nut and sand details with TW

Table V.1: pH levels experiment with sand, tea, and nut with TW

Days	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
1	7.34	7.22	6.72	7.21	7.22	7.67	7.42	6.94	6.95
4	7.41	6.32	7.05	7.93	6.77	7.25	7.04	6.73	6.95
8	7.51	6.79	7.38	7.76	7.28	7.46	7.13	7.29	7.13
9	7.47	6.71	7.10	7.58	7.05	7.24	7.56	7.06	7.26
11	7.58	6.65	7.05	7.56	7.07	7.24	7.31	6.97	7.05
12	7.85	6.60	7.06	7.67	7.04	7.10	7.41	7.01	6.90
15	7.68	6.95	7.54	7.44	7.95	7.36	7.05	7.01	6.99
16	7.74	6.87	7.20	7.59	7.23	7.21	7.23	6.81	7.16
17	7.68	7.06	7.27	7.47	7.40	7.30	7.26	7.03	7.09
18	7.55	6.95	7.36	7.37	7.30	7.32	7.30	7.19	7.28
19	7.55	6.98	7.34	7.27	7.29	7.33	7.40	7.06	7.28
20	7.63	7.20	7.28	7.36	7.49	7.53	7.34	7.24	7.28
22	7.65	7.20	7.57	7.49	7.57	7.63	7.32	7.47	7.36
24	7.64	7.15	7.24	7.50	7.45	7.48	7.43	7.33	7.38
26	7.42	7.14	7.25	7.28	7.31	7.47	7.38	7.36	7.49
27	7.22	7.01	7.54	7.22	7.21	7.28	7.29	7.25	7.24
29	7.43	7.32	7.40	7.45	7.49	7.61	7.44	7.44	7.34
30	7.51	7.22	7.17	7.35	7.35	7.58	7.29	7.25	7.43
31	7.52	7.28	7.29	7.41	7.31	7.49	7.43	7.34	7.50
33	7.50	7.51	7.52	7.54	7.69	7.72	7.55	7.39	7.45
36	7.31	7.56	7.62	7.76	7.69	7.80	7.86	7.42	7.72
37	7.61	7.60	7.60	7.66	7.64	7.65	7.81	7.69	7.83
39	7.74	7.66	7.77	7.94	7.73	7.83	7.52	7.42	7.69
40	7.63	7.57	7.57	7.75	7.58	7.72	7.54	7.70	7.59
43	7.67	7.74	7.71	7.84	7.68	7.86	7.77	7.50	7.61
45	7.51	7.75	7.72	7.99	7.83	7.94	7.65	7.51	7.69
47	7.51	7.70	7.71	7.89	7.70	7.77	7.51	7.56	7.60
50	7.50	7.69	7.63	7.97	7.77	7.92	7.72	7.53	7.68
51	7.50	7.60	7.62	7.80	7.61	7.67	7.77	7.45	7.61

Table V.2: Conductivity levels experiment with sand tea, nut with TW

µS/cm	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
9	340.99	574.56	364.74	113.56	602.27	363.75	89.70	246.97	198.47
11	402.04	535.08	340.42	100.76	575.18	342.37	107.12	232.81	190.75
12	336.85	523.30	336.85	104.41	553.56	344.66	297.80	236.30	217.75
15	341.79	699.87	389.60	259.83	561.25	449.11	347.64	353.49	293.98
16	347.21	552.21	338.46	104.01	576.50	366.64	651.32	509.46	372.47
17		680.07	316.98	195.03	756.34	452.22	568.59	298.68	368.12
18	393.21	510.95	381.53	177.77	634.54	418.51	429.21	394.18	525.55
19		610.36	515.07	559.35	991.82	592.07	358.52	387.06	989.58
20		461.96	559.53	336.65	555.71	385.43	388.30	326.12	307.95
22	330.91	516.49	361.52	364.39	573.88	397.87	399.78	347.17	333.78
24	320.62	445.09	353.88	359.58	562.92	382.38	397.58	343.42	339.62
26		438.09	355.26	365.61	550.11	385.38	401.38	345.85	340.20
27	323.20	431.71	360.00	376.04	584.58	414.73	399.63	343.02	347.73
29		443.65	381.77	411.24	581.18	636.19	433.83	361.14	385.70
30	366.10	440.75	381.82	411.28	596.92	414.23	415.21	360.21	371.01
31		436.29	379.96	414.55	576.60	413.56	413.56	358.23	360.20
33	509.20	472.76	401.84	448.13	609.67	420.55	442.22	395.93	383.12
36	364.26	467.94	421.53	466.95	628.88	452.14	477.81	381.05	401.78
37		446.80	413.15	444.82	607.11	438.88	421.07	397.32	394.35
39	363.66	457.42	441.63	452.48	626.18	450.51	409.06	390.31	413.01
40		444.22	425.60	426.58	609.77	440.30	422.67	381.52	407.97
43	348.90	462.81	458.88	431.38	644.48	459.86	414.69	376.39	405.85
45	358.78	457.31	466.09	441.70	634.87	454.38	438.78	385.12	400.73
47	346.69	433.86	455.65	427.91	609.19	429.90	434.85	375.41	388.29
50	352.38	468.97	474.85	475.83	640.41	464.07	452.31	394.51	396.47
51	348.63	457.06	451.20	427.75	599.67	424.82	437.52	380.87	395.52

Table V.3: Redox potential levels experiment with sand tea, nut with TW

mV	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
4		116.75	175.99	134.55	166.50	166.30	220.59	198.34	322.86
8		269.76	251.76	241.18	242.57	252.95		226.35	
9	276.85	191.44	221.58	188.58	221.39	222.27	284.83	231.93	189.96
11	320.77	234.73	247.39	308.11	242.94	253.92	310.19	226.23	243.63
12	303.73	202.92	218.82	193.94	217.34	279.44	310.64	231.16	301.16
15	320.01	208.37	292.79	315.77	242.89	297.13	326.23	222.57	289.93
16	274.32	170.27	173.34	191.00	212.62	257.36	311.51	257.06	255.87
17		230.22	279.65	254.94	243.42	273.94	272.07	247.26	304.37
18	349.48	237.20	238.49	325.40	209.85	237.50	342.25	218.77	268.62
19		278.07	266.21	264.81	273.28	255.94	282.85	304.18	263.92
20		269.85	303.72	296.55	303.13	312.06	272.40	270.34	270.44
22	288.11	258.17	261.31	258.46	243.73	268.77	283.79	245.50	277.80
24	240.73	204.71	230.09	277.40	234.38	278.98	206.39	206.86	206.67
26		206.46	215.24	211.30	226.43	217.65	272.88	246.39	264.38
27	198.29	184.86	183.42	188.37	190.54	210.01	195.40	195.22	194.68
29		175.68	182.74	178.35	186.65	191.86	221.14	200.07	239.61
30	240.52	168.64	163.71	180.57	185.58	177.63	241.24	198.38	241.48
31		169.65	170.97	169.32	175.34	220.39	191.56	169.90	169.74
33	177.66	170.19	169.86	172.24	175.20	170.44	170.44	170.11	169.45
36	167.67	165.29	162.92	168.26	167.24	165.72	166.73	168.09	170.04
37		177.45	175.33	180.74	182.35	180.40	183.36	184.46	183.11
39	171.19	171.11	177.45	177.11	178.12	175.16	173.47	176.18	176.35
40		180.91	181.08	181.33	179.57	180.41	181.00	182.51	182.26
43	180.83	182.64	183.54	183.87	182.88	180.75	182.55	182.55	167.12
45	188.58	182.66	185.58	185.58	185.99	171.08	170.99	177.16	168.33
47	176.58	174.12	179.20	177.81	178.22	175.76	180.52	178.88	178.14
50	169.01	167.08	166.74	168.17	169.77	171.28	176.33	171.70	172.12
51	185.06	177.50	184.25	182.30	178.80	175.79	171.57	181.57	169.94

Table V.4: TOC levels experiment with sand tea, nut with TW

ppm	Initial	Sand-L	Sand-H	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	1.75	2.05	1.98	3.83	6.67	9.86	30.00	86.91	120.14
4	2.55	2.51	1.76	3.27	8.39	7.65	10.62	46.93	83.01
8	3.15	2.96	2.11	4.29	8.24	9.32	0.61	67.48	113.69
11	3.00	3.10	2.32	4.02	8.91	12.09	3.45	52.15	85.21
12	1.76	2.38	1.75	3.52	5.48	6.69	6.45	47.09	74.90
15	1.77	2.35	2.60	3.40	5.85	6.52	5.68	46.02	66.21
16	2.21	2.21	1.58	4.20	6.98	7.39	6.99	35.22	59.16
18	2.07	3.26	4.19	4.10	6.32	7.56	7.25	31.72	60.67
20	2.45	3.30	4.06	4.16	6.27	6.93	6.80	50.28	78.58
22	2.16	2.93	3.44	4.95	7.86	9.04	8.11	14.70	34.36
24	1.46	2.16	2.29	3.61	7.15	7.20	5.93	30.46	47.26
27	2.30	3.44	3.15	4.09	5.86	6.69	6.96	27.16	46.76
30	1.72	2.99	3.73	4.68	6.25	8.52	7.61	37.96	38.86
33	5.85	4.13	4.72	6.30	7.08	9.06	10.02	35.27	27.34
36	6.66	7.63	6.85	6.46	9.15	10.29	7.77	30.57	21.70
39	3.88	4.58	4.86	6.51	8.34	8.23	6.89	21.88	18.06
43	2.10	3.26	4.20	4.18	5.70	6.96	5.83	33.41	19.78
45	1.39	3.62	5.80	1.72	8.18	6.69	5.64	38.31	20.78
47	3.39	2.91	4.49	6.66	3.96	5.23	3.58	28.04	12.07
51	2.27	3.12	4.12	5.02	3.74	6.11	6.30	12.77	18.69

Table V.5: Nitrate levels experiment with sand tea, nut with TW

mmol/l* 10^-3	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	516.64	132.31	245.41	0.00	0.81	0.00	0.00	0.00	0.00
4	509.71	294.46	339.00	0.00	0.81	0.00	0.00	0.00	0.00
9	526.49	360.78	363.85	0.00	0.65	0.00	0.00	0.00	0.00
12	506.96	380.46	355.13	0.00	0.65	0.00	0.00	0.00	0.00
15	522.13	399.67	376.91	0.97	0.00	0.00	0.00	0.00	0.00
16	523.10	450.01	366.59	0.00	0.00	0.00	0.00	0.00	8.07
18	524.87	420.64	339.97	0.00	0.00	0.00	0.00	0.00	0.16
20	510.19	427.58	343.68	0.00	0.00	0.00	0.00	0.00	0.65
22	502.12	450.49	407.09	0.00	0.00	0.00	0.00	0.00	0.32
24	503.74	439.20	337.87	0.00	0.00	0.16	0.00	0.32	0.48
27	507.77	422.74	335.12	0.16	0.00	2.90	0.16	0.32	0.65
30	492.60	414.03	331.58	0.00	0.00	1.94	0.48	0.16	0.16
33	529.55	425.16	365.46	0.00	0.00	5.16	0.16	0.00	0.00
36	492.12	417.25	325.28	0.00	0.16	0.65	0.16	0.00	0.65
37	484.37	326.57	296.56	0.00	0.00	0.16	0.00	0.00	0.00
40	509.54	306.57	389.02	0.00	0.00	0.32	0.32	0.00	0.00
43	530.52	364.97	385.14	0.00	0.00	0.16	0.16	0.00	0.48
45	486.79	332.70	371.27	0.00	0.16	0.97	1.29	0.65	0.16
47	518.10	329.32	368.52	0.00	0.16	0.00	0.00	0.32	1.94
51	524.55	361.75	318.99	0.00	0.00	0.16	0.00	2.58	0.16

Table V.6: Nitrite levels experiment with sand tea, nut with TW

mmol/l * 10^-3	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	3.24	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	19.60	0.00	0.00	0.00	0.00	0.00	2.55
18	0.00	0.00	89.04	0.00	0.00	0.00	0.00	0.00	2.67
20	0.00	4.10	247.26	3.30	2.84	2.90	2.44	2.32	3.98
22	0.00	3.93	84.87	3.24	2.78	2.84	3.01	5.41	4.04
24	0.00	4.15	311.84	3.35	3.01	2.78	2.55	4.04	4.27
27	2.55	2.78	5.70	2.55	2.38	2.32	2.44	2.55	2.44
30	3.81	3.01	5.93	2.72	2.38	2.27	2.50	2.44	2.32
33	3.01	8.85	4.27	2.78	2.44	2.32	2.32	2.67	2.27
36	2.67	2.50	344.67	2.21	2.55	2.55	2.44	2.38	2.44
37	2.21	22.29	176.04	2.80	2.38	2.32	2.44	2.55	2.32
40	0.00	2.15	19.54	0.00	0.00	2.78	2.27	0.00	0.00
43	2.32	19.60	12.39	0.00	0.00	0.00	0.00	0.00	2.32
45	3.87	3.30	2.21	0.00	2.21	2.67	2.55	2.55	2.32
47	0.00	0.00	153.28	0.00	0.00	0.00	0.00	3.24	2.32
51	0.00	2.67	309.38	0.00	0.00	0.00	0.00	0.00	0.00

Table V.7: Ammonium levels experiment with sand tea, nut with TW

mmol/l * 10^-3	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	6.14	0.00	0.00	0.00	2.54	0.00
9	0.61	0.00	0.00	0.00	0.00	0.00	0.00	2.16	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.09	0.00
15	0.00	0.00	0.00	1.46	0.00	0.00	2.30	2.09	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.89	0.75	0.75
18	0.00	0.00	0.47	0.00	0.00	2.58	0.75	0.00	1.03
20	0.00	6.25	5.61	0.00	0.00	0.00	0.00	0.00	3.01
22	0.00	5.96	6.39	0.00	0.00	0.00	1.81	5.82	4.98
24	0.00	6.67	4.41	0.00	0.00	0.00	0.00	10.96	3.85
27	1.74	1.67	3.01	4.48	19.28	7.87	10.05	14.70	1.17
30	0.75	4.84	5.12	0.00	14.42	6.60	7.16	5.05	0.75
33	1.60	1.31	3.71	0.00	23.22	6.32	3.71	3.99	0.00
36	0.00	1.53	1.74	2.37	19.98	1.67	4.55	0.00	0.00
37	0.75	0.00	11.10	0.00	13.57	2.86	5.47	0.61	1.60
40	0.00	0.00	4.70	0.00	0.00	1.10	0.00	0.00	0.00
43	0.33	0.00	1.67	0.00	10.82	2.44	7.44	0.61	1.57
45	4.13	4.27	0.00	0.00	18.78	0.00	0.00	0.00	0.00
47	0.00	1.46	1.74	0.00	16.46	2.09	1.74	0.61	0.00
51	0.00	0.00	1.60	0.00	10.33	1.53	0.89	1.17	1.31

Table V.8: TN levels experiment with sand tea, nut with TW

mmol/l * 10^-3	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	516.64	132.31	245.41	0.00	0.81	0.00	0.00	0.00	0.00
4	509.71	294.46	339.00	6.14	0.81	0.00	0.00	2.54	0.00
9	527.10	360.78	363.85	0.00	0.65	0.00	0.00	2.16	0.00
12	506.96	380.46	355.13	0.00	0.65	0.00	0.00	2.09	0.00
15	522.13	399.67	380.15	2.42	0.00	0.00	2.30	2.09	0.00
16	523.10	450.01	386.19	0.00	0.00	0.00	0.89	0.75	11.37
18	524.87	420.64	429.48	0.00	0.00	2.58	0.75	0.00	3.86
20	510.19	437.92	596.55	3.30	2.84	2.90	2.44	2.32	7.63
22	502.12	460.38	498.34	3.24	2.78	2.84	4.82	11.24	9.34
24	503.74	450.02	654.12	3.35	3.01	2.94	2.55	15.33	8.60
27	512.06	427.19	343.83	7.20	21.66	13.09	12.65	17.57	4.26
30	497.17	421.87	342.62	2.72	16.80	10.80	10.14	7.65	3.24
33	534.16	435.32	373.44	2.78	25.66	13.80	6.20	6.66	2.27
36	494.79	421.27	671.69	4.58	22.69	4.87	7.15	2.38	3.08
37	487.34	348.86	483.71	2.80	15.95	5.35	7.91	3.16	3.92
40	509.54	308.72	413.25	0.00	0.00	4.21	2.59	0.00	0.00
43	533.17	384.57	399.20	0.00	10.82	2.60	7.60	0.61	4.38
45	494.80	340.27	373.48	0.00	21.15	3.64	3.84	3.20	2.49
47	518.10	330.77	523.54	0.00	16.62	2.09	1.74	4.17	4.26
51	524.55	364.42	629.97	0.00	10.33	1.69	0.89	3.76	1.48

Table V.9: Phosphate levels experiment with sand tea, nut with TW

mg/l	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1		0.47							
4	0.67	0.43							
9	0.71	0.29							
12	0.72	0.22							
15	0.22	0.07							0.15
16	0.23	2.07	2.32						4.52
18	0.24	5.07	12.59						12.55
20	3.79	18.00	17.97	2.15	0.90	0.13	0.26		28.41
22	3.37	18.56	18.61	4.22	5.29	1.26	1.25		27.80
24	3.30	17.85	18.63	14.26	15.26	4.26	2.36		26.43
27	3.02	18.44	19.24	55.35	39.06	8.53	6.62	0.34	24.62
30	0.93	18.20	18.89	0.27	0.25	33.44	3.69	0.23	25.53
33	0.25	18.84	19.40	0.59	1.41	1.17	43.25	13.57	37.09
36	2.61	31.16	24.87	4.96	14.16	5.97	17.71	42.25	48.64
37	0.25	17.37	18.18	9.32	0.35	1.18	15.43	17.09	22.69
40	1.55	19.27	19.31	0.24	8.60	0.58	34.40	13.43	21.42
43	2.66	17.68	18.34	0.36	10.38	7.91	30.33	10.69	20.79
45	1.78	16.07	18.55	47.28	25.24	34.40	7.78	8.86	23.25
47	1.69	17.86	19.03	0.39	11.72	16.63	8.23	5.20	21.10
51	2.92	18.51	19.03	24.10	10.22	26.93	6.57	3.28	20.85

Table V.10: Potassium levels experiment with sand tea, nut with TW

mg/l	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	30.25	1.93	1.47	3.52	3.41	1.65	6.10	9.02	2.11
4	31.66	2.04	1.84	2.17	2.02	1.92	4.26	7.34	2.59
9	31.43	1.59	2.44	2.13	1.77	1.79	3.74	6.69	2.54
12	31.46	5.40	8.34	2.32	4.77	8.99	3.77	6.29	3.25
15	31.35	11.47	13.34	1.43	7.33	3.72	9.43	8.76	3.75
16	32.23	12.18	15.56	6.05	15.85	7.16	4.48	6.57	4.37
18	34.13	12.40	14.60	10.40	19.78	6.68	6.30	8.45	12.46
20	32.58	12.05	16.25	11.27	18.93	11.34	7.16	28.71	10.46
22	30.41	11.51	15.55	11.33	13.62	11.56	7.88	15.85	15.83
24	30.16	11.90	22.14	11.43	12.58	11.74	9.82	16.43	20.11
27	30.87	13.11	24.43	20.06	16.06	25.00	14.63	19.68	23.10
30	36.43	9.50	25.28	20.47	18.96	27.30	15.47	13.31	25.61
33	33.93	18.00	27.32	22.17	22.14	30.33	18.37	17.08	27.83
36	33.33	22.58	28.78	23.80	22.75	28.94	20.63	19.76	28.95
37	32.50	23.63	26.70	22.63	21.58	28.66	20.31	20.12	27.96
40	31.54	25.77	26.07	23.51	22.54	30.48	21.67	21.80	28.73
43	30.41	26.66	26.15	25.09	24.49	29.97	22.73	24.43	28.65
45	30.13	27.37	28.89	25.95	25.17	30.69	23.66	26.36	29.60
47	31.22	28.16	30.27	25.23	25.19	31.29	23.21	27.03	30.31
51	31.47	31.33	30.80	27.50	27.26	31.94	25.19	28.46	30.91

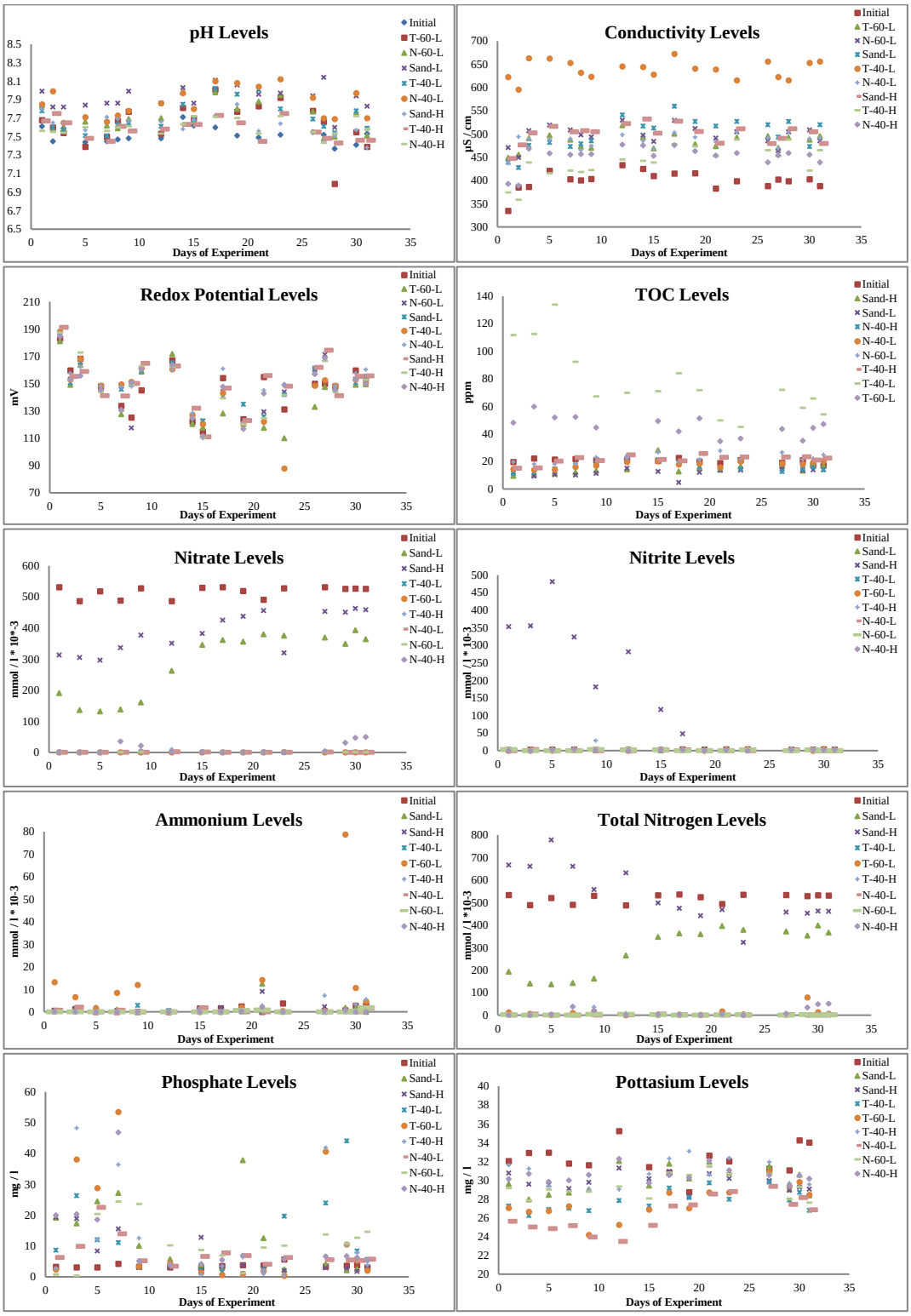


Figure V.2: Columns experiment with tea, nut and sand details with GW

Table V.11: pH levels experiment with sand tea, nut with GW

Days	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
1	7.61	7.68	7.84	7.99	7.78	7.85	7.80	7.67	7.56
2	7.45	7.60	7.58	7.82	7.60	7.99	7.65	7.75	7.55
3	7.59	7.54	7.58	7.82	7.57	7.65	7.65	7.65	7.54
5	7.44	7.39	7.66	7.84	7.51	7.71	7.57	7.48	7.60
7	7.45	7.50	7.62	7.86	7.51	7.66	7.71	7.45	7.56
8	7.47	7.67	7.59	7.86	7.63	7.73	7.69	7.61	7.60
9	7.48	7.77	7.69	7.99	7.65	7.78	7.67	7.56	7.61
12	7.48	7.53	7.70	7.86	7.61	7.86	7.66	7.58	7.53
14	7.71	7.81	7.98	8.03	7.85	7.97	7.58	7.63	7.63
15	7.62	7.70	7.68	7.86	7.68	7.80	7.68	7.63	7.72
17	7.60	8.01	7.98	8.11	8.02	8.10	7.73	7.73	7.71
19	7.51	7.77	7.80	8.06	7.96	8.08	7.85	7.65	7.70
21	7.49	7.83	7.88	7.96	8.01	8.04	7.56	7.45	7.53
23	7.52	7.92	7.95	7.97	7.80	8.12	7.64	7.75	7.72
26	7.55	7.78	7.77	7.94	7.69	7.92	7.54	7.55	7.55
27	7.52	7.66	7.48	8.14	7.61	7.70	7.45	7.48	7.43
28	7.37	6.99	7.50	7.60	7.54	7.69	7.51	7.43	7.53
30	7.41	7.55	7.76	7.94	7.78	7.97	7.57	7.46	7.72
31	7.53	7.39	7.51	7.83	7.59	7.70	7.38	7.46	7.58

Table V.12: Conductivity levels experiment with sand tea, nut with GW

µS/cm	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
1	334.73	448.86	470.32	439.10	622.48	437.15	446.91	373.75	392.28
2	384.98	455.10	449.26	427.83	595.36	494.06	476.53	357.70	388.87
3	386.14	490.66	507.11	475.17	662.91	488.72	502.27	438.40	468.40
5	420.66	497.11	519.37	481.63	662.61	488.40	516.47	414.85	458.40
7	402.27	487.44	508.73	472.92	652.93	489.37	504.86	420.66	455.50
8	400.33	472.92	498.08	478.72	631.64	465.18	506.79	417.75	456.47
9	403.20	470.01	493.24	485.50	622.93	474.85	504.86	421.62	456.47
12	433.24	518.40	530.02	540.66	645.19	498.08	522.28	444.85	476.79
14	424.53	492.62	497.42	516.60	644.14	484.95	531.94	441.79	475.36
15	409.67	468.96	484.26	512.95	627.71	467.05	501.47	438.36	452.70
17	414.86	500.72	529.34	559.87	672.44	502.63	527.44	477.83	475.92
19	415.35	478.38	511.80	527.08	640.71	492.70	505.11	472.65	463.10
21	382.59	473.26	491.00	516.63	638.84	483.12	480.16	451.58	453.55
23	398.21	492.51	504.30	526.89	615.30	504.30	511.17	487.60	459.11
26	387.92	495.19	485.35	519.80	655.62	491.26	479.45	464.68	439.09
27	401.74	469.87	464.03	493.23	622.68	468.90	490.31	452.35	454.30
28	398.21	492.51	504.30	526.89	615.30	504.30	511.17	487.60	459.11
30	402.27	487.44	508.73	472.92	652.93	489.37	504.86	420.66	455.50
31	387.92	495.19	485.35	519.80	655.62	491.26	479.45	464.68	439.09

Table V.13: Redox potential levels experiment with sand tea, nut with GW

mV	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
1	182.94	180.97	187.37	187.37	188.27	185.56	191.13	187.12	184.42
2	159.67	149.11	153.37	150.61	152.89	157.54	155.34	152.26	152.81
3	168.31	163.77	165.60	165.84	168.15	162.26	158.92	172.61	155.65
5	147.83	146.43	148.28	146.35	148.57	148.35	141.10	143.69	145.61
7	133.70	127.63	148.13	145.98	149.53	147.76	140.95	130.00	130.81
8	125.19	150.20	117.57	149.39	151.31	147.98	150.20	149.09	150.72
9	145.20	158.61	161.45	159.53	161.45	162.30	164.91	162.76	160.30
12	166.99	171.59	163.91	165.22	160.53	160.15	162.84	162.30	163.61
14	122.19	120.33	124.66	125.35	127.21	128.60	132.00	124.74	124.50
15	114.85	117.80	122.52	122.83	120.43	110.21	110.98	110.36	111.68
17	154.03	128.22	144.13	140.78	143.19	160.97	146.62	139.06	147.72
19	124.01	120.46	122.75	134.91	120.75	121.49	122.98	120.08	116.82
21	154.90	117.50	129.35	125.81	122.20	145.05	155.82	124.58	142.74
23	131.14	109.98	143.83	141.95	87.89	148.38	148.06	141.56	149.08
26	149.83	132.85	160.25	161.23	148.52	157.21	161.97	157.29	156.88
27	149.84	147.59	171.20	167.99	152.41	168.31	174.49	166.38	168.87
28	147.83	146.43	148.28	146.35	148.57	148.35	141.10	143.69	145.61
30	159.67	149.11	153.37	150.61	152.89	157.54	155.34	152.26	152.81
31	153.98	149.53	155.11	155.34	150.36	160.31	155.79	152.77	149.98

Table V.14: TOC levels experiment with sand tea, nut with GW

ppm	Initial	Sand-H	Sand-L	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	19.72	9.50	12.19	11.43	14.58	19.74	15.11	111.55	48.17
3	22.31	10.33	9.29	12.40	13.93	18.07	15.34	112.43	59.81
5	21.47	11.54	10.40	14.60	14.22	18.04	20.21	133.70	51.87
7	21.86	12.23	10.13	17.49	16.12	18.25	22.85	92.22	52.35
9	20.87	13.34	11.45	19.15	17.15	22.98	20.65	67.12	44.65
12	21.26	14.40	15.07	20.00	19.62	24.20	24.72	69.68	22.98
15	20.44	28.09	12.82	21.34	20.18	26.78	21.52	70.89	49.31
17	22.64	12.83	4.92	19.42	17.95	19.64	20.45	83.96	41.88
19	20.10	15.24	12.22	16.27	18.53	21.43	25.72	71.72	51.29
21	18.81	13.92	13.86	15.05	15.67	27.79	23.01	49.64	34.61
23	21.12	16.41	13.74	14.48	20.14	21.94	23.24	45.06	36.76
27	19.08	16.67	14.54	12.67	18.60	26.57	23.27	71.87	43.67
29	21.15	13.25	13.59	14.71	18.14	21.56	23.39	58.89	35.00
30	17.22	17.65	13.95	16.19	19.57	21.98	20.99	65.64	44.31
31	17.17	17.01	14.06	13.89	20.18	24.91	22.51	54.00	47.20

Table V.15: Nitrate levels experiment with sand tea, nut with GW

mmol/l * 10-3	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	530.73	189.91	313.31	0.00	0.00	0.00	0.00	0.15	1.42
3	486.05	136.18	304.90	0.00	0.00	0.00	0.56	0.00	0.00
5	518.02	131.68	296.17	0.00	0.00	0.98	0.00	0.00	0.23
7	488.23	137.42	336.90	0.00	0.00	0.00	0.00	0.34	34.87
9	527.94	160.09	376.98	0.00	0.00	8.37	0.00	0.58	20.80
12	486.39	261.77	350.76	0.00	0.00	9.42	2.10	2.02	2.31
15	529.26	345.34	382.11	0.00	0.00	0.98	0.92	0.87	1.47
17	531.38	361.41	425.46	0.00	0.00	0.00	0.00	0.69	0.45
19	518.82	355.57	437.61	0.00	0.29	0.23	0.19	0.00	0.00
21	490.60	379.54	455.96	0.00	0.11	1.27	0.16	0.23	1.15
23	527.89	375.28	319.57	0.00	1.11	0.00	0.21	1.29	0.00
27	531.38	369.27	452.85	0.00	0.35	1.34	1.29	0.15	3.76
29	525.45	349.21	450.59	0.00	0.00	0.00	0.00	0.48	30.53
30	526.68	392.65	462.79	0.00	0.13	1.34	0.00	0.00	46.63
31	525.92	363.94	458.22	0.00	0.21	0.81	0.21	0.45	48.78

Table V.16: Nitrite levels experiment with sand tea, nut with GW

mmol/l* 10^-3	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	2.32	3.01	352.68	0.00	0.00	0.00	0.00	3.01	0.00
3	2.55	3.12	355.20	0.00	0.00	0.00	3.12	0.00	0.00
5	2.61	4.15	480.87	0.00	0.00	2.10	0.00	0.00	2.10
7	2.44	4.61	323.28	0.00	2.21	0.00	0.00	0.00	3.35
9	2.32	2.55	181.54	0.00	2.38	28.26	0.00	3.09	1.09
12	2.21	4.15	281.52	0.00	0.00	0.00	0.00	2.15	0.00
15	2.32	2.32	116.73	0.00	2.55	2.10	2.32	2.32	2.32
17	3.70	2.44	47.57	0.00	2.78	0.00	2.21	0.00	2.80
19	3.07	2.72	3.01	0.00	0.00	0.00	0.00	0.00	0.00
21	3.58	3.93	3.35	0.00	3.12	0.00	0.00	0.00	2.38
23	3.24	3.93	3.12	0.00	2.55	0.00	0.00	2.10	0.00
27	2.55	2.44	2.32	0.00	2.78	0.34	2.21	0.00	2.55
29	2.55	2.67	2.32	0.00	0.00	0.00	0.00	2.32	2.55
30	3.75	4.50	0.00	0.00	2.55	2.21	0.00	0.00	2.21
31	2.55	3.70	2.21	0.00	2.32	2.38	0.00	0.00	2.44

Table V.17: Ammonium levels experiment with sand tea, nut with GW

mmol/l * 10^-3	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	0.47	0.47	0.68	0.00	13.15	0.00	0.68	0.00	0.00
3	1.17	1.74	1.46	0.00	6.53	0.00	2.02	0.00	0.00
5	0.00	1.95	1.60	0.00	1.60	0.00	0.00	0.00	0.00
7	0.00	0.96	0.61	0.00	8.50	0.00	0.68	0.00	0.00
9	0.00	0.00	0.00	2.86	11.95	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.61	0.00	0.00	0.00	0.00	0.00
15	1.60	0.00	0.00	0.00	0.00	1.71	1.85	0.00	0.00
17	1.57	0.00	1.99	0.00	0.00	0.00	0.00	0.00	0.00
19	2.37	1.17	1.10	0.00	1.88	0.00	0.00	0.61	0.33
21	0.00	12.44	9.06	0.00	14.20	2.79	0.00	0.89	2.16
23	3.78	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00
27	0.33	0.00	2.30	0.00	0.33	7.25	0.00	0.00	0.33
29	0.74	1.85	0.61	0.00	78.79	0.00	0.00	0.00	0.74
30	2.65	1.39	0.33	0.00	10.68	3.22	0.00	0.33	0.00
31	3.01	0.00	0.75	0.00	4.55	5.54	1.60	1.85	0.00

Table V.18: TN levels experiment with sand tea, nut with GW

mmol/l * 10^-3	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	533.52	193.39	666.67	0.00	13.15	0.00	0.68	3.16	1.42
3	489.78	141.04	661.56	0.00	6.53	0.00	5.71	0.00	0.00
5	520.63	137.78	778.64	0.00	1.60	3.08	0.00	0.00	2.32
7	490.67	143.00	660.79	0.00	10.71	0.00	0.68	0.34	38.22
9	530.26	162.64	558.51	2.86	14.33	36.63	0.00	3.67	21.88
12	488.60	265.93	632.28	0.61	0.00	9.42	2.10	4.17	2.31
15	533.18	347.66	498.84	0.00	2.55	4.79	5.09	3.20	3.79
17	536.65	363.85	475.02	0.00	2.78	0.00	2.21	0.69	3.25
19	524.26	359.47	441.73	0.00	2.17	0.23	0.19	0.61	0.33
21	494.18	395.91	468.38	0.00	17.44	4.07	0.16	1.12	5.69
23	534.91	379.21	323.02	0.00	4.00	0.00	0.21	3.39	0.00
27	534.26	371.71	457.47	0.00	3.47	8.93	3.50	0.15	6.64
29	528.75	353.73	453.52	0.00	78.79	0.00	0.00	2.81	33.82
30	533.09	398.53	463.11	0.00	13.36	6.77	0.00	0.33	48.84
31	531.48	367.64	461.18	0.00	7.09	8.73	1.81	2.30	51.21

Table V.19: Phosphate levels experiment with sand tea, nut with GW

Days mg/l	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	3.24	19.17	19.33	8.61	2.36	2.68	6.21	0.71	19.97
3	3.04	17.29	18.85	26.33	38.04	48.19	9.89	0.36	20.33
5	3.06	24.41	8.36	12.02	28.77	12.04	22.51	20.26	18.48
7	4.17	27.16	15.50	11.12	53.40	36.41	13.96	24.33	46.79
9	3.27	10.00	3.39	2.96	3.31	12.52	5.20	23.54	5.06
12	3.08	5.71	2.93	3.98	4.26	4.22	3.38	10.11	3.95
15	2.95	3.95	12.80	1.98	1.28	0.69	6.56	8.59	4.11
17	3.51	2.86	3.28	1.54	0.56	3.38	7.75	6.87	5.42
19	3.72	37.81	3.72	1.00	0.96	6.30	6.85	1.07	6.82
21	3.65	12.50	2.94	2.34	1.78	0.97	4.05	9.45	1.82
23	5.60	2.35	1.97	19.68	0.33	0.39	6.22	10.05	5.74
27	3.30	4.06	2.87	23.89	40.59	41.89	5.54	13.63	6.62
29	3.57	2.09	2.82	44.10	10.45	10.26	5.43	10.71	6.64
30	3.77	2.56	1.76	8.38	5.31	7.85	5.27	12.63	6.26
31	3.08	2.78	2.81	5.59	1.97	3.82	5.74	14.57	5.30

Table V.20: Potassium levels experiment with sand tea, nut with GW

Days mg/l	Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
1	32.02	29.60	30.74	27.25	27.05	31.58	25.61	29.12	30.12
3	32.90	27.95	29.56	26.24	26.64	31.22	25.00	27.83	30.67
5	32.93	28.44	29.49	26.87	26.72	29.38	24.83	28.98	29.81
7	31.76	28.67	29.11	27.02	27.22	30.01	25.16	28.82	29.98
9	31.60	29.02	29.76	26.74	24.18	29.00	23.94	28.74	30.56
12	35.22	32.04	31.27	27.81	25.25	29.02	23.47	29.30	32.27
15	31.39	29.42	30.15	27.24	26.87	30.65	25.19	28.03	29.68
17	30.82	31.74	30.93	29.16	28.66	32.29	27.24	30.77	30.53
19	28.73	30.16	28.30	28.15	27.01	33.08	27.37	30.53	30.25
21	32.60	31.93	30.69	29.71	28.69	30.57	28.53	31.41	32.07
23	32.00	30.80	30.16	27.97	28.68	32.35	28.78	30.69	31.05
27	31.22	31.45	29.94	29.78	30.84	31.90	29.34	30.64	30.52
29	31.05	29.00	28.92	27.87	29.36	29.68	27.42	27.98	29.36
30	34.24	30.58	29.24	28.69	29.80	29.28	28.15	29.27	30.49
31	33.99	28.38	29.04	26.78	28.44	29.54	26.83	27.60	30.15

Appendix VI
COLUMNS EXPERIMENT WITH TEA WASTE MATERIALS, HAZELNUT HUSK WASTES AND PERLITE

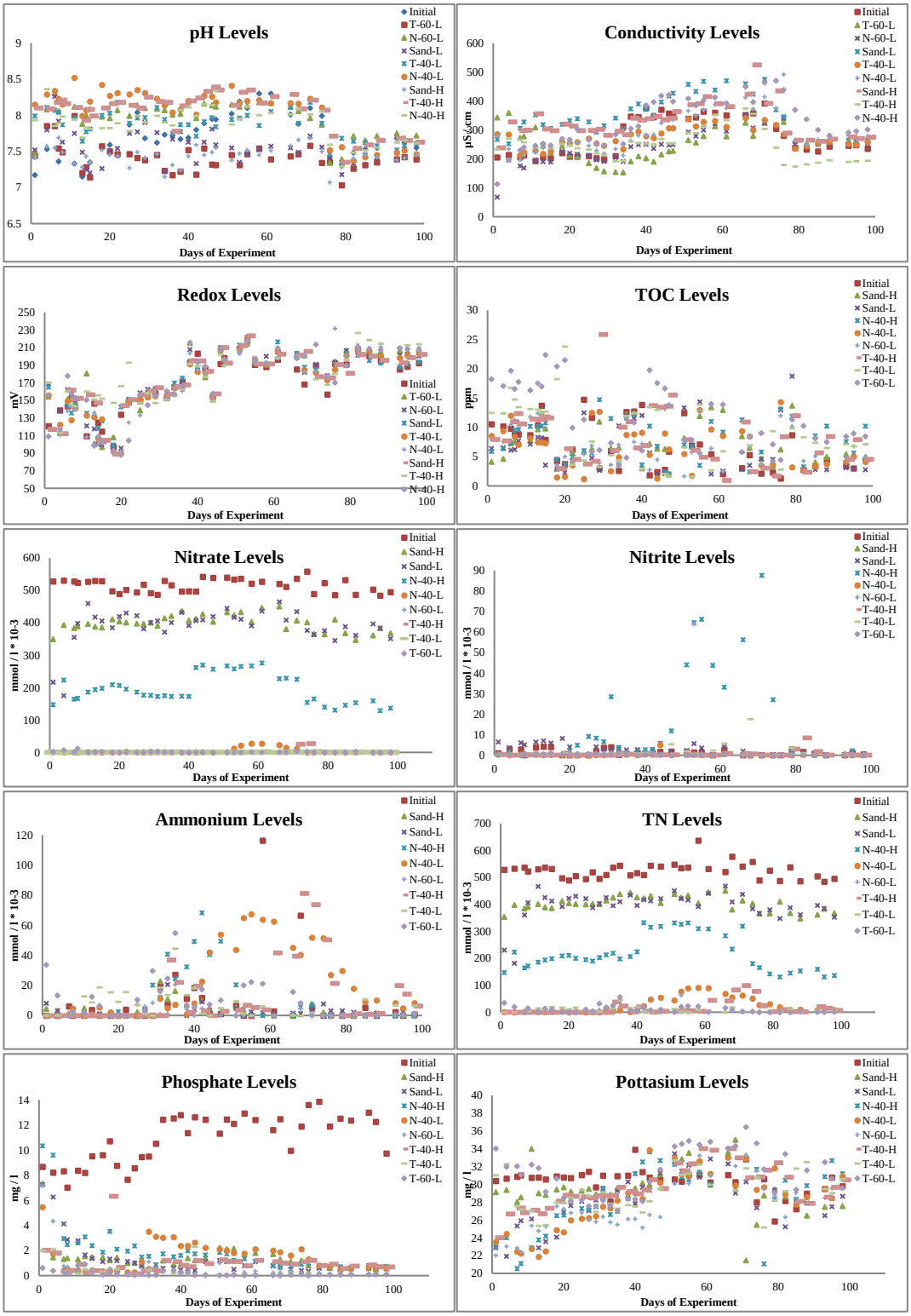


Figure VI.1: Columns experiment with tea, nut, sand and perlite details with GW

Table VI.1: pH levels experiment with sand tea, nut and perlite with GW

Days	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
1	7.17	7.44	7.46	7.52	7.99	8.15	7.41	8.10	7.93
4	7.53	7.85	8.08	7.80	8.11	8.29	7.57	8.09	8.36
6		7.85	8.31	8.26	8.07	8.34	7.55	8.18	7.85
7	7.36	7.54	8.06	7.92	8.04	8.23	7.69	8.21	7.89
8		7.49	8.16	7.63	7.87	8.15	7.49	8.16	7.98
11	7.33	7.99	8.12	7.75	7.96	8.52	7.33	8.11	7.94
13	7.15	7.20	7.88	7.49	7.64	7.99	7.28	7.93	7.69
14		7.28	8.00	7.25	7.77	8.07	7.43	8.08	7.83
15	7.35	7.14	8.09	7.20	7.81	8.19	7.35	7.99	7.81
18	7.56	7.57	7.98	7.26	8.00	8.42	7.52	8.10	7.82
20	7.59	7.50	7.96	7.51	8.03	8.27	7.45	8.16	7.82
22	7.44	7.46	8.07	7.54	8.18	8.31	7.44	8.18	7.89
25	7.87	7.46	7.99	7.75	7.89	8.29	7.27	8.14	7.78
27	8.04	7.41	8.14	7.77	7.96	8.35	7.49	8.10	7.99
29	7.68	7.38	8.10	7.90	7.97	8.31	7.41	8.25	7.90
32	7.62	7.45	8.09	7.76	8.11	8.23	7.46	8.19	7.99
34	7.73	7.23	8.07	7.22	7.94	8.14	7.15	8.18	7.90
36	7.72	7.17	8.01	7.67	7.75	8.04	7.31	7.78	7.86
38	7.67	7.22	8.02	7.77	7.87	8.08	7.24	8.13	8.07
40	7.69	7.52	8.18	7.63	7.87	8.20	7.43	8.20	8.11
42	7.80	7.18	7.96	7.33	7.71	8.01	7.59	8.24	7.99
44	7.89	7.54	8.16	7.63	7.91	8.27	7.45	8.33	8.15
46	7.95	7.32	8.14	7.81	7.97	8.26	7.49	8.39	8.14
47	7.94	7.31	8.12	7.59	7.84	8.18	7.55	8.35	8.34
51	8.02	7.45	8.15	7.55	7.94	8.41	7.38	8.14	7.87
53	8.19	7.31	8.12	7.35	7.96	8.17	7.47	8.32	7.95
55	8.10	7.46	8.13	7.46	8.00	8.20	7.52	8.35	8.12
58	8.30	7.54	8.18	7.52	7.86	8.21	7.45	8.17	8.00
61	8.30	7.39	8.17	7.57	8.05	8.17	7.51	8.26	8.03
66	8.01	7.43	8.12	7.55	8.11	8.17	7.58	8.29	8.01
68	7.88	7.46	8.06	7.49	7.89	8.16	7.66	8.09	8.09
71	8.10	7.58	7.96	7.71	8.18	8.23	7.68	8.21	8.01
74	7.99	7.34	7.87	7.56	7.89	8.11	7.50	8.07	8.05
76	7.42	7.35	7.34	7.42	7.46	7.52	7.07	7.71	7.59
79	7.32	7.03	7.39	7.18	7.68	7.56	7.28	7.35	7.29
82	7.48	7.26	7.71	7.33	7.64	7.37	7.40	7.54	7.48
85	7.53	7.31	7.65	7.45	7.62	7.42	7.45	7.59	7.51
88	7.55	7.35	7.71	7.42	7.58	7.45	7.42	7.65	7.55
93	7.68	7.39	7.75	7.48	7.52	7.48	7.38	7.68	7.62
95	7.62	7.42	7.68	7.53	7.58	7.51	7.41	7.65	7.65
98	7.55	7.39	7.72	7.45	7.61	7.46	7.45	7.63	7.61

Table VI.2: Conductivity levels experiment with sand tea, nut and perlite with GW

Days µS/cm	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
1	204.84	342.87	67.31	267.13	285.07	279.09	238.23	231.25	112.26
4	213.71	358.82	196.47	252.18	284.07	234.24	327.92	269.12	196.17
7	206.24	273.11	175.54	306.00	212.71	195.47	297.03	265.13	229.26
8	212.58	277.09	168.36	327.92	222.08	212.02	299.02	257.16	240.22
11	192.78	308.99	194.48	351.84	227.26	221.88	355.83	238.23	245.20
13	205.74	245.20	189.59	317.95	216.90	238.23	303.01	250.19	253.18
15	201.75	221.48	189.29	305.00	225.27	237.23	297.03	237.23	246.20
18	220.49	244.21	225.27	318.95	258.16	267.13	319.95	251.18	269.12
20	207.43	212.51	219.49	331.91	254.17	257.16	312.97	245.20	266.13
22	207.33	207.03	215.90	338.88	258.16	256.17	297.03	236.23	303.01
25	209.33	185.01	210.42	327.92	270.12	245.20	298.02	231.25	266.13
27	202.55	168.46	196.47	293.04	253.18	228.26	302.01	218.10	252.18
29	198.56	157.30	190.59	313.97	245.20	218.39	281.08	213.71	244.21
32	209.72	154.01	204.84	340.88	261.15	234.24	295.03	191.19	284.07
34	312.54	152.62	190.49	304.00	233.24	211.92	283.07	191.59	251.18
36	346.10	190.35	241.54	372.73	284.94	262.26	341.17	259.30	303.69
38	344.83	208.84	248.89	389.33	294.39	280.54	333.95	253.84	312.19
40	375.19	202.43	250.37	371.23	278.11	282.07	338.54	251.36	385.09
42	344.86	190.19	236.23	350.84	272.11	284.07	346.86	249.19	396.69
44	369.98	215.18	250.68	396.16	287.54	323.42	335.06	268.14	418.47
46	356.98	225.35	247.04	417.30	305.42	333.63	340.44	252.88	421.19
47	353.99	227.25	249.27	426.42	306.03	335.40	363.78	253.19	398.03
51	338.48	264.47	293.08	457.88	337.49	366.11	386.83	289.14	408.54
53	343.34	255.50	278.93	433.12	312.11	350.17	391.15	287.71	385.30
55	362.92	278.40	298.80	467.84	327.95	368.75	415.38	311.43	401.78
58	359.16	312.50	322.22	437.91	330.97	388.33	390.27	352.36	412.63
61	349.98	277.13	295.59	469.47	310.16	375.24	380.10	333.47	391.75
66	351.26	324.40	352.63	459.68	340.95	411.99	423.67	374.04	448.97
68	357.46	279.88	307.04	423.40	299.28	372.98	524.25	354.55	396.25
71	391.40	324.64	323.67	474.61	334.31	414.62	391.50	303.35	463.00
74	303.95	317.86	314.94	370.34	316.88	375.20	435.46	238.35	469.47
76	279.99	327.23	339.92	345.78	285.24	491.26	288.68	178.23	438.54
79	237.63	263.44	249.01	236.29	234.73	263.44	264.55	173.00	368.66
82	232.28	248.83	244.96	243.50	243.99	248.25	264.26	180.49	337.31
85	225.37	254.80	252.89	248.90	254.25	265.85	260.13	185.75	300.25
88	242.56	267.45	258.78	256.60	258.65	275.85	267.80	194.24	280.45
93	245.89	262.78	250.45	264.55	252.36	280.63	275.62	188.62	290.47
95	247.80	270.45	245.78	260.47	248.65	275.45	270.15	190.50	295.78
98	235.26	265.78	253.86	264.86	257.60	278.69	274.69	192.60	301.45

Table VI.3: Redox potential levels experiment with sand tea, nut and perlite with GW

Days mV	Initial	T-60-L	N-60-L	Sand-L	T-40-L	N-40-L	Sand-H	T-40-H	N-40-H
1	120.83	154.26	168.91	165.25	155.97	155.89	116.92	169.82	109.03
4	138.80	113.43	137.98	112.45	122.43	110.65	112.20	113.19	117.28
6	143.58	151.32	162.52	143.73	149.37	138.17	161.84	160.04	177.84
7	151.99	152.58	157.09	135.68	127.64	142.62	151.92	144.54	157.68
8	142.52	147.27	150.44	144.54	140.85	139.74	152.29	143.06	152.95
11	109.41	180.55	121.15	135.43	132.75	108.28	156.44	159.76	150.71
13	146.42	100.92	117.74	131.33	131.07	136.14	147.05	150.77	98.57
14	124.78	102.80	100.15	118.40	107.28	100.37	102.94	155.52	108.68
15	114.73	96.79	99.71	126.76	128.68	101.41	104.69	151.84	103.05
18	97.35	108.03	97.28	99.25	92.04	91.29	88.98	147.21	92.86
20	133.90	92.53	95.71	142.52	89.95	92.13	144.18	166.07	87.76
22	148.55	147.27	148.36	151.62	147.84	124.92	150.92	192.91	104.30
25	149.44	138.85	158.73	152.33	151.09	133.07	156.39	147.10	156.18
27	156.45	155.46	162.81	153.70	153.63	156.17	160.76	157.94	144.58
29	157.77	157.98	162.04	163.65	155.04	155.74	164.42	146.99	155.67
32	152.87	156.40	159.35	158.47	155.74	152.72	157.14	150.51	160.53
34	165.17	166.06	165.91	169.83	163.91	163.76	165.47	154.81	157.00
36	171.62	165.46	171.14	175.33	163.49	160.80	167.67	162.54	166.25
38	192.65	215.54	207.83	194.54	191.10	203.56	195.28	191.26	215.86
40	203.19	186.29	184.44	184.52	182.42	184.92	194.98	188.62	193.53
42	185.49	176.35	183.66	182.23	181.20	180.72	183.18	176.67	188.74
44	154.41	150.98	152.98	156.77	152.63	151.13	157.34	148.77	157.84
46	191.67	207.05	210.09	209.26	207.70	206.41	210.00	201.98	210.00
47	198.43	196.88	194.61	196.88	196.15	189.87	192.51	189.60	196.24
51	208.93	209.21	206.61	212.93	208.09	203.07	212.38	205.77	206.05
53	217.92	214.67	218.76	221.92	220.62	216.16	223.59	216.90	217.83
55	190.66	193.17	197.04	192.72	196.23	198.47	191.55	193.71	194.16
58	188.13	190.63	200.25	190.90	191.09	191.83	191.64	192.20	192.85
61	196.55	205.53	207.23	216.77	206.47	197.87	202.60	197.69	209.40
66	185.24	202.51	202.60	203.64	198.95	197.39	201.47	199.91	199.73
68	168.15	201.30	181.86	187.76	184.29	183.08	205.90	200.69	201.12
71	189.66	184.20	190.77	185.48	181.73	182.93	176.96	173.72	213.53
74	156.91	174.96	178.22	173.60	173.23	175.68	175.86	167.52	177.86
76	191.77	194.45	193.38	187.30	185.07	231.88	190.88	180.15	170.06
79	190.83	206.72	206.62	203.85	201.08	201.18	180.95	196.00	189.54
82	208.44	212.13	204.84	201.49	207.30	208.44	202.37	226.46	210.72
85	200.48	208.56	202.35	195.65	206.58	209.45	200.45	218.45	208.75
88	195.20	205.56	200.54	192.35	200.45	210.78	195.66	214.56	207.56
93	185.34	206.78	201.45	188.50	198.25	208.47	192.88	210.25	209.65
95	188.56	204.45	202.56	191.56	201.45	207.56	198.65	212.56	208.54
98	192.54	208.99	200.45	193.67	203.56	209.56	202.35	213.78	207.42

Table VI.4: TOC levels experiment with sand tea, nut and perlite with GW

Days ppm	Initial	Sand-H	Sand-L	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	10.55	4.12	6.41	5.88	8.55	7.88	7.95	12.45	18.22
4	10.19	4.64	6.54	6.38	9.38	7.56	7.70	12.37	17.06
6	9.80	7.31	6.15	7.32	12.00	10.51	10.98	16.73	19.69
7	8.93	7.57	6.22	9.51	8.42	11.32	12.38	14.74	16.58
8	8.80	7.29	7.70	9.64	7.07	10.37	10.62	13.43	17.76
11	8.70	7.76	7.17	10.16	8.15	11.80	11.45	13.11	16.33
13	10.39	9.66	8.30	10.17	7.49	12.41	11.41	13.03	17.52
14	13.74	12.41	8.22	10.21	7.42	11.96	11.83	12.52	16.97
15	7.88	9.83	3.59	10.79	7.29	11.78	11.63	12.69	22.39
18	4.32	2.98	2.90	4.61	1.47	3.58	2.98	18.23	20.40
20	3.76	3.37	2.29	3.66	1.57	5.00	6.37	23.77	21.50
22	6.21	5.67	4.31	4.95	3.52	4.18	4.55	3.60	9.96
25	14.76	5.85	12.52	6.04	1.21	5.45	3.77	4.32	11.36
27	11.69	6.38	4.80	5.61	12.53	6.88	4.22	7.56	5.84
29	3.03	10.97	2.69	14.72	12.70	3.92	25.85	3.24	6.12
32	5.94	6.43	5.88	11.51	2.54	3.62	6.04	5.24	7.35
34	2.57	6.54	10.28	3.66	4.90	7.00	10.04	4.32	3.63
36	12.66	12.27	11.98	11.62	8.76	11.59	10.99	11.94	4.83
38	12.72	12.46	11.54	12.25	8.85	7.53	6.50	12.40	10.82
40	13.88	3.03	12.05	3.61	9.13	7.20	6.91	2.87	6.03
42	1.85	7.19	5.68	10.21	5.34	2.74	13.73	13.42	19.76
44	2.40	6.38	2.03	6.77	1.27	4.51	13.45	12.99	17.58
46	2.79	6.33	2.07	2.36	8.99	13.18	13.61	2.04	16.67
47	5.92	2.03	1.87	2.09	6.27	1.81	15.54	1.63	13.81
51	12.45	8.16	13.33	7.04	5.46	1.68	7.89	6.53	7.94
53	6.93	6.67	6.85	6.71	1.74	5.75	6.14	1.56	7.85
55	7.10	11.93	14.37	3.53	12.96	12.93	4.94	13.85	5.64
58	5.42	13.42	2.05	11.03	9.66	6.42	4.60	2.65	13.99
61	2.02	12.96	2.54	9.19	8.56	2.49	0.96	5.91	13.90
66	3.08	8.50	2.83	11.30	9.38	5.89	10.92	5.65	5.79
68	5.28	6.50	3.51	2.39	8.46	6.69	2.43	6.91	8.45
71	2.15	4.68	4.58	5.82	3.51	3.74	3.08	9.00	9.00
74	2.31	3.39	4.57	3.63	3.66	4.20	1.73	9.35	7.10
76	1.29	6.23	12.98	13.18	14.32	11.98	8.39	2.78	4.44
79	8.71	13.73	18.74	11.88	3.20	10.20	12.00	9.81	11.33
82	2.73	3.09	2.73	3.27	2.65	4.28	2.36	8.31	10.27
85	3.45	4.05	2.81	8.56	3.45	5.12	5.65	7.35	8.14
88	4.45	4.98	2.78	10.25	3.75	7.45	8.45	6.45	7.52
93	3.42	5.45	3.12	7.56	4.12	4.89	4.56	7.12	8.45
95	3.01	5.12	2.96	5.62	4.99	6.78	7.89	6.78	8.99
98	4.45	4.98	2.78	10.25	4.12	4.89	4.56	7.12	8.45

Table VI.5: Nitrate levels experiment with sand tea, nut and perlite with GW

mmol/l *10-3	Initial	Sand-H	Sand-L	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	527.42	348.81	215.89	147.51	0.26	0.00	0.34	0.06	0.98
4	529.86	393.10	174.87	222.49	0.00	0.02	0.02	0.11	6.41
7	527.79	383.26	354.76	163.90	0.39	0.02	0.00	0.10	0.02
8	522.95	388.64	397.81	166.80	0.19	0.00	0.02	0.08	11.58
11	527.07	396.13	459.33	185.46	0.45	0.00	0.00	0.29	0.02
13	529.18	387.92	417.20	193.25	0.29	0.02	0.02	0.02	0.90
15	528.00	385.32	405.23	197.99	0.18	0.02	0.02	0.29	1.26
18	497.10	410.27	384.24	208.77	0.15	0.02	0.37	1.05	0.18
20	488.97	403.96	418.61	206.38	0.00	0.02	0.02	0.79	0.10
22	501.88	400.76	430.19	195.20	1.16	0.00	0.02	0.71	0.10
25	494.18	397.41	421.82	185.54	0.00	0.02	0.02	0.02	0.18
27	517.35	396.23	380.71	176.73	0.02	0.02	0.00	0.03	0.02
29	491.20	400.79	400.70	175.81	0.00	0.02	0.05	1.63	2.15
31	485.96	390.40	405.54	172.24	1.29	0.00	0.02	0.11	1.37
33	529.52	414.30	371.24	175.03	0.47	0.02	0.06	0.02	0.03
35	515.69	420.93	400.73	172.14	0.08	0.02	0.74	0.18	0.66
38	496.06	435.99	431.76	172.89	0.02	0.02	0.02	0.32	0.00
40	496.96	407.04	391.53	172.14	0.02	0.02	0.00	0.03	0.15
42	496.83	412.35	405.96	261.76	0.15	0.02	0.11	0.02	0.37
44	541.49	426.71	408.65	268.75	0.71	0.00	0.08	0.15	0.05
47	538.35	402.81	418.62	256.63	0.03	0.00	0.18	0.50	0.06
51	539.67	432.60	444.08	267.00	0.48	0.02	0.10	0.52	0.19
53	533.75	422.90	416.12	257.63	11.86	0.02	0.00	0.10	0.06
55	535.80	432.18	410.48	265.32	20.70	0.05	0.00	0.00	0.02
58	520.71	403.21	390.45	266.23	26.56	0.00	0.00	0.48	0.45
61	526.65	445.59	435.53	275.91	26.62	0.05	1.29	0.05	0.05
66	520.37	449.93	464.62	227.50	22.59	0.69	1.45	0.00	0.02
68	510.63	380.03	408.20	229.12	14.52	0.02	1.13	0.16	0.02
71	535.93	406.38	434.44	225.05	10.99	0.48	25.24	0.27	3.10
74	557.95	400.91	376.27	153.98	0.24	1.57	27.12	0.29	2.19
76	488.89	365.44	363.60	165.14	0.11	0.00	2.57	0.31	0.11
79	522.69	364.17	374.93	139.71	0.03	0.44	0.18	0.24	0.00
82	485.66	410.14	344.55	130.27	0.08	0.08	0.21	0.68	0.03
85	532.13	367.25	387.50	145.33	0.03	0.02	0.06	0.10	0.00
88	486.60	346.58	360.73	153.28	0.00	0.44	0.18	0.27	2.19
93	502.61	360.57	396.28	158.96	0.11	0.08	0.21	0.29	0.11
95	483.45	383.53	383.05	128.92	0.03	0.02	0.06	0.31	0.00
98	494.67	367.56	350.94	136.42	0.08	0.00	0.00	0.24	0.03

Table VI.6: Nitrite levels experiment with sand tea, nut and perlite with GW

mmol/l *10-3	Initial	Sand-H	Sand-L	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	1.01	0.75	6.36	0.13	0.24	0.02	0.33	0.00	0.35
4	2.84	1.47	3.57	0.62	0.00	0.00	0.00	0.00	0.00
7	2.85	0.31	5.96	0.41	0.25	0.09	0.00	0.00	0.00
8	0.00	0.51	5.12	0.30	0.38	0.00	0.15	0.62	0.54
11	3.77	0.55	6.35	0.38	0.28	0.00	0.00	0.47	0.36
13	4.20	0.47	6.91	0.24	0.00	0.00	0.00	0.28	0.34
15	3.96	0.71	5.92	0.61	0.15	0.00	0.00	0.39	0.94
18	0.03	0.72	8.10	0.25	0.17	0.00	1.01	0.86	0.33
20	0.03	0.57	4.00	2.14	0.00	0.00	0.00	0.88	0.49
22	0.03	0.62	0.04	4.81	0.17	0.00	0.00	0.96	0.44
25	0.03	0.38	0.04	9.08	0.00	0.00	0.07	0.50	0.62
27	1.83	0.43	3.96	8.32	0.00	0.00	0.00	0.54	0.70
29	3.67	0.25	2.83	6.57	0.00	0.00	0.07	0.42	0.47
31	3.92	1.66	2.63	28.38	0.75	0.10	0.00	0.64	0.66
33	0.12	0.53	2.96	3.72	0.71	0.09	0.65	0.24	0.32
35	0.66	0.99	2.56	1.17	0.27	0.20	0.49	0.56	0.87
38	0.99	0.50	2.66	1.71	0.08	0.26	0.07	0.82	0.18
40	0.84	0.53	1.10	2.67	0.57	0.22	0.70	0.90	0.46
42	0.96	0.37	2.03	2.81	0.39	0.14	0.00	0.40	0.54
44	1.59	0.33	0.60	5.74	4.90	0.00	0.16	0.20	0.20
47	1.79	0.67	1.67	11.88	0.00	0.00	0.16	5.26	0.00
51	1.54	0.35	2.25	43.92	0.47	0.00	0.00	1.88	0.00
53	1.39	0.38	5.55	64.56	0.23	63.72	0.00	0.54	0.00
55	1.37	0.33	3.48	66.13	0.19	0.00	0.00	0.00	0.36
58	0.00	0.00	0.00	43.73	0.00	0.00	0.00	2.10	0.00
61	2.68	0.60	3.95	33.13	0.83	0.10	0.88	5.66	0.23
66	0.00	0.69	1.87	56.23	0.44	0.00	0.74	0.00	0.00
68	0.00	0.00	0.00	0.00	0.00	0.00	0.52	17.36	0.00
71	0.00	0.00	0.00	87.48	0.00	0.00	0.00	0.00	0.00
74	0.00	0.00	0.00	26.96	0.00	0.00	0.00	0.00	0.00
76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
79	2.83	0.20	2.13	2.20	0.07	0.09	2.91	3.53	0.00
82	1.68	0.26	0.73	0.82	0.34	0.00	8.44	0.82	0.00
85	0.70	0.13	0.29	0.10	0.11	0.00	1.77	0.07	0.00
88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93	0.00	0.73	0.00	0.07	0.07	0.00	0.23	0.23	0.00
95	1.03	0.00	0.00	2.20	0.00	0.10	0.00	0.00	0.23
98	0.27	0.00	0.73	0.00	0.00	0.00	0.00	0.00	0.00

Table VI.7: Ammonium levels experiment with sand tea, nut and perlite with GW

mmol/l *10-3	Initial	Sand-H	Sand-L	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	0.00	4.48	8.15	0.00	0.00	0.00	0.00	0.00	33.64
4	0.00	3.26	3.26	0.00	0.00	0.00	0.00	0.00	13.25
7	6.07	1.22	0.00	0.00	0.82	0.00	0.00	0.00	0.00
8	0.00	1.63	3.87	4.48	4.69	0.00	0.00	2.04	2.85
11	0.00	4.89	1.43	0.00	0.00	0.00	0.00	12.64	1.83
13	3.66	0.82	1.22	0.00	0.82	0.00	0.00	8.56	12.44
15	0.00	0.00	1.22	0.00	5.30	0.00	0.00	18.55	6.52
18	0.00	1.63	0.00	0.00	2.04	0.00	0.00	15.29	0.00
20	0.00	0.00	0.00	2.45	0.00	0.00	0.00	8.97	0.00
22	3.94	0.00	0.00	0.00	0.00	0.00	0.00	15.49	0.00
25	0.00	2.24	0.00	0.00	0.00	7.54	0.00	6.73	10.40
27	0.00	5.10	3.87	4.48	1.02	5.91	0.00	1.22	3.26
29	0.00	0.00	0.00	20.18	0.00	8.36	0.00	0.00	29.76
31	19.37	22.83	17.74	12.03	11.21	1.02	0.00	18.35	16.11
33	7.54	10.80	20.59	40.77	4.89	1.22	36.90	13.45	24.67
35	27.11	16.31	6.93	24.46	7.14	20.59	22.02	44.44	54.84
38	11.21	6.93	0.00	32.41	0.82	4.28	2.04	13.45	6.93
40	18.35	19.37	3.87	49.33	8.15	0.00	19.57	18.14	5.50
42	11.62	9.99	8.97	68.29	22.63	4.69	3.06	1.22	17.33
44	1.22	4.28	3.67	40.57	42.00	0.00	0.00	0.00	7.34
47	1.02	2.24	1.22	49.54	53.82	0.00	2.85	4.89	9.99
51	6.52	5.71	4.89	20.39	43.63	0.00	3.47	9.38	0.00
53	0.00	2.04	0.00	4.08	64.83	0.00	6.73	1.22	19.98
55	0.00	0.82	2.45	0.00	67.48	0.00	5.30	0.00	22.22
58	116.40	1.22	1.83	0.00	63.74	5.32	3.67	5.91	21.06
61	2.85	0.00	1.22	0.00	62.59	0.00	41.59	0.00	0.00
66	0.00	0.00	1.63	0.00	45.05	0.82	39.55	0.00	15.70
68	66.52	0.78	1.39	5.54	40.46	4.71	81.14	0.00	8.31
71	5.30	7.75	3.67	6.93	51.78	0.00	73.80	1.63	0.00
74	0.00	2.04	7.75	0.00	51.37	0.00	50.35	0.00	0.00
76	0.00	0.00	3.26	0.00	26.91	0.00	21.41	0.00	0.00
79	0.00	2.04	3.26	0.41	29.76	0.00	0.00	0.00	0.00
82	0.00	0.00	2.04	0.00	17.94	0.00	1.22	0.00	0.00
85	5.10	0.00	0.00	0.00	8.97	0.00	0.82	10.60	0.00
88	0.00	1.43	1.46	0.00	10.09	0.00	1.44	0.00	0.00
93	2.22	0.00	0.00	0.00	8.30	0.00	19.95	6.65	0.67
95	0.00	0.71	2.04	0.25	4.88	0.62	14.25	0.00	0.00
98	0.67	0.00	0.96	0.00	8.18	0.00	6.21	6.29	1.42

Table VI.8: TN levels experiment with sand tea, nut and perlite with GW

mmol/l *10^-3	Initial	Sand-H	Sand-L	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	528.43	354.04	230.41	147.64	0.50	0.02	0.67	0.06	34.97
4	532.70	397.83	181.70	223.10	0.00	0.02	0.02	0.11	19.66
7	536.71	384.78	360.72	164.31	1.45	0.11	0.00	0.10	0.02
8	522.95	390.79	406.80	171.59	5.26	0.00	0.17	2.74	14.98
11	530.83	401.57	467.11	185.84	0.73	0.00	0.00	13.40	2.21
13	537.04	389.21	425.34	193.49	1.11	0.02	0.02	8.86	13.68
15	531.96	386.03	412.38	198.60	5.63	0.02	0.02	19.23	8.72
18	497.14	412.62	392.34	209.02	2.36	0.02	1.38	17.19	0.51
20	489.01	404.53	422.61	210.97	0.00	0.02	0.02	10.64	0.59
22	505.85	401.38	430.24	200.01	1.34	0.00	0.02	17.17	0.53
25	494.22	400.03	421.86	194.62	0.00	7.56	0.08	7.24	11.20
27	519.18	401.76	388.54	189.53	1.04	5.93	0.00	1.80	3.98
29	494.87	401.04	403.53	202.56	0.00	8.37	0.11	2.05	32.38
31	509.25	414.90	425.91	212.65	13.26	1.12	0.02	19.10	18.14
33	537.18	425.63	394.78	219.52	6.07	1.33	37.61	13.71	25.02
35	543.47	438.23	410.22	197.77	7.48	20.81	23.25	45.18	56.37
38	508.26	443.41	434.42	207.01	0.91	4.56	2.12	14.60	7.11
40	516.15	426.94	396.51	224.15	8.74	0.23	20.27	19.08	6.11
42	509.41	422.71	416.96	332.86	23.16	4.85	3.17	1.64	18.24
44	544.30	431.32	412.92	315.06	47.60	0.00	0.24	0.34	7.58
47	541.16	405.72	421.51	318.05	53.85	0.00	3.19	10.65	10.05
51	547.73	438.66	451.23	331.31	44.58	0.02	3.56	11.77	0.19
53	535.14	425.31	421.67	326.27	76.92	63.74	6.73	1.86	20.04
55	537.16	433.33	416.40	331.45	88.37	0.05	5.30	0.00	22.60
58	637.11	404.43	392.28	309.96	90.30	5.32	3.67	8.49	21.52
61	532.18	446.19	440.70	309.03	90.04	0.15	43.76	5.70	0.28
66	520.37	450.62	468.13	283.74	68.08	1.51	41.74	0.00	15.71
68	577.14	380.80	409.59	234.66	54.99	4.73	82.79	17.52	8.33
71	541.23	414.12	438.11	319.46	62.77	0.48	99.03	1.91	3.10
74	557.95	402.95	384.02	180.94	51.62	1.57	77.48	0.29	2.19
76	488.89	365.44	366.87	165.14	27.02	0.00	23.97	0.31	0.11
79	525.53	366.41	380.32	142.32	29.86	0.53	3.09	3.77	0.00
82	487.34	410.40	347.32	131.10	18.36	0.08	9.87	1.50	0.03
85	537.93	367.38	387.79	145.43	9.11	0.02	2.65	10.76	0.00
88	486.60	348.01	362.19	153.28	10.09	0.44	1.62	0.27	2.19
93	504.82	361.30	396.28	159.03	8.48	0.08	20.39	7.18	0.78
95	484.48	384.24	385.09	131.37	4.91	0.74	14.31	0.31	0.23
98	495.60	367.56	352.63	136.42	8.26	0.00	6.21	6.53	1.46

Table VI.9: Phosphate levels experiment with sand tea, nut and perlite with GW

Days mg/l	Initial	Sand-H	Sand-L	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	8.65	7.32	7.25	10.33	5.45	7.15	2.02	1.97	0.61
4	8.21	1.45	6.25	9.60	1.73	4.34	1.80	2.08	0.38
7	8.31	1.32	4.12	2.94	0.38	0.76	0.28	0.31	0.28
8	7.00	1.39	2.85	2.46	0.51	0.69	0.41	0.16	0.14
11	8.36	1.29	2.78	2.61	0.40	0.41	0.89	0.14	0.82
13	8.18	1.54	1.64	3.07	0.32	0.23	0.24	0.50	0.90
15	9.50	1.47	1.37	2.39	0.43	0.48	0.38	0.16	0.30
18	9.59	1.45	1.10	1.87	0.34	0.27	0.28	0.30	0.06
20	10.71	1.22	1.31	3.52	0.40	0.56	6.32	0.22	0.18
22	8.74	1.43	1.19	2.11	0.35	0.27	0.65	0.49	0.07
25	7.63	0.99	1.12	1.94	0.25	0.11	0.13	0.08	0.07
27	8.54	1.02	0.99	2.36	0.25	0.17	0.14	0.08	0.04
29	9.45	0.89	0.76	1.47	1.10	0.07	0.38	0.08	0.10
31	9.49	0.55	0.47	1.53	3.50	1.10	0.49	0.43	0.04
33	10.50	0.64	0.46	0.87	3.10	0.51	0.36	0.07	0.04
35	12.42	1.18	0.57	1.72	3.02	0.35	1.18	0.33	0.37
38	12.53	0.89	0.58	1.50	3.05	0.44	1.09	0.19	0.40
40	12.78	0.88	0.46	1.60	2.36	0.17	0.81	0.19	0.24
42	11.36	1.38	0.53	1.97	2.35	0.16	0.71	0.11	0.28
44	12.61	1.22	0.40	1.74	2.63	0.15	1.18	2.28	0.04
47	12.42	1.38	1.10	1.65	2.20	0.12	0.94	0.07	0.02
51	11.30	2.21	1.20	1.85	2.10	0.99	1.26	0.08	0.01
53	12.44	2.10	1.10	1.72	2.00	0.02	1.13	0.05	0.01
55	12.08	2.15	0.50	1.22	1.80	0.15	1.13	0.07	0.06
58	12.91	1.70	0.80	1.34	1.75	0.25	0.78	0.07	0.02
61	12.40	1.80	1.10	1.15	2.10	0.35	1.20	0.09	0.01
66	11.60	1.70	0.75	0.81	2.00	0.20	1.10	0.06	0.01
68	12.47	1.30	0.82	0.65	1.87	0.25	1.00	0.10	0.00
71	9.95	1.10	0.50	0.75	1.60	0.35	0.90	0.22	0.05
74	11.87	1.20	0.40	0.90	2.10	0.25	1.10	0.07	0.08
76	13.58	0.80	0.35	1.00	1.30	0.20	1.20	0.16	0.00
79	13.85	0.75	0.58	0.80	0.80	0.21	0.80	0.11	0.11
82	11.88	0.60	0.65	0.60	0.70	0.15	0.91	0.07	0.05
85	12.50	0.50	0.55	0.70	0.50	0.18	0.55	0.09	0.06
88	12.36	0.55	0.62	0.65	0.55	0.16	0.75	0.09	0.08
93	12.99	0.59	0.65	0.79	0.68	0.13	0.85	0.08	0.08
95	12.25	0.49	0.61	0.64	0.58	0.16	0.72	0.10	0.09
98	9.73	0.58	0.58	0.73	0.59	0.18	0.68	0.08	0.07

Table VI.10: Potassium levels experiment with sand tea, nut and perlite with GW

Days mg/l	Initial	Sand-H	Sand-L	N-40-H	N-40-L	N-60-L	T-40-H	T-40-L	T-60-L
1	30.36	29.10	22.85	23.00	23.53	22.00	24.00	30.97	34.02
4	30.63	29.35	21.90	24.00	24.42	23.00	26.66	32.24	31.95
7	30.84	28.02	25.30	20.54	22.44	22.82	27.34	27.55	32.01
8	31.02	28.53	25.89	21.08	22.23	22.37	26.80	27.11	31.08
11	30.72	33.99	26.12	22.87	22.74	25.35	27.08	26.98	32.20
13	30.76	28.98	22.85	23.75	21.84	24.77	26.68	25.31	31.79
15	30.54	28.79	23.56	24.18	22.47	25.26	27.31	25.39	29.15
18	30.88	29.42	24.05	26.47	24.86	27.53	28.09	27.92	30.64
20	30.77	29.66	26.83	26.53	24.62	27.25	28.76	27.86	28.47
22	30.71	29.18	27.62	28.21	25.96	26.78	28.64	28.47	28.11
25	31.05	29.26	28.07	27.26	26.13	26.80	28.59	29.35	28.23
27	31.40	29.49	27.02	27.15	26.20	25.98	28.47	28.67	27.54
29	29.65	28.88	28.23	27.47	26.40	25.81	28.70	26.86	27.56
31	30.98	28.50	29.40	29.56	27.48	26.35	28.70	29.07	28.19
33	28.06	28.83	27.47	26.59	27.29	25.74	28.74	27.80	27.55
35	30.90	29.54	29.30	26.95	28.15	25.45	29.66	27.24	27.03
38	30.98	28.73	30.27	28.40	29.05	26.07	29.21	27.63	28.37
40	33.89	29.55	27.82	31.18	29.01	26.11	28.91	27.49	28.32
42	31.38	30.12	29.66	32.52	28.33	25.12	29.37	26.82	28.11
44	30.75	29.98	29.33	33.65	33.84	26.57	30.52	28.06	30.29
47	30.52	31.11	31.24	32.66	30.17	26.31	29.51	29.49	31.64
51	30.54	32.54	33.43	32.24	32.95	30.05	32.40	32.70	34.24
53	30.31	32.80	31.47	30.85	31.10	31.20	31.52	31.05	34.52
55	31.06	33.52	32.14	30.51	30.91	30.08	32.28	31.19	34.88
58	31.08	31.32	30.80	31.35	32.59	33.21	34.00	32.21	34.45
61	30.22	29.92	32.85	29.96	31.15	30.99	33.81	29.94	34.76
66	31.04	33.58	32.29	33.00	33.00	34.00	34.00	34.06	34.02
68	30.23	35.00	29.03	29.55	29.83	32.04	32.96	29.30	34.27
71	32.80	21.45	30.26	32.88	32.87	33.30	30.72	31.76	36.42
74	27.98	25.44	27.35	31.68	29.37	31.50	30.81	30.74	34.60
76	30.59	28.74	30.49	21.04	29.65	29.47	31.59	25.14	31.68
79	25.84	29.46	29.33	28.31	31.81	28.74	32.43	28.20	29.05
82	28.12	28.63	25.21	30.47	28.87	26.32	30.80	29.20	33.41
85	27.21	27.75	28.09	28.32	27.74	29.06	28.08	31.74	29.39
88	28.95	26.47	27.88	29.85	28.55	27.85	27.85	32.47	31.55
93	29.47	27.45	26.48	30.85	29.45	28.45	28.55	29.45	32.47
95	30.75	28.47	27.47	32.65	28.47	29.55	29.65	28.99	30.65
98	29.78	27.55	28.65	31.18	30.87	30.45	30.48	30.55	29.56

Appendix VII
SAMOS CASE STUDY

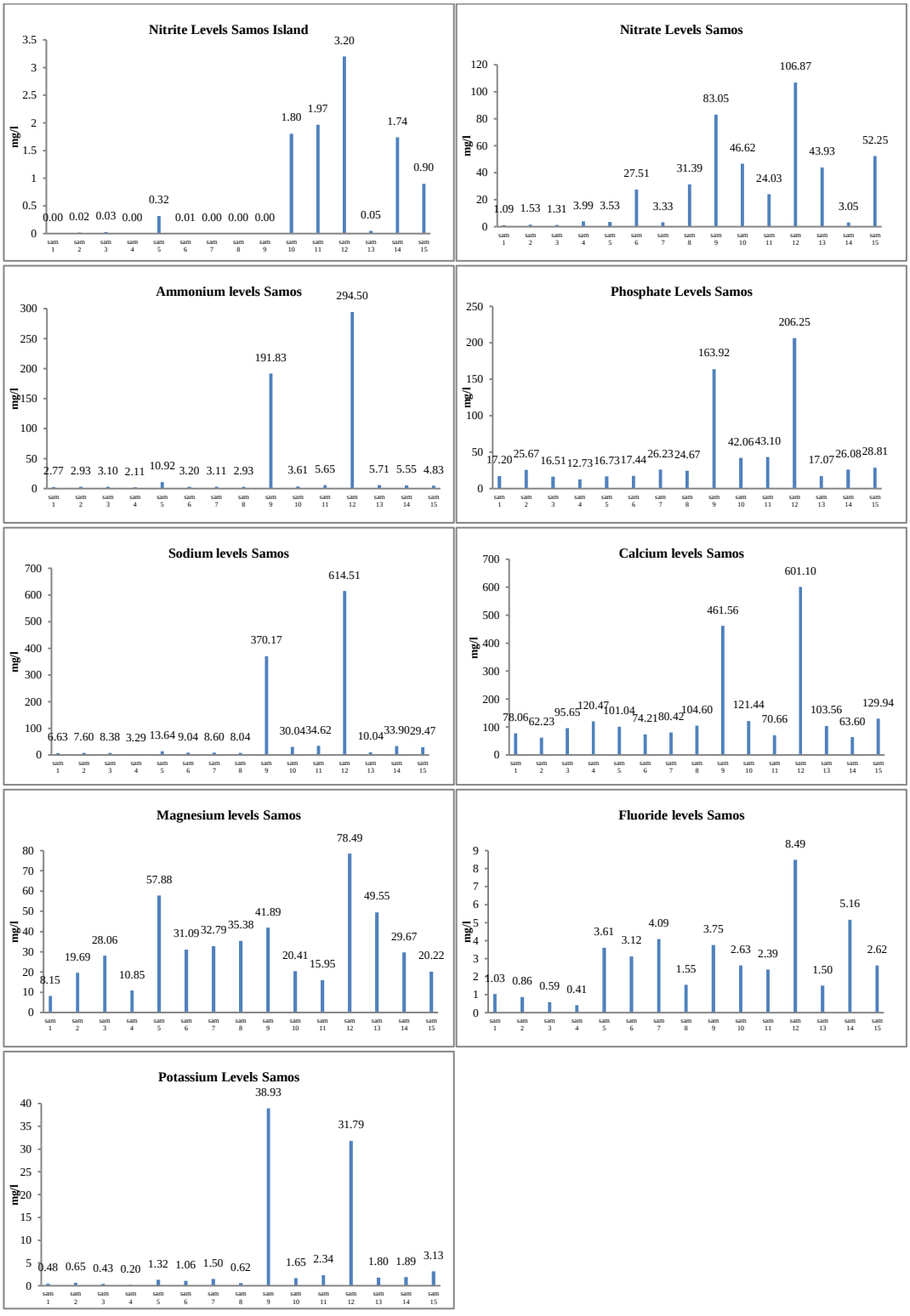


Figure VII.1: Anions and Cations Samos Island

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Record high °C VATHY													
Record high °C AIRPORT 1	20	20.4	22	28	33.2	39.4	41	38	37.2	31.6	25	21	41
Record high °C AIRPORT 2	21.8	21.9	26.8	30.7	35.8	37.1	38.7	38.2	37	33.4	30	25.9	38.7
Average high °C VATHY													
Average high °C AIRPORT 1	13.4	13.2	15.6	19.5	24.6	29.7	32.5	32.2	28.6	23.3	17.8	14.9	22.11
Average high °C AIRPORT 2	13.7	14.3	16.1	19.6	24	27.8	30	30	27	22.7	19.3	15.6	21.7
Daily mean °C VATHY	11.1	10.6	12.7	15.1	19.9	25.2	26.8	26.6	22.3	18.7	13.4	10.9	17.8
Daily mean °C AIRPORT 1	10.3	10	12.1	15.9	20.6	25.5	28.4	27.9	24.3	19.4	14.5	11.9	18.4
Daily mean °C AIRPORT 2	10.9	11.2	12.8	16.3	20.4	24.4	26.5	26.4	23.8	19.7	16.1	12.8	18.4
Average low °C VATHY													
Average low °C AIRPORT 1	6.9	6.5	7.9	10.5	14.3	18.6	22.2	22.1	18.8	15	10.7	8.5	13.5
Average low °C AIRPORT 2	8.1	8.2	9.3	12.5	16.3	20.1	22.1	22.2	19.6	16.3	13.3	10.1	14.8
Record low °C VATHY													
Record low °C AIRPORT 1	-2.4	-3.4	-1	5	7.4	8.8	14.8	16.4	12.2	7	1	-1.4	-3.4
Record low °C AIRPORT 2	-4.3	-3.8	-0.2	4.2	8.2	14	16.4	17.6	10.2	5.5	0.4	-2	-4.3
Precipitation VATHY	17.3	26.7	40.7	3	44.8	0	0	0	6.2	80.8	85.7	0.9	306.1
Precipitation AIRPORT 1	148.5	102.8	85.9	31.8	15.5	2.7	0.7	1.1	22.7	28.9	110.4	163.7	714.7
Precipitation AIRPORT 2	204.7	137.5	100.9	49.8	37.8	4.8	0.2	0.4	9.2	50.5	112.4	210.2	918.4
Avg. precipitation days VATHY													
Avg. precipitation days AIRPORT 1	12.4	10.4	8.6	7.4	4	1.1	0.2	0.1	1.4	4.6	9.3	13.7	73.2
Avg. precipitation days AIRPORT 2													
% Humidity VATHY	69.9	74.3	69.4	59.8	70.4	57	52.5	59.8	67.1	79.1	75.2	72.8	62.3
% Humidity AIRPORT 1	70.2	68.1	67.5	64.4	59.1	50.5	43.7	46	51.6	62.2	68.6	72.6	60.4
% Humidity AIRPORT 2	72	70	66	67	66	61	58	61	64	69	72	73	66.5
Wind Direction VATHY	SE	SE	SE	NW	SE	NW	NW	NW	NW	NW	SE	N	NW
Wind Direction AIRPORT 1													
Wind Direction AIRPORT 2	NW	SE	NW	NW	NW	NW	NW	NW	NW	NW	NW	SE	NW
Solar Radiation VATHY	67337.2	80263.5	130860.2	187456.4	204729.5	216899.8	229655.5	200308.2	154148.2	106866.1	78766.8	64126.1	1721418
Solar Radiation AIRPORT 1													
Solar Radiation AIRPORT 2													
Soil Temperature VATHY	10.4	10.8	12.8	15.9	19.9	24.9	27.4	25.6	25.8	19.8	13.3	11.2	18.2
Soil Temperature AIRPORT 1													
Soil Temperature AIRPORT 2													
Wind Speed VATHY	2.6	2.4	2.9	2.6	1.5	1.4	1.7	2	2.5	1.6	1.9	2.1	2.1
Wind Speed AIRPORT 1													
Wind Speed AIRPORT 2	2.9	3.1	3.1	3.1	2.7	3.1	3.5	3.3	2.9	2.5	2.6	3	3
Atmospheric pressure VATHY	1005.3	998.1	998.9	999.5	995.3	994.5	992.9	992.9	997.5	998.2	1003	1008.4	998.7
Atmospheric pressure AIRPORT 1													
Atmospheric pressure AIRPORT 2													

Table VII.1: Meteorological Data 2006-7 Samos Island (Hellenic National Meteorological Service)

Appendix VIII
PUBLISHED PAPERS

Enhanced denitrification process using readily available materials: columns studies

S.A. Gkiouzepas*, C.W. Knapp, R.M. Kalin
Department of Civil and Environmental Engineering, University of Strathclyde, James Weir Building, Level 5, 75 Montrose Street, Glasgow, G1 1XJ

*Corresponding author: E-mail: stylianos.gkiouzepas@strath.ac.uk, Tel +447749051570

Abstract
This paper presents results showing denitrification processes in column experiments with natural groundwater. Outcomes show the relation between the media used and the denitrification rates achieved. The groundwater was pre-treated in artificial wetland with reed beds (*Phragmites australis*). The selected media are readily available: sand, straw, mulch and perlite. The pre-treated groundwater was spiked by potassium nitrate (KNO_3) to achieve initial concentration 25 mg/l NO_3^--N. The results show reduction of nitrates more than 70% and reduction in total nitrogen and phosphate (PO_4^-) compounds. A correct combination of media supports denitrification process and phosphorus removal.

Keywords: Groundwater, denitrification, mulch, perlite.

1. INTRODUCTION

Agriculture often contributes to groundwater contamination via nitrogen and phosphorus fertilizers and uncontrolled land discharges of treated wastewater and sludge. The sources of groundwater include point sources such as animal feeds, waste lagoons and septic fields and non-point sources like applied chemical fertilizers and manure [15]. The European Nitrate Directive limits nitrate levels from agricultural sources (Nitrate Directive 91/676/EEC at 50 mg/l NO_3^--N and 10 mg/l NO_3^- N in drinking water [2,11]. This directive also contains restrictions for all nitrogen compounds, and it sets the same values with WHO. The health-related consequences with nitrates include methemoglobinemia (blue baby syndrome at infants), oesophagus problems, and cancers of the digestion system [4].
Denitrification processes reduce the quantity of available nitrates in soil leachate [6]. In particular, microbiological denitrification is an anoxic process [2] that involves the reduction of nitrates by anaerobic bacteria into dinitrogen gas. There are two different denitrification routes: heterotrophic denitrification requires an environment that is rich at organic carbon [20]; autotrophic denitrification utilises inorganic carbon source [4,17].
The present research reports the results of column experiments using different substrate material and focused on the use of perlite at the University of Strathclyde. The design of the experiment focused on inexpensive and easily found materials to reduce the nitrogen compounds in groundwater to acceptable limits. The denitrification process investigated was heterotrophic denitrification and was based field study on Samos Island in Greece.

2. MATERIALS AND METHODS

2.1 Substrate material

Four materials were selected to represent different media sources: sand (granulated sand with grains less than 0.66mm; to represent nutrient-poor conditions), wheat straw (chopped to less than 2 cm), mulch (mixture (60%-20%-20%) of compost soil, wheat straw and sawdust, respectively) and

Editors: A. Liakopoulos, A. Kungolos, C. Christodoulatos, A. Koutsopsyros
ISBN 978-960-88490-6-8

perlite (amorphous glass less than 1cm grains). The selection of all substrates was made based on their availability at the study area and low cost.
Of particular interest, perlite is an inexpensive product that is used in the construction sector. It is an amorphous volcanic glass that has relatively high water content, typically formed by the hydration of obsidian. It occurs naturally in Greece, with an annual production about 525,000 t. Perlite is used to many agriculture experiments to investigate the increasing rate growth of in small plants [16], and it is used in artificial wetlands and groundwater to remove heavy metals[7,13,14].

2.2. Column experiment design

Two experiments were undertaken. The first focused on the sand and wheat straw with different percentages in the substrate media in six different columns (Table 1). The second experiment focused on different substrate media and the inclusion of perlite (Table 2).
Groundwater was pretreated in an artificial wetland tank (150cm x 10cm x 60cm), which consisted of sand and reed bed (*Phragmites australis*). This was done to help condition the water and restore microbial activity. The 3-day hydraulic retention time (HRT) in the tank was maintained with a peristaltic pump, and the pre-treated groundwater was stored at tanks of 25 litres.
The columns consisted of Perspex cylinders columns with diameter 7 cm and length 65cm, with five longitudinal sample ports. Potassium nitrate (KNO_3) was added to conditioned groundwater to 25 mg/l NO_3-N. The temperature of the experiment was stable room temperature, 20±5 °C, as it mention in other researchers [9,10]. The measurements were taken at specified times, and the samples were harvested by sterilized syringes. The water samples were kept frozen at -80 °C until analyses.
The sand was sieved to use particles less than 0.66mm. The wheat straw was taken from local farmers, chopped in pieces less than 2 cm, and packed at the columns uniformly with the sand [7,17]. The design of the columns is the visible at table 1. The hydraulic retention time (HRT) in the first experiment was seven hours, and the samples were collected over 18 days.

Table 1: Design of columns first experiment with varying substrate concentrations.

Column 1	100% (v/v) sand
Column 2	20% (v/v) straw, 80% (v/v) sand
Column 3	40% (v/v) straw, 60% (v/v) sand
Column 4	60% (v/v) straw, 40% (v/v) sand
Column 5	80% (v/v) straw, 20% (v/v) sand
Column 6	100% (v/v) straw

In the second experiment, the same particles sizes were used for the sand, and the perlite was also sieved to less than 1cm. The mulch was a mixture of compost soil, wheat straw (< 1cm) and sawdust. The design of columns is shown in table 2. The HRT was 16 hours, and the duration of the experiment was 57days.

Table 2: Design of second experiment, along the columns with varying compositions of perlite and mulch.

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
30% perlite	25% perlite	25% perlite	25% sand	20% perlite	20% perlite	25% perlite
20% mulch	25% mulch	25% sand	25% mulch	30% mulch	30% sand	25% perlite
20% sand	25% mulch	25% sand	25% mulch	30% mulch	20% mulch	25% perlite
30% perlite	25% perlite	25% perlite	25% sand	20% sand	30% sand	25% perlite

2.3 Methodology

The findings of the study shows that the nitrogen compounds reduced more than 70%.

Measurements were made for NO_3^-, NO_2^-, NH_4^+ and PO_4^-. For the NO_2^--N, NH_4^+-N and PO_4^-, a micro plate was used for the measurements in a spectrometer, the NO_3^--N measurement became at spectrophotometer with each sample alone. TOC and TN analysis was undertaken with a TOC analyzer (Tekman Dohrmann Apollo 9000) and pH with pH meter (Meter Toledo). Results for all the nitrogen compounds, anions and cations received also from IC chromatography (Metter Toledo). All the measurements were made according ASTM methods. Total organic carbon was determined by means of a high-temperature aqueous TOC analyzer Tekman Dohrmann Apollo 9000. Before the analysis, all samples filtered through 0.45 μm membrane filters.

3. RESULTS

3.2 pH results

The pH ranged between 6.5 and 8.5, which is the optimal pH range for biological denitrification. The lowest pH (6.7) was in column that contains only straw and the highest pH (8.1) in the column with sand only. With perlite the pH range was the same, achieving the lowest pH (7.4) in the column that contains 60% perlite, 20% mulch, 20% sand and the highest pH (7.9) in the column that contain 50% perlite and 50% sand.

3.3 First experiment results

Nitrate removal was observed in all columns (Figure 1). The reduction was more than 70%. The initial concentration was 25 mg/l NO_3^--N, and treatment achieved final levels around 7 mg/l NO_3^--N. Nitrite was formed in some columns with the highest level in the column with 60% straw and 40% sand of 12 mg/l NO_2^--N and at the second highest levels in column with 80% sand and 20% straw with 2.5 mg/l NO_2^--N. The transformation rates followed zero and first order kinetics with the best degradation rates achieved in column with wheat straw. The denitrification rate was 0.041 mg/l $d^{-1}g^{-1}NO_3$-N.The amount of ammonium found was as high as 1.6 mg/l at column 80% straw and 20% sand and only 0.1 mg/l at the column with 100% sand. The phosphate levels along the experiment were between 2 and 5 mg/l PO_4^- without adding at the initial solution any phosphate compound and with initial concentration at 15 mg/l.

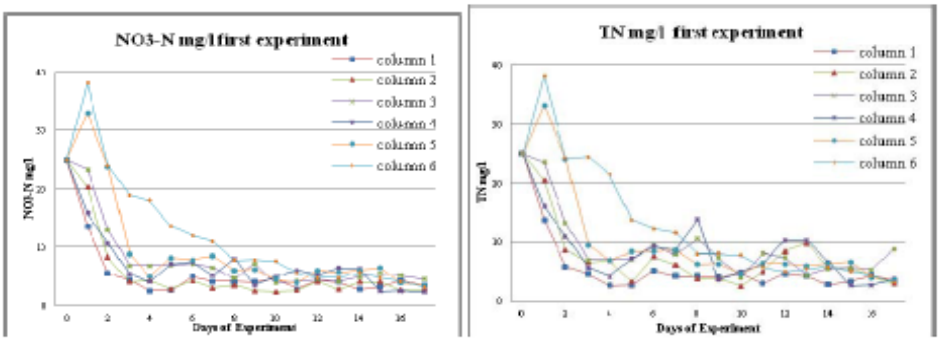


Figure 1: Nitrogen compounds during the first experiment

3.4 Second experiment results

Nitrate levels (Figure 2) during the second experiment were different with perlite than previously with only organic substrates. There is an initial increase in N, which is noticed in other studies

[1,14,18], and then there is the reduction. The initial NO_3^--N concentration was 25mg/l. Reduction of nitrate was observed in columns with mulch as expected (7 mg/l NO_3^-N), and also in column with perlite and sand, there was noticeable reduction (12.1 mg/l NO_3^-N). The denitrification rates follow the first order kinetics and the highest degradation rate noticed in column with mulch, perlite and sand. The denitrification rate was 0.025 mg NO_3-N dm^{-3} $d^{-1}g^{-1}$.
The output nitrite levels from the columns below 0.3 mg/l at all the duration of experiment at all columns, and ammonium levels were also very low, less than 0.35 mg/l NH_4-N during the duration of the experiment. Output levels of phosphates were very low 0.02 mg/l and the reduction is noticeable especially in the part with perlite columns achieving level more than 85%.

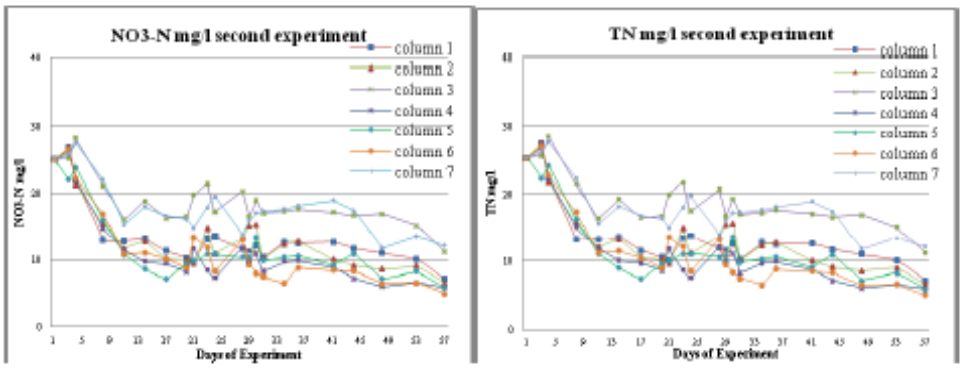


Figure 2: Nitrogen compounds along the second experiment

It is noticed that is the TOC concentration peaked (12.5 ppm) initially in first week and remained stable when the amount of water that pass through the columns increased. The average amount of TOC was 6.6 ppm with the highest price achieved in the column that contains 60% mulch, 20% perlite, 20% sand (average 8.12 ppm).

4. SAMOS ISLAND FIELD OBSERVATIONS

An investigation of the area of Samos Island Greece took place to investigate the water quality of the area. At the east part of the island water samples were collected from many water points (sources, distribution system and river basins) and analyzed according to ASTM. All samples showed that the amount of nitrates (40 mg/l), phosphates (25 mg/l), sulfates (150 mg/l) were high especially at the areas with agricultural production and next to the river basins, and in some of those areas were higher than the acceptable limits. Along the east coast line the area is plain and there are many vegetables and fruits fields that are used to deliver the main production for the whole island. The last years, there is a turn of the farmers to compact agricultural production of vegetables with hydroponics factories that discard their wastes (liquids/fertilizers/feed solution) to the local river basin without any treatment, increasing the levels of pollution in water quality causing eutrophication. Except from that the levels of fertilizers that contain N and P compounds are used from all the farmers of the island.
With the data that was collected from the area, an investigation into the applicability of the denitrification approach was undertaken. The reactions observed in the laboratory would face two main obstacles for implementation at full scale. The first is the phsical filters, made from sand, mulch, perlite, that can be applied in small scale even in the sources of water even in the distribution channels before the field applications.
As NITRABAR (EU Cost Project) has shown, areas next to river basins in the southeast area of the island before the flow in the rivers, big filter areas can be constructed to treat the water from agricultural fields and agricultural factories that discard huge water amounts. It is essential and very important to reduce the nitrogen and phosphate levels that are high due to the fertilizers that

are used. Also drainage systems with a filter level with the same substrates, in larges scales in areas with vegetable production can be designed and applied to achieve even better results even at the production of the vegetables even for the fertilizers levels that are used and finally affect the quality of agricultural water.

5. CONCLUSIONS

The results show there is significant reduction of nitrogen compounds found during previous investigations using different substrate material and different hydraulic retention time, using even columns, even sequence batch reactor [1,5,12,14,18]. The significant point of this study focused on the investigation of the perlite as a new media at column studies. There are investigations for perlite studies that reduce heavy metals like As, Cd, Cr, Cu and Zn [3]. There are also some studies that perlite can reduce N, P and COD in biofilters but in percentages of the treatment process with Zeolite and Bentonite combination [8]. The reduction at these systems for nitrates achieved levels of 80%.
In combination with specific microbial media, there is also reduction at nitrates [19].The results shown here indicate that perlite is viable additional substrates with organic matter to not only reduce the nitrogen burden in agricultural settings, but also to reduce the phosphorous burden.

Acknowledgements

The authors would like to acknowledge the support of the Scottish Funding Council GRPE grant for infrastructure and the University of Strathclyde.

References

1. Aslan S., Turkman A. (2005). Combined biological removal of nitrate and pesticides using wheat straw as substrates. *Process Biochemistry* **40**, 935-943.
2. Blowes, D., Robertson, W., Ptacek, C., Merkley, C. (1994). Removal of agricultural nitrate from tile-drainage effluent water using in-line bioreactors. *Journal of contaminant Hydrology*, **15**, 207-211.
3. Cruz Viggi, C., Pagnanelli, F., Cibati, A., Uccelletti, D., Palleschi, C., Toro, L.(2010). Biotreatment and bioassessment of heavy metal removal by sulphate reducing bacteria in fixed bed reactors. *Water Research* **44**, 151-158.
4. Della Rocca, C., Belgiorno, V., Meric, S. (2007). Overview of in-situ applicable nitrate removal processes. *Desalination* **204**, 46-62.
5. Gibert, O., Pomierny, S., Rowe, I., Kalin, R. (2008). Selection of organic substrate as potential reactive materials for use in a Denitrification permeable reactive barrier (PRB). *Biosource Technology* **99**, 7587-7596.
6. Jarvis, S.C. (2000). Progress in studies of nitrate leaching from grassland soils. *Soil Use Management* **16**, 152–156.
7. Lowengart A., Diab S., Kochba M., Avnimelech Y. (1993). Development of Biofilter for turbid and Nitrogen-rich irrigation water; An Organic carbon degradation and nitrogen removal process. *Bioresource Technology* **44**, 131-135.
8. Ozel, U., Akdemir, A., Nuri Ergun, O.(2012). Utilization of natural zeolite and perlite as landfill liners for in situ leachate treatment in landfills. *Int. J. Environ. Res. Public Health* **9**, 1581-1592.
9. Rivett, M., Buss, S., Morgan, P., Smith, J., Bemment, C. (2008). Nitrate attenuation in groundwater: A review of biochemical controlling processes. *Water Research* **42**, 4215-4232.
10. Rust, C.M., Aelion, C.M., Flora, J.R.V. (2000). Control of pH during denitrification in sub-surface sediment microcosms using encapsulated phosphate buffer. *Water Research* **34 (5)**, 1447-1454.

11. Saeed T., Sun, G. (2012). A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. *Journal of Environmental Management* **112**, 429-448.
12. Saliling,W.J., Westerman, P., Losordo, T. (2007). Wood chips and wheat straw as alternative biofilter media for Denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. *Aquaculture Engineering* **37**, 222-233.
13. Shrimali, M., Singh, K. (2001). New methods of nitrate removal from water. *Environmental Pollution* **112**, 351-359.
14. Soares M., Abeliovich A. (1998). Wheat straw as substrate for water denitrification. *Water Resources* **32**, 3790-3794
15. Su, S., Puls, R. (2007). Removal of added nitrate in cotton burr compost, mulch compost, and peat: Mechanisms and potential use for groundwater nitrate remediation. *Chemosphere* **66**, 91-98.
16. U.S. Geological Survey (2011). Mineral Commodity Summaries 2011. Retrieved from http://minerals.usgs.gov/minerals/pubs/mcs/2011/mcs2011.pdf. Last accessed 13/02/2013.
17. Van Rijn, J., Tal, Y., Schreier, H. (2006). Denitrification in recirculating systems: theory and applications. Aquaculture Eng. 34, 364-376.
18. Warneke, S., Schipper, L., Bruesewitz D., Troy Baisden, W. (2011). A comparison of different approaches for measuring Denitrification rates in a nitrate removing bioreactor. Water research **45**, 4141-4151.
19. Yamashita, T., Yokoyama, H., Kanafusa, S., Ogino, A., Ishida, M., Osada, T., Tanaka, Y. (2011). Nitrate-removal activity of a biofilm attached to a perlite carrier under continuous aeration conditions. *Biosci. Biotechnol. Biochem* **75**, 578-581.
20. Zhou, W., Sun, Y., Wu, B., Zhang, Y., Huang, M., Miyanaga T., Zhang, Z. (2011). Autotrophic Denitrification for nitrate and nitrite removal using sulfur-limestone. *Journal of Environmental Science* **23 (11)**, 1761-1769.

A COMPARATIVE DENITRIFICATION PROCESS UNDER ANOXIC CONDITIONS USING WASTE MATERIALS

GKIOUZEPAS S.*, KNAPP C. and KALIN R.

University of Strathclyde, Department of Civil and Environmental Engineering, University of Strathclyde, James Weir Building, Level 5, 75 Montrose Street, Glasgow, G1 1XJ, stylianos.gkiouzepas@strath.ac.uk

Keywords: Tea factory wastes, Hazelnut husk.

Abstract

The denitrification process is a process to reduce nitrogen compounds. The denitrification process was used in batch experiments. Factory tea wastes and hazelnut husk were added in different percentages in Duran bottles without any light connection. The duration of experiments was 8 days. It was noticed clear reduction of nitrate compound with factory tea waste bottles achieving reduction levels of more than 95%. In hazelnut husk bottles the reduction was between 55 and 95%. The batch tests were repeated 3 times in both trials with tap water solution in the first stage and with groundwater in the second stage. It was noticed that denitrification process was working faster in tap water solution. Except from the instrumental measurements with ion chromatography and total organic carbon analyzer, quantitative PCR analysis identified that denitrification microbial colonies grew up in all bottles mixtures and accelerated the denitrification process.

1. Introduction

The components of the nitrogen cycle affect life in various ways (Bothe *et al.* 2007; Rodriguez 2011). Nitrate is one of the most widespread pollutants of groundwater in many developed and developing countries and it is the most oxidized contaminant which is found in groundwater worldwide (Lee *et al.* 2006; Nancharaiah *et al.* 2011).

Groundwater is extensively used as one of the main sources of drinking water in many areas around the world. Also the levels of water pollution enlarged in developed countries where the standards of living are increased. Specifically, groundwater supplies are becoming contaminated when nitrates concentrations above overtakes the drinking water standard of 10 mg/l nitrogen (Moon *et al.* 2006; Qambrani *et al.* 2013).

Intensive use of chemical fertilizers, manure in crop production, septic tanks poor, wastewater treatment and collection systems are the main sources of nitrate contamination in groundwater. Industrial actions, like the combustion of fossil fuels and the production of fertilizers, explosives, glass, plastics and cured meat can also result to nitrate pollution (Robinson-Lora *et al.* 2009)

Natural levels of nitrate in groundwater are usually less than 3 mg L^{-1} as N. However, in contaminated areas, nitrate concentrations can exceed 200 mg L^{-1} as N (ITRC 2002). World-wide, nitrate is among the most common groundwater contaminants, with primary source the agricultural activities that are related use of nitrate fertilizers and manure (Misiti *et al.* 2011; Rivett *et al.* 2008).

Nitrate and nitrite concentrations higher than the international limit present health concerns as they are toxic to humans and livestock (US EPA 2009). Nitrate removal from water sources is a topic of intense research due to its implications on human health and water quality.

The existence of nitrate in drinking water is a possible danger to human health, the reason being that nitrate is reduced to nitrite in the digestion system and then absorbed by blood, which in turn is combined with haemoglobin, preventing the oxygen uptake of blood. This can lead to methemoglobinemia (blue baby syndrome) in infants. Moreover, the formation of nitrate compounds in the stomach has been shown to be carcinogenic (Hekmatzadeh 2012; WHO 2004).

The presence of nitrate causes a problem of eutrophication of rivers and lakes which followed by hypoxia and fish-kill, acidification and other environmental effects (Carrera *et al.* 2004). In incomplete denitrification processes the gas nitrous oxide (N_2O) can transform (Pynaert *et al.* 2003). This gas is responsible for global warming (Bothe *et al.* 2007).

The organisms involved in these processes can be classified according to the source of carbon in autotrophic and heterotrophic species. The autotrophs are those organisms able to synthesize organic matter from minerals and the heterotrophs are those in need of organic matter for their development and maintenance (Rodriguez *et al.* 2011).

Several techniques for nitrate removal have been used (biological denitrification, reverse osmosis, electro dialysis, ion exchange, adsorption, chemical reduction using zero valent iron, and catalytic reduction) (Xing *et al.* 2011). Biological denitrification is a natural process that is part of the nitrogen cycle, and preferred in wastewater treatment plants for the removal of excess nitrogen (Soares 2000). Biological denitrification is the proposed method to remove nitrate from surface waters (Kapoor and Viraraghavan 1997) and the most frequently used method for municipal wastewaters. Few studies have shown effective nitrate removal at concentrated wastes with biological denitrification (Healy *et al.* 2012; Liu *et al.* 2013; Schipper *et al.* 2010; Xing *et al.* 2011).

The process of denitrification is the reduction of nitrate to nitrite and subsequently the reduction of nitrite to nitric oxide (NO), then to nitrous oxide (N_2O) and finally to molecular nitrogen (N_2), which is released into the atmosphere (Knowles 2005). Denitrification is mainly carried out by heterotrophic and some autotrophic bacteria. Denitrification removes nitrate from the aquatic environment, recovering some of the alkalinity consumed in nitrification, and increasing the pH.

The present research reports the results of batch experiments in Duran bottles using factory tea wastes and hazelnut husk, both of them from Black Sea Region in Turkey, as substrate materials to reduce the nitrogen compounds to acceptable limits under anoxic conditions and without adding any carbon source. These materials are used either as fuel in local production areas or as fertilizers in the agricultural fields. The research investigation took place in University of Strathclyde, Glasgow Scotland. Batch tests were carried out to determine the ability of wastes to provide dissolved organic carbon and stimulate the activity of denitrifying bacteria, accelerating the denitrification process in the bottles.

2. Batch experiments

2.1 Design of batch experiments

Batch experiments were designed to study the denitrification patterns using as carbon sources factory tea wastes and hazelnut husks. The reactive materials were obtained from the Turkey from the region next to Black Sea. The husk was cleaned of non-husk impurities, washed, dried at 105° C for 3 h. All Tea and Hazelnut wastes were air dried and screened to obtain a fraction below 4 mm. Batch experiments were accomplished to describe the denitrification kinetics of the enriched culture of denitrification microorganisms at the end of the eight days experiment. Duran bottles 100 mL fit with modified screw-on caps were used for the experiments. Triplicate tests were run with 8 days duration. All flasks were sterilised in an autoclave (105 °C) for an hour before their usage.

Each material was first mixed carefully to get a homogeneous sample and then the desired volume of solid was placed in 1000 mL flask to achieve saturation conditions. The flasks were then saturated with tap water and kept in the fridge at temperatures below 4° C till the time of experiments.

Batch tests were carried out in a series of eight 100 mL glass anaerobic flasks. Eight flasks were filled with one solid material (tea wastes, hazelnut husk and sand) in different percentages, while the eighth flasks were filled with a solution of $NaNO_3$ achieving an initial concentration of NO_3^--N equal to 32.2 mg L^{-1}. The experiment was separated in two stages. At the first stage,

tap water was used in the bottles as solution, and at the second stage, groundwater from the industrial rural area outside from Glasgow, Scotland was used as solution.

For all eight bottles the experimental protocol was same. A solid: liquid ratio of 200:800 (cm^3: cm^3) was used. The flasks were sealed to create anaerobic conditions and covered with an opaque material without having any light connection. All experiments were took place at room temperature (20 ± 2 °C).

2.2 Analytical techniques

Water samples were obtained with a syringe and filtered through a 0.45-μm cellulose membrane filter. Samples were analysed for TOC, pH, oxidation–reduction potential (ORP) and conductivity. The dissolved oxygen (DO) levels in the flasks were tested using a digital DO meter (Model 9143, Hanna, Italy). The pH and ORP measured with Mettler Toledo Seven Multi multimeter. Analysis of anions and cations completed by ion chromatography (IC) (Metrohm 850 Professional) to monitor NO_3^--N, NO_2^--N and NH_4^+-N concentrations. TOC was measured with TOC analyser Apollo 9000 (Teledyne Tekmar). All the measurements were done according to ASTM. Control tests were also carried out in two stages with tap water and groundwater, respectively. Total Nitrogen (TN) concentrations were determined by summing the concentrations of nitrate, nitrite ans ammonium. Special care was given to all sampling and measurement procedure to minimise aeration and assurance of homogenous sampling.

Quantitative real-time PCR (qPCR) was used for gene detection because it is rapid, detects genes in both culturable and non-culturable bacteria, and it is quantitative, which allows statistical analysis between gene levels and experimental treatments. Specifically genes were quantified, which were associated with the non-haeme containing (*nirS*) and copper-containing (*nirK*) nitrite-reductases that encode the key enzyme classes responsible for the conversion of nitrite (NO_2^-) to nitric oxide (NO) within the denitrification pathway (Philippot 2002). Targeting such genes has been used to determine denitrifier community composition (Braker *et al.* 1998; Philippot and Hallin 2005; Henry *et al.* 2004; Graham *et al.* 2010) in environmental samples. The ultimate goal is to quantify gene abundances of the two key *nir* genes as a measure of denitrifier population numbers, and explain treatment differences in nitrate reduction.

3. Results

3.1 Experiment with tap water solution

Tap water was used in the first stage of batch tests with initial concentration in NO_3^--N (0.01 mg L^{-1}), NO_2^--N (0.001 mg L^{-1}), and NH_4^+-N (0.001 mg L^{-1}). Afterward, the tap water was spiked with amount of $NaNO_3$ to achieve a concentration of 32.2 mg L^{-1} NO_3^--N.

3.1.1 Factory Tea wastes Bottles measurements with tap water

The pH levels in the first stage were measured directly after the sampling time in the remaining solution of the bottles. At the first stage, the pH range in factory tea waste bottles was between 5 in 100% Tea flask and 6.2 in 40% Tea flask. The ORP levels had wide range with negative values (60% Tea) and positive values at 100%Tea and 40% Tea flasks. The DO levels in all the bottles were lower than 5 ppm with the lowest level in 40% Tea flask (1.65 ppm). The conductivity in the tea bottles was lower than hazelnut husk bottles and it was between 225 and 342 μS/cm, with the highest value at 100% Tea flask (Figure 2). The TOC levels in tea bottles were much higher than the hazelnut husk bottles. The highest values had noticed in 100%Tea flask with 532.53 ppm and the lowest value in 40%Tea flask with 176.93 ppm (Table 1).

The nitrogen levels were lower than the initial concentration of NO_3^--N that it was spiked. The total nitrogen (TN) in the tea flask was between 4.06 and 20.84 mg L^{-1}.The highest content observed in 40%Tea flask and the lowest in 100%Tea flask. The NO_3^--N levels were in all bottles at the same level and below 1.5 mg L^{-1}. The NO_2^--N levels were noticeable in 40% and 60% Tea flasks with 18.96 and 4.86 mg L^{-1}, respectively. Finally, the NH_4^+-N levels were very low with

the highest concentration 2.79 mg L^{-1} in the 100% Tea flask (Figure 1).

The pH was lower than the best suitable conditions for growing up denitrification bacteria colonies but even with this acidic environment the results were very acceptable. The reduction of nitrate achieved level higher than 95%. The results show that the denitrification process took place in the bottles with tap water.

3.1.2 Hazelnut Husk Bottles measurements with tap water

The pH range in nut bottles was between 6.8 and 7.3, which is the best range for denitrification process. The lowest value was in 100% Nut flask and the highest in 40% Nut flask. The ORP levels had wide range. Negative and positive values were noticed, much lower than Tea bottles as it is visible to the Table 1. The DO levels at all the bottles were lower than 3 ppm. The conductivity was between 290 and 345 μS/cm with the peak price at 100% Nut flask .The TOC levels were much lower than the Tea bottles and all cases less than 100 ppm. As it is noticed at Tea part with tap water, also at hazelnut husk part the lowest value was at 40% Nut flask (29.5 ppm) and the highest at 100% Nut flask (78.2 ppm). The TOC levels were higher than the initial tap water solution, showing that denitrification process is going on.

In contrast to the tea bottles, at the hazelnut bottles the nitrogen levels were higher. The TN levels were between 7.7 and 22.56 mg/l with the lowest concentration in 100% Nut flask and the highest in 40% Nut flask. The NO_3^--N levels were higher than tea bottles and between 1.91 and 14.92 mg/l with the highest and lowest concentrations in the 40% and 100% flasks, respectively with reduction between 55 and 95%. The NO_2^--N levels were lower than NO_3^--N and the lowest concentration was 4.01mg/l in 100% Nut flask and the highest 7.17 mg/l in the 60% Nut flask. The NH_4^+-N levels were lower than 1.2 mg/l at all bottles (Figure 1).

3.2 Experiment with groundwater solution

At the second stage of experiment, groundwater was used as solution for batch tests to simulate the conditions of the ground. The groundwater was periodically collected on site (spring close to Glasgow, Scotland) and stored at 4 °C until the use in the laboratory. Groundwater initial concentrations were: NO_3^--N (0.01 mg L^{-1}), NO_2^--N (0.01 mg L^{-1}) and NH_4^+-N (0.02 mg L^{-1}). Afterwards, it was spiked with an appropriate mass of $NaNO_3$ to achieve a nitrate concentration of 32.2 mg L^{-1} NO_3^--N.

3.2.1 Factory Tea Wastes Bottles measurements with groundwater solution

The pH levels at the second stage of experiment were measured directly from bottles, using the same procedure with tap water bottles. In contrast to first stage of experiments, the pH values were higher achieving better conditions to grow up denitrification bacteria. The levels were between 5.8 and 6.6 with the lowest value, again at the column with 100%Tea flask and the highest value at the 40%Tea flask. The ORP levels were negative at all bottles with the lowest value in the 100% Tea flask (-336.3 mV) and the highest in 60% Tea flask (-99.8mV). The oxidation-reduction potential that achieves values below -200 mV, it is possible to occur due to excessive nitrite and sulphide formation (Saliling *et al.* 2007). The DO level at all the bottles were lower than 3 ppm with the lowest level in 40%Tea flask (DO=1.51 ppm). In contrast to the first stage lower levels noticed at 40 and 60%Tea flaks except from the 60%Tea flask. Also, the conductivity levels were higher than the first stage achieving values between 309 and 558 μS/cm. The TOC levels in 40% and 60% flasks were lower than the tap water bottles with values of 56.8 ppm and 70 ppm, respectively. In 100%Tea flasks the TOC was higher with groundwater solution, achieving levels of 681 ppm. The TOC levels shows that in groundwater solution the denitrification bacteria can grow up colonies (Table 1).

It is visible from TOC levels that denitrification bacteria activity exists. The TN levels remained below 50 mg L^{-1} at all bottles. The NO_3^--N concentration was less than 0.03 mg L^{-1} at all flasks, achieving reduction more than 99%. The NO_2^--N also was lower than 2.65 mg L^{-1} at all flasks

with the highest level at the 60% Tea flask. The NH_4^+-N levels were increased and produced the high level of TN with 41.84 mg L^{-1} in the bottles of 100% Tea, 45.22 mg L^{-1} in the bottles with 60% Tea and 46.85 mg L^{-1} in 40% Tea (Figure 1). Accumulation of ammonium observed in the bottles could be due to Dissimilatory Nitrate Reduction to Ammonium (DNRA) in groundwater. DNRA is a further anaerobic reduction reaction that competes with denitrification and converts nitrate to ammonium rather than N_2 (Zhang *et al.* 2012).

In hazelnut bottles was noticeable that the TN levels were higher in the bottles where groundwater was used as solution. The NO_3^--N and NO_2^--N levels were close to zero in the second experiment with the groundwater solution and shown that the denitrification process takes places faster than in the tap water solution bottles.

3.2.2 Hazelnut Husk Bottles measurements with groundwater solution

At the hazelnut husk bottles the pH values were again higher than the first stage. The pH range in hazelnut bottles was between 7.3 and 7.6, perfect conditions for denitrification process. The ORP levels had varied range with negative (100%Nut = -105.8 mV) and positive prices (40%Nut = +7.78mV and 60%Nut = +130mV). The DO levels at all the bottles were lower than 2 ppm. The conductivity was higher than the first stage, with values between 298 and 538 μS/cm. The highest value was observed in 100%Nut flask. The TOC levels were much lower than the Tea bottles filled in with groundwater solution and in all cases less than 100 ppm. At the 60% and 100%Nut flaks the amount of organic carbon was lower than the tap water bottles with values of 32 ppm and 58.5 ppm, respectively. In 40%Nut flask the TOC level was higher than the tap water flask achieving a value of 34.5 ppm (Table 1).

The hazelnut husk bottles reduced the nitrogen levels but not as much as the tea bottles. The NO_3^--N levels were higher than the tea bottles and achieved levels from 6.79 till 16.07 mg L^{-1} and reduction between 55 and 85% with the lowest value at the 100%Nut flask and the highest in 40%Nut flask. The NO_2^--N levels were lower than 1.6 mg/l at all cases. The NH_4^+-N levels were lower than tea bottles. The lowest levels were at 100%Nut flask with 34.27 mg L^{-1} NH_4^+-N and the highest level at 40%Nut flask with 39.19 mg L^{-1} NH_4^+-N. The TN levels in 100%Nut flask was 42.3 mg L^{-1} and in the 60% and 40%Nut flasks the TN levels were higher achieving 53.1 and 59.7 mg L^{-1}, respectively (Figure 1).

Table 1: Summary of pH, Redox, DO, Conductivity and TOC levels

	40% TEA	60% TEA	100% TEA	40% NUT	60% NUT	100% NUT
pH groundwater	6.55	6.39	5.81	7.64	7.40	7.31
pH tap water	6.16	5.91	5.00	7.31	7.28	6.83
Redox groundwater (mV)	103.68	-99.87	-336.35	130.00	7.78	-105.80
Redox tap water (mV)	34.01	-250.71	122.27	62.00	-24.33	83.38
DO groundwater (ppm)	1.51	1.79	2.56	1.79	1.86	2.00
DO tap water (ppm)	1.65	2.50	5.10	1.85	1.75	2.75
Conductivity groundwater (μS/cm)	309.92	315.54	558.48	298.35	305.62	537.99
Conductivity tap water (μS/cm)	229.86	225.67	341.65	290.42	304.63	345.95
TOC groundwater (ppm)	56.79	70.07	681.07	34.21	32.25	58.54
TOC tap water (ppm)	176.93	252.03	532.53	29.47	43.53	78.19

In contrast to the first experiment with tap water solution, the TN levels were higher in groundwater solution bottles. The difference was noticeable in NO_2^--N and NH_4^+-N. The NO_2^--N levels were much lower than the bottles with the tap water solution. The NH_4^+-N levels in groundwater solution bottles were much higher. The high levels of NH_4^+-N show that even here the DNRA

process was noticed along the denitrification process, as it was noticed in Tea bottles with groundwater solution.

Table 2: Summary of Nitrogen Compounds mg L^{-1}

	40% TEA	60% TEA	100% TEA	40% NUT	60% NUT	100% NUT
NO_3-N tap water	1.57	0.18	1.26	14.92	8.50	1.91
NO_3-N groundwater	0.01	0.02	0.03	16.07	14.54	6.76
NO_2-N tap water	18.96	4.86	0.03	6.58	7.17	4.01
NO_2-N groundwater	0.05	2.65	0.03	1.57	1.19	0.83
NH_4-N tap water	0.31	0.23	2.79	1.05	1.2	1.25
NH_4-N groundwater	46.85	45.22	41.84	39.19	37.27	34.27
TN tap water	20.84	5.27	4.08	22.55	16.87	7.17
TN groundwater	46.90	47.89	41.89	56.83	53.00	41.86

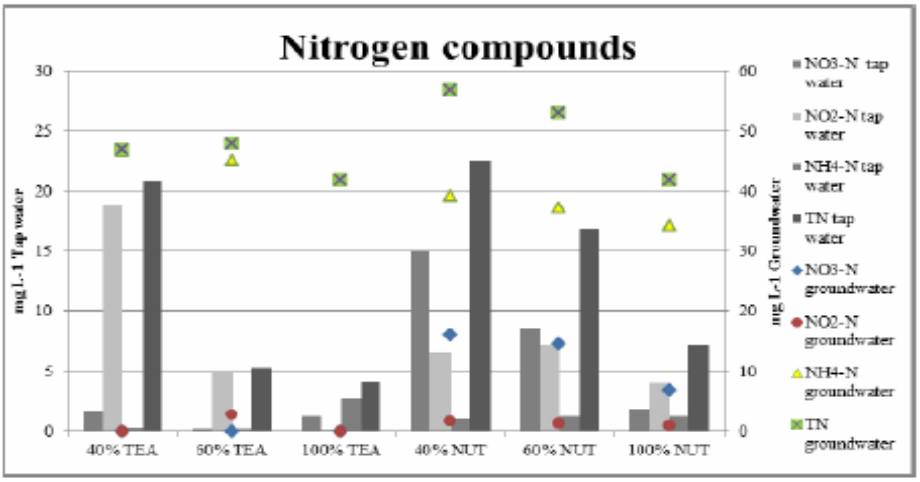


Figure 1: Nitrogen compounds: nitrate, nitrite, ammonium and total nitrogen levels in Duran bottles after eight days experiment

4. Discussion

Good removal of NO_3^--N and NO_2^--N was observed in the batch studies. In both experiments with tap water solution and with groundwater solution, the reduction that it was noticed was separated in two phases.

In the first phase the denitrification process take place in the tea bottles and the amount of NO_3^--N was less than 1.6 mg L^{-1} in all cases. This shows that the denitrification process is working and achieving with all mixtures the appropriate results. The NO_2^--N levels were higher in the first experiment with tap water and close to zero at the second. In the first experiment with the tap water solution the NO_2^--N concentration was higher between 0.03-18.96 mg L^{-1}. This shows that the denitrification procedure is still on process. That is following the denitrification process of nitrogen conversion that from NO_3^- is converted to NO_2^- and finally in N_2.

In the second experiment with groundwater, the denitrification process had totally finished and achieved the lower levels of NO_2^--N. The DNRA process was noticed in the groundwater bottles.

The second phase was the hazelnut husk bottles. At the hazelnut bottles in both experiments the reduction of NO_3^--N had not achieved the best reduction in 8 days experiments.

In the first part with tap water was noticed the best reduction in 100% Nut bottle with only 1.91 mg L^{-1} in contrast to the other two bottles, where the NO_3^--N concentration was much higher: 8.5 and 14.92 mg L^{-1} in 60 and 40 flasks, respectively. Parallel the NO_2^--N levels were observable between 4-7.2 mg L^{-1} that shows that the conversion from NO_3^- to NO_2^- is still on process.

On the second experiment with groundwater solution the NO_3^--N levels were between 6.76-16.07 mg L^{-1} with the best removal again in 100% Nut flask. In contrast to the first experiment, the NO_2^--N levels were much lower at all bottles less than 1.6mg L^{-1}. The denitrification process is working better and faster in tap water solution. The NH_4^+-N concentration again was noticeable, showing that DNRA process was happening in the bottles.

The presence of concentrations of NH_4^+-N, NO_2^--N to NO_3^--N in the final solution, indicate that the process of nitrification–denitrification was incomplete, and therefore more time was required to achieve the best results in hazelnut husk bottles.

In the hazelnut husk bottles the pH was higher than factory tea wastes bottles. In all cases the pH range is an indicator that the denitrification bacteria are working. Both of the materials can provide the suitable amount of organic carbon to accelerate the denitrification procedure. Additionally to the measurements that completed with the instruments (ion chromatography and TOC analyzer) to find out that the denitrification process is taking place, samples from syringe cellulose filter were analysed for denitrifier bacteria. Quantitative real-time PCR (qPCR) was used for gene detection, and the results shown that the denitrifier bacteria exist in the mixtures of all bottles. qPCR results represented the denitrifying bacteria that in tea factory wastes were more than nine times higher than the hazelnut husk wastes. A neutral pH in the batch tests with our mixtures could suggest a more favourable condition for microbial activity to increase our denitrification rates. In contrast to other researches (Rivett 2008; Zang *et al.* 2012), it is noticed that only in bottles with tea the complete denitrification was achieved within 8 days. The other bottles with tea and hazelnut husk materials need more time to complete the whole process.

All the measurements for DO and ORP potential became at the end of the experiment with triplicate results and under stable conditions in all cases (room temperature 20±2 °C and stable conditions). There is a concern to the range of ORP potential, that in some cases mentioned as critical with the negative prices that received. Additionally, no other carbon source existed, except from the waste materials that added in the start of the experiment. Oxidation reduction potential (ORP) with prices below -200 mV might cause to excessive nitrite and sulphide formation (Saliling *et al.* 2007). Nitrate and nitrite accumulation was related with mineralization of ammonium which is connected with DO concentration (Lee *et al.* 2014).

5. Conclusion

Denitrification process is appreciable in the batch tests. In both cases, it is noticeable the reduction in nitrate compounds with tap water and groundwater as solution. It is observed that the results are more successful with factory tea waste bottles at all percentages and specifically in the bottles with 100% tea, a reduction of more than 99% was observed. At the hazelnut husk bottles, the decrease was observable but not with the same efficiency like tea bottles, achieving levels between 55-95%. Also in the hazelnut husk bottles the best results were detected in 100% nut bottles. In all cases the results with tap water are lower and more successful than groundwater, where the denitrification process was incomplete or DRNA developed producing ammonium.

6. References

Bothe, H., Ferguson, S.J., Newton, W.E. (2007). Biology of the nitrogen cycle. Primera edición. Elsevier.

Braker, G. Fesefeldt, A., Witzel, K.P. (1998). Development of PCR primer systems for amplification of nitrite reductase genes (nirK and nirS) to detect denitrifying bacteria in environmental samples. Applied and Environmental Microbiology 64: 3769-3775.

Carrera, J., Baeza, J.A., Vicent, T., Lafuente, J. (2003). Biological nitrogen removal of high-strength ammonium industrial wastewater with two-sludge system. Water Research 37 (17): 4211-4221.

Graham, D.W., Trippett, C., Dodds, W.K., O'Brien J.M., Banner, E.B.K., Head, I.M., Smith, M.S., Yang, R.K., Knapp, C.W. (2010). Correlations between in situ denitrification activity and nir-gene abundances in pristine and impacted prairie streams. Environmental Pollution 158: 3225-3229.

Healy, M., Ibrahim, T.G., Lanigan, G.J., Serrenho, A.J., Fenton, O. (2012). Nitrate removal rare, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors. Ecological Engineering 40: 198-209.

Hekmatzadeh, A.A.,Karimi-Jashani A., Talebbeydokhti N., Kløve B. (2012). Modeling of nitrate removal for ion exchange resin in batch and fixed bed experiments. Desalination 284: 22-31.

Henry, S., Baudoin, E., Lopez-Gutierrez, J.C., Matin-Laurent, F., Brauman, A., Philippot, L. (2004). Quantification of denitrifying bacteria in soils by *nir*K gene targeted real-time PCR. Journal of Microbiological Methods 59: 327-335.

ITRC (2002). A systematic approach to in situ bioremediation in groundwater: decision trees on in situ bioremediation for nitrates, Carbon Tetrachloride, and Perchlorate. ISB-8. Accessed at <http://www.itrcweb.org/user/isb-8r.pdf>.

Kapoor, A., Viraraghavan, T. (1997). Nitrate removal from drinking water. Journal of Environmental Engineering 123: 371-380.

Knowles, R. (2005). Denitrifiers associated with methanotrophs and their potential impact on the nitrogen cycle. Ecological Engineering 24 (5): 441-446.

Lee, S., Maken, S., Jang, J.H., Park, K., Park, J.W. (2006). Development of physicochemical nitrogen removal process for high strength industrial wastewater. Water Res. 40: 975-980.

Lee. B.S., Lee. K., Um, J.Y., Nam, K. (2014). Slowly released molasses barrier system for controlling nitrate plumes in groundwater: A pilot-scale tank study. Chemosphere 97: 135-139.

Liu S.J., Zhao Z.Y., Li, J., Wang, J., Qi, Y. (2013). An anaerobic two layer permeable reactive biobarrier for the remediation of nitrate-contaminated groundwater. Water Research 47: 5977-5985.

Misiti, T.M., Hajaya, M. G., Pavlostathis, S. G. (2011). Nitrate reduction in a simulated free - water surface wetland system. Water Research 45: 5587-5598.

Moon, H. S., Chang, S.W., Nam, K., Choe, J., & Kim, J. Y. (2006). Environmental Pollution, 144: 802– 807.

Nancharaiah, Y. V., Venugopalan, P. V. (2011). Denitrification of synthetic concentrated nitrate wastes by aerobic granular sludge under anoxic conditions. Chemosphere 85: 683–688.

Philippot, L. (2002). Dentrifying genes in bacterial and archaeal genomes. Biochimica et Biophysica Acta-Gene Structure and Expression. 1577: 355-376.

Philippot, L., Hallin, S. (2005). Finding the missing link between diversity and activity using denitrifying bacteria as a model functional community. Current Opinions in Microbiology 8:234-239.

Pynaert, K., Smets, B., Wyffels, S., Beheydt, D., Siciliano, S.D., Verstraete, W. (2003). Characterization of an autotrophic nitrogen-removing biofilm from a highly loaded lab-scale rotating biological contactor. Applied and Environmental Microbiology 69 (6): 3626-3635.

Qambrani, N. A., Oh, S. (2013). Effect of dissolved oxygen tension and agitation rates on sulfur-utilizing autotrophic denitrification: batch tests. Appl Biochem Biotechnol 169:181-191.

Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.W.N., Bemment, C.D. (2008). Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. Water Research 42 (16): 4215-4232.

Robinson-Lora, M.A., Brennan, R.A. (2009). The use of crab-shell chitin for biological denitrification: batch and column tests. Bioresource Technology 100: 534-541.

Rodríguez, D.C., Pino, N., Peñuela, G. (2011). Monitoring the removal of nitrogen by applying a nitrification-denitrification process in a Sequencing Batch Reactor (SBR). Bioresource Technology 102: 2316-2321.

Saliling, W.J., Westerman, P.W., Losordo, T.M. (2007). Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. Aquacultural Engineering, 37: 222-233.

Schipper, L.A., Robertson, W.D., Gold, A.J., Jaynes, D.B., Cameron, S.D. (2010). Denitrifying bioreactors-An approach for reducing nitrate loads to receiving waters. Ecological Engineering 36: 1532-1543.

Soares, M.I.M., Abeliovich, A. (1998). Wheat straw as substrate for water denitrification. Water Res. 32: 3790-3794.

United States Environmental Protection Agency (US EPA). (2009). Drinking Water Contaminants. http://water.epa.gov/drink/ contaminants/index.cfm (accessed 31.01.13).

WHO. (2004). Guidelines for Drinking-water quality – 3rd edition. Chapter 12: Chemical Fact Sheets. pp 417-420.

Xing, X., Gao, B.-Y., Zhong, Q.-Q., Yue, Q.-Y., Li, Q. (2011). Sorption of nitrate onto amine-cross linked wheat straw: characteristics, column sorption and desorption properties. J. Hazard. Mater. 186: 206-211.

Zhang, J., Feng, C., Hong, S., Hao, H., Yang, Y. (2012). Behaviour of solid carbon sources for biological denitrification in groundwater remediation. Water Science and Technology 65 (9): 1696-1704.

USE OF TEA AND HAZELNUT HUSK WASTES ON DENITRIFIACTION PROCESS TO CLEAR GROUNDWATER

Stylianos Gkiouzepas, Charles Knapp, Robert Kalin
University of Strathclyde, Department of Civil and Environmental Engineering, James Weir Building, Level 5, 75 Montrose Street, Glasgow, G1 1XJ, E-mail: stylianos.gkiouzepas@strath.ac.uk

ABSTRACT

The high nitrogen levels in water, is a problem that exists worldwide due to the high levels of fertilizers that used in agriculture. Denitrification is the process that is used to clear groundwater under biological procedure. The experiments focus on the reuse of groundwater for agricultural purposes. Denitrification process is under investigation in column studies that the environment is controlled and all conditions can be changed to simulate the nature. The substrate materials that used were tea waste materials and hazelnut husk wastes without any other addition of carbon source. All materials received from Mediterranean area (Greece and Turkey). The initial amount of nitrogen levels remains stable by adding specific amount of potassium nitrate to receive initial amount of nitrate levels 32.2 mg/l. The substrate materials used first time to denitrification process. The columns were separated in 2 categories, one with high flow rate and one with low flow rate. The flow rate is ensured by the use of peristaltic pump. The columns had 40% and 60% w/w substrate materials combined with sand. The results that received were very promising. The reduction that received in Tea and Nut columns was more than 97% at all cases. A concerning issue noticed in phosphate levels of experiment.

1. INTRODUCTION

Nitrogen cycle is connecting with human life with several ways. Nitrogen compounds are part of nitrogen cycle (Bothe et al., 2007). Nitrate is one of the most important nitrogen compounds and the most widespread pollutants (Rodriguez et al., 2011). It is the most oxidized contaminant in groundwater worldwide in many developed and developing countries. Groundwater is the main sources of drinking water in many areas around the world (Nancharaiah et al., 2011).

Worldwide, nitrate is the most common groundwater contaminant, with primary source all the agricultural activities. The main sources of groundwater contamination includes point sources such as animal feeds, waste lagoons and septic tanks and non-point sources like applied chemical fertilizers (nitrogen and phosphorus based) and manure (Robinson Lora et al., 2009, Su and Puls, 2007).

Several industrial activities like the combustion of fossil fuels, fertilizers production, explosives, glass, plastics and cured meat can also result to nitrate pollution (Misiti et al., 2011).

Groundwater contains nitrate levels less than 3 mg L^{-1} as N. However, in contaminated areas, nitrate concentrations can exceed 200 mg L^{-1} as N. The levels of water pollution enlarged in developed countries where the standards of living are increased (ITRC, 2002).

The European Nitrate Directive limits nitrate levels from agricultural sources (Nitrate Directive 91/676/EEC) at 50 mg/l NO_3^--N and 10 mg/l NO_3-N in drinking water (Blowes et al., 1994, Saeed and Sun, 2012).

Nitrate and nitrite concentrations higher than international limit present health concerns as they are toxic to humans and livestock. There are health-related consequences in waters with high levels of nitrogen compounds and include methemoglobinemia (blue baby syndrome at infants), oesophagus problems, and cancers of digestion system (Hekmatzadeh et al., 2012).

Except from the problems in health, high nitrate levels cause several problems in nature. The main problem on rivers and lakes is eutrophication which followed by hypoxia and fish-kill, acidification and other environmental effects (Carrera et al., 2004).

In nature the incomplete denitrification processes (part of nitrogen cycle) the gas nitrous oxide (N_2O) can be transformed, and it is responsible for global warming (Bothe et al., 2007).

Several techniques have been used for nitrate reduction such as biological denitrification, reverse osmosis, electrodialysis, ion exchange, adsorption, chemical reduction using zero valent iron, and catalytic reduction (Knowles et al., 2005, Xing et al., 2011).

The process of denitrification is the reduction of nitrate to nitrite and subsequently the reduction of nitrite to nitric oxide (NO), then to nitrous oxide (N_2O) and finally to molecular nitrogen (N_2), which is released into atmosphere. Denitrification is mainly carried out by two routes: heterotrophic denitrification which requires an environment that is rich at organic carbon and autotrophic denitrification where autotrophs organisms are able to synthesize organic matter from minerals (Della Rocca et al., 2007, Van Rijn et al., 2006, Zhou et al., 2011).

Biological denitrification is a natural process and used in several fields like wastewater treatment plants for the removal of excess nitrogen, removal of nitrate from surface waters and it is the most frequently method for municipal wastewaters. Effective nitrate removal at concentrated wastes in few studies is achieved by biological denitrification. Biological denitrification removes nitrate from the aquatic environment, recovering some of the alkalinity consumed in nitrification, and increasing the pH (Healy et al., 2012, Liu et al., 2013, Schipper et al., 2010).

The present research reports the results of column experiments using tea wastes materials and hazelnut husk wastes as substrate materials to reduce nitrogen compounds to acceptable limits under anoxic conditions, without any light connection and any other carbon source. These materials are from the area of Greece and Turkey and they are used either as fertilizers in agricultural fields or as a fuel in local production areas. The research investigation took place in University of Strathclyde, Glasgow Scotland. Column experiments were carried out to determine the ability of wastes to provide dissolved organic carbon and stimulate the activity of denitrifying bacteria and the effectiveness in short and long term time periods.

2. MATERIALS AND METHODS

2.1 Substrate materials

Halzelnut husk wastes was obtained from local farms in Ordu (Middle Black Sea Region) after harvesting and from local farms in Veria (Macedonia). The husk was cleaned of non-husk impurities, washed, dried at 105° C for 3 h (Albanis et al., 1998).
The tea factory wastes were otained from the Caykur tea factory located in Cayeli Rize, the East Black Sea Region of Turkey and from the area of Chalkidiki in Macedonia, Greece direct from the nature. All wastes were air dried and screened to obtain a fraction below 4 mm (Jing et al., 2010).
In column experiments saturated samples were utilized to avoid the presence of trapped oxygen. Saturation of wastes was carried out by filling 1000 mL Duran bottles with hazelnut husk wastes or tea factory wastes and adding tap water until the bottles were totally full. These bottles were stored at temperatures below 4° C.

2.2. Column experiment design

Lab-scale barrier system (Gilbert et al., 2008, Omirou et al., 2012) was set up using eight PVC columns of 50 cm length and 5 cm internal diameter. These columns were filled with 40% (w/w) organic substrate and 60% (w/w) sand, two with 60% (w/w) organic substrate and 40% (w/w) sand and two with only sand (100%) (w/w). All materials were placed as saturated materials into the columns full with water to avoid the presence of trapped oxygen. The porosity of reactive mixtures was determined approximately from the volume of displaced water. The columns were fed with a synthetic groundwater (32.2 mg L^{-1}/ 0.526 mmol L^{-1} NO_3^--N) in up-flow mode.
Two different flow rates were applied using two multichannel peristaltic pumps. The hydrodynamic characteristic, flow rate and hydraulic retention time of columns were investigated with a tracer test using 1000 mg L^{-1} chloride solution. The determined flow rates and retention times were HRT 3.25 d for the fast columns and HRT 6.10 d for the slow columns, respectively.
All columns were sealed to obtain anoxic conditions and covered with aluminium folio to avoid light penetration. Experiments were carried out at stable room temperature (20±2 ^oC). Effluents were taken four to five times per week and analysed.
Two types of solution used. In the first part tap water from laboratories of Strathclyde University received and in the second part groundwater from the suburban area outside of Glasgow, UK used.

2.3 Methodology

Water samples were obtained at the output of each column every 2 days and filtered through a 0.45-μm cellulose membrane filter. Samples were analysed for TOC, pH, oxidation–reduction potential (ORP) and conductivity levels. The dissolved oxygen (DO) levels in the initial flasks were tested using a digital DO meter (Model 9143, Hanna, Italy). The pH and ORP measured with Mettler Toledo Seven Multi multi-meter. Analysis of anions and cations completed by ion chromatography (IC) (Metrohm 850 Professional) to monitor NO_3^--N, NO_2-N, NH_4^+-N and PO_4-P concentrations. TOC was measured with TOC analyser Apollo 9000 (Teledyne Tekmar). All measurements

were done according to ASTM. Control tests were also carried out in two stages with tap water and groundwater, respectively. Total Nitrogen (TN) concentrations were determined by summing the concentrations of nitrate, nitrite and ammonium.

3. RESULTS

The experiment is separated in two phases. The first one had duration 51 days and the solution that used was tap water from Glasgow. The tap water analysed to find out the initial condition and then specific amount of potassium nitrate spiked to the initial solution to receive the expected nitrate levels (32.2 mg L^{-1} - 0.526 mmol L^{-1} NO_3^--N).
The sand columns used as control columns. The one with high flow rate (Sand-H) and the second with low flow rate (Sand–L) to examine the two situations. The next part was the Tea columns with 40% and 60% substrate materials in high (T-40-H) and low flow rate (T-40-L and T-60-L). The last group was the Nut columns that follow the same design like Tea columns.

3.1 Results with tap water as solution

All the results of the first experiment are given in the Table 1.
The pH levels at all columns remain in neutral levels with the highest prices received in N-40-L column with pH=7.53 and the lowest levels in T-60-L column with pH=7.21.
Conductivity levels have a wide spread and the highest and lowest levels along the experiment achieved in Tea columns in T-40-L and T-40-H, respectively. In Nut columns the spread was smaller with the same attitude in columns, lower in N-40-H and higher in N-40-L column.
Redox potential levels remain in the same levels at Tea and Nut columns with slightly lower levels in Tea columns achieving the lowest level in T-60-L column and the highest in N-40-H column.

TABLE 1: Tap water column results

		Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
pH	mean	7.55	7.59	7.44	7.44	7.21	7.27	7.53	7.39	7.36
	st dev	*0.14*	*0.24*	*0.22*	*0.28*	*0.39*	*0.25*	*0.24*	*0.26*	*0.26*
	% change		*0.54*	*-1.43*	*-1.44*	*-4.51*	*-3.61*	*-0.20*	*-2.09*	*-2.44*
Conductivity µS/cm	mean	360.86	349.91	401.93	617.06	494.98	357.79	430.56	404.92	381.92
	st dev	*42.58*	*134.05*	*110.81*	*87.35*	*74.29*	*57.10*	*64.81*	*59.47*	*143.07*
	% change		*-3.03*	*11.38*	*71.00*	*37.17*	*-0.85*	*19.32*	*12.21*	*5.84*
Redox mV	mean	234.97	212.71	233.44	207.03	197.38	209.63	218.60	208.68	223.78
	st dev	*62.87*	*52.99*	*58.36*	*35.61*	*38.47*	*35.26*	*46.45*	*43.10*	*51.75*
	% change		*-9.47*	*-0.65*	*-11.89*	*-16.00*	*-10.78*	*-6.96*	*-11.19*	*-4.76*
TOC ppm	mean	2.69	3.24	3.50	38.72	52.36	7.62	6.82	7.90	4.45
	st dev	*1.38*	*1.22*	*1.46*	*17.28*	*32.44*	*5.70*	*1.51*	*1.65*	*1.25*
	% change		*20.41*	*29.89*	*1336.87*	*1843.27*	*182.97*	*153.07*	*193.25*	*65.10*
NO3 mmol/l 10^-3	mean	511.09	372.83	348.12	0.06	0.17	0.63	0.14	0.22	0.69
	st dev	*14.23*	*74.59*	*35.93*	*0.22*	*0.30*	*1.31*	*0.30*	*0.58*	*1.79*
	% change		*-27.05*	*-31.89*	*-99.99*	*-99.97*	*-99.88*	*-99.97*	*-99.96*	*-99.86*
NO2 mmol/l 10^-3	mean	1.02	3.97	89.46	1.15	1.15	1.29	1.25	1.51	1.81
	st dev	*1.48*	*6.24*	*122.14*	*1.46*	*1.31*	*1.33*	*1.29*	*1.69*	*1.49*
	% change		*288.11*	*8653.72*	*12.28*	*12.38*	*25.98*	*22.11*	*47.50*	*77.40*
NH4 mmol/l 10^-3	mean	0.50	1.70	2.56	0.72	7.34	1.75	2.34	2.66	1.00
	st dev	*1.01*	*2.43*	*2.96*	*1.70*	*8.80*	*2.47*	*3.05*	*3.93*	*1.43*
	% change		*242.68*	*417.36*	*45.81*	*1381.94*	*253.78*	*371.85*	*436.33*	*102.02*
PO4 mg/l	mean	1.63	12.82	17.66	12.58	10.99	10.95	13.68	11.49	22.87
	st dev	*1.27*	*9.27*	*4.85*	*18.62*	*11.11*	*12.71*	*13.93*	*12.25*	*11.31*
	% change		*688.12*	*985.78*	*673.04*	*575.40*	*573.27*	*741.08*	*606.52*	*1305.48*
TN mmol/l 10^-3	mean	512.61	378.50	440.15	1.93	8.66	3.67	3.72	4.38	3.51
	st dev	*13.36*	*75.99*	*119.22*	*2.27*	*9.33*	*4.22*	*3.72*	*4.98*	*3.42*
	% change		*-26.16*	*-14.14*	*-99.62*	*-98.31*	*-99.28*	*-99.27*	*-99.14*	*-99.32*

Total Organic Carbon (TOC) levels are higher than the initial solution (2.7 ppm) as it was expected showing that the two new substrate materials are working and can provide the carbon source to the system for denitrification process. In Tea columns the increase was really noticeable and especially in low flow rate columns achieving levels higher than 35 ppm till 80 ppm. In Nut columns the increase was in lower levels between 4.5-8 ppm. At all cases the suitable environmental conditions in columns can provide the best results in denitrification process (Hamersley et al., 2002, Rivett et al., 2008).

In nitrogen compounds, the reduction is unexpected. The results that received are really promising. In details, nitrate levels at all columns with Tea and Nut had reduction more than 99.8% showing that denitrification process is working on columns. Nitrite and ammonium levels increased from the initial solution but in contrast to nitrate levels the concentrations are negligible. Total Nitrogen (TN) levels in all columns reduced more than 98.3%. The best reduction in Tea columns received in T-40-L column (99.6%) and the best reduction in Nut columns in N-40-H column (99.3%)

Finally phosphate levels are the concerning point of research. The levels are increased at all columns from 11 till 22 times higher than the initial solution. In Tea columns phosphate levels were lower than Nut columns between 10.9-12.6 mg/l. In Nut columns phosphate levels were between 11.5-22.9 mg/l with the highest levels in N-40-H column.

3.2 Results with groundwater as solution

Groundwater used as solution in the second part of experiment and received from suburban area outside of Glasgow, UK where a lot of farms and heavy industries are located in the close area. Groundwater solution received at the middle of autumn where water levels expect from groundwater are affected by rain volumes. The duration of experiment was 32 days. The system remain the same and the calibration of lab barrier became for 10 days before the application of potassium nitrate. Groundwater analysed before the application to find out the initial concentrations in anions and cations. The groundwater spiked with potassium nitrate to receive the same initial quantity of nitrate levels. The design and flow rate remain the same as in tap water experiment. Groundwater from collection day till the time of application remain in temperature less than 4ºC to keep alive all the microcosm of nature. All the results are given in Table 2.

TABLE 2: Ground water column results

		Initial	Sand-L	Sand-H	T-40-L	T-60-L	T-40-H	N-40-L	N-60-L	N-40-H
pH	mean	7.52	7.92	7.63	7.70	7.64	7.58	7.86	7.71	7.60
	st dev	*0.08*	*0.13*	*0.11*	*0.16*	*0.23*	*0.11*	*0.16*	*0.16*	*0.08*
	% change		*5.38*	*1.46*	*2.51*	*1.60*	*0.82*	*4.57*	*2.63*	*1.10*
Conductivity µS/cm	mean	399.41	499.69	500.18	638.81	483.53	439.44	485.03	496.95	451.58
	st dev	*21.06*	*34.13*	*20.60*	*20.09*	*16.88*	*34.91*	*16.47*	*20.95*	*23.83*
	% change		*25.11*	*25.23*	*59.94*	*21.06*	*10.02*	*21.44*	*24.42*	*13.06*
Redox mV	mean	146.95	149.65	151.08	145.01	141.48	146.98	151.49	148.48	147.93
	st dev	*17.84*	*16.33*	*18.01*	*21.76*	*19.80*	*19.42*	*17.08*	*18.59*	*17.53*
	% change		*1.84*	*2.81*	*-1.32*	*-3.72*	*0.02*	*3.09*	*1.04*	*0.66*
TOC ppm	mean	20.35	12.15	14.83	77.22	44.26	21.53	17.64	22.26	15.94
	st dev	*1.68*	*2.63*	*4.40*	*25.27*	*9.10*	*2.99*	*2.24*	*3.24*	*2.98*
	% change		*-40.30*	*-27.13*	*279.52*	*117.50*	*5.82*	*-13.31*	*9.39*	*-21.67*
NO3 mmol/l 10^-3	mean	516.32	287.28	388.28	0.00	0.15	1.65	0.38	0.48	12.83
	st dev	*18.23*	*104.59*	*63.74*	*0.00*	*0.29*	*3.00*	*0.61*	*0.56*	*18.31*
	% change		*-44.36*	*-24.80*	*-100.00*	*-99.97*	*-99.68*	*-99.93*	*-99.91*	*-97.52*
NO2 mmol/l 10^-3	mean	2.78	3.35	143.71	0.00	1.55	2.49	0.66	1.00	1.59
	st dev	*0.54*	*0.82*	*170.01*	*0.00*	*1.33*	*7.20*	*1.15*	*1.29*	*1.25*
	% change		*20.31*	*5062.16*	*-100.00*	*-44.35*	*-10.46*	*-76.39*	*-64.10*	*-43.03*
NH4 mmol/l 10^-3	mean	1.18	1.46	1.39	0.23	10.17	1.37	0.46	0.25	0.24
	st dev	*1.26*	*3.13*	*2.24*	*0.74*	*19.70*	*2.33*	*0.75*	*0.52*	*0.57*
	% change		*24.19*	*17.69*	*-80.38*	*762.01*	*15.94*	*-61.39*	*-79.20*	*-79.88*
PO4 mg/l	mean	3.53	11.65	6.89	11.57	12.89	12.77	7.64	11.13	10.89
	st dev	*0.67*	*11.17*	*6.41*	*12.16*	*17.85*	*15.85*	*4.84*	*7.50*	*11.62*
	% change		*229.56*	*94.93*	*227.33*	*264.78*	*261.46*	*116.11*	*214.81*	*208.07*
TN mmol/l 10^-3	mean	520.28	292.10	533.38	0.23	11.86	5.51	1.49	1.73	14.65
	st dev	*18.88*	*105.00*	*120.94*	*0.74*	*19.30*	*9.36*	*1.91*	*1.54*	*18.87*
	% change		*-43.86*	*2.52*	*-99.96*	*-97.72*	*-98.94*	*-99.71*	*-99.67*	*-97.18*

The comparison between the two solutions that used are in conductivity levels that are higher in groundwater about 50 µS/cm, redox levels are lower about 90 mV, TOC levels are higher 10 time in groundwater and all nitrogen compounds are slightly higher than tap water. Finally phosphate levels are double than tap water.

The pH levels as it is happening in tap water remain in neutral levels at all the duration of experiment at all columns. These levels are the most suitable levels for denitrification process (Rivett et al., 2008). In details in contrast to tap water pH levels are higher. The

lowest levels received again in Tea columns with the lowest levels in T-40-H column pH=7.58 and the highest levels in N-40-L column with pH=7.86.
Conductivity levels follow the same attitude like tap water part. The highest and lowest levels received in Tea columns, T-40-L and T-40-H, respectively and more average levels in Nut columns.
Redox potential levels at all columns are much lower than tap water part. The lowest levels noticed in Tea columns with the lowest level in T-60-L column (141.48 mV). In Nut columns the levels are higher and the highest levels are in N-40-L column (151.49 mV).
TOC levels are higher than the part with tap water. The higher level received in Tea columns between 21-77 ppm with the highest level in T-40-L column and lower level in Nut columns between 16-22 ppm. The lowest level noticed in N-40-H column.
In nitrogen compounds the reduction that is noticed is in the same levels as tap water. Nitrate levels at all columns removed more than 99.6%. The only column that the reduction levels were lower 97.5% was N-40-H column. Nitrite and ammonium levels are slightly higher than tap water part but again negligible. TN levels in Tea columns removed more than 97.7% at all columns with the highest levels in T-40-L column (99.96%). In Nut columns the reduction is more than 97.1%. The best reduction is noticed in N-40-L column (99.71%).
Phosphate levels remain in the same levels as in tap water part. The highest levels noticed in Tea columns with levels between 11-13 mg/l and the highest level in T-60-L column. In Nut columns phosphate levels were lower between 7-12 mg/l. The lowest levels noticed in N-40-L column.

4. DISCUSSION

At both parts of experiment, denitrification process takes place and it is visible in detail at all results. The reduction is achieved in the first two days at both experiments. This phase is the initial face and the microbial activity follows the first order kinetics. The second phase is the stable phase. The degradation rates follow the zero order kinetics (Tang et al., 2010). It is noticeable that retention time and degradation rates are changing along the experiment. The environmental conditions and the carbon source which are the substrate material that are used are connected with denitrification process. The half-life that is noticed in the columns is less than 1.5 days at all the columns. The best half-life noticed in high flow rate columns with 11.47 hours in Tea and Nut column in tap water part. The highest half-life noticed in T-60-L column with 24.52 hours. In the second part with groundwater the half-life follow the same attitude with lowest levels in T-40-H column with half-life 12.55 hours. Finally the highest half-life noticed in T-60-L column with 25.39 hours. The half-lives that receive are in the limits that other researcher used and even faster degradation rates are met in that research (Robertson, 2010, Patterson et al., 2005, 2009).
Denitrification process is taking place and the bacteria colonies that noticed in the columns were the genes denitrifiers. The identification done with q-PCR and that was the evidence that there are denitrifiers bacteria (*Thiobacillus denitrificans* and *Pseudomonas*) as it is noticed at several researchers in denitrification process (Munoz-leoz et al., 2011, Rivett et al., 2008).

The issue that is concerning in both parts is the high phosphate levels. The levels are increased in both cases more than 3 times (12 mg/l). The levels are noticeable and the reductions of phosphate levels parallel with nitrate must succeed.

5. CONCLUSION

Denitrification process investigation in columns studies with tea waste materials and hazelnut husk wastes is successful. The HRT was 3.25 for fast flow rate and 6.1 days for slow flow rate. The nitrogen removal at all cases was more than 97% even with tap water or groundwater as solution. The half-lives for the denitrification rates were fast between 11.4 and 25.4 hours. The concerning point is the high levels of phosphates that are visible in both experiment. In long term studies and in combination with the suitable substrate material like perlite this problem will not exist. In all cases the reduction that achieved in slow flow rate and in Tea and Nut columns is more successful than in fast flow rate.

REFERENCES

Albanis, T., D.G. Hela, T.M. Sakellarides, I.K. Konstantinou (1998) 'Monitoring of pesticide residues and their metabolites in surface and underground waters of Imathia (N. Greece) by means of solid-phase extraction disks and gas chromatography' **Journal of Chromatography A,** Vol. 823, pp. 59-71.

Blowes, D., W. Robertson, C. Ptacek, C. Merkley (1994) 'Removal of agricultural nitrate from tile-drainage effluent water using in-line bioreactors' **Journal of Contaminant Hydrology**, Vol. 15, pp. 207-211.

Bothe, H., S.J. Ferguson, W.E. Newton (2007) 'Biology of the nitrogen cycle' Primera edición. Elsevier.

Carrera, J., J.A. Baeza, T. Vicent, J. Lafuente (2004) 'Biological nitrogen removal of high-strength ammonium industrial wastewater with two-sludge system' **Water Research,** Vol. 37, No. 17, pp. 4211-4221.

Della Rocca, C., V. Belgiorno, S. Meric (2007) 'Overview of in-situ applicable nitrate removal processes' **Desalination** Vol. 204, pp. 46-62.

Gibert, O., S. Pomierny, I. Rowe, R. Kalin (2008) 'Selection of organic substrate as potential reactive materials for use in a Denitrification permeable reactive barrier (PRB)' **Biosource Technology,** Vol. 99, pp. 7587-7596.

Hamersley, M.R., B.L. Howes (2002) 'Control of denitrification in a septage-treating artificial wetland: The dual role of particulate organic carbon' **Water Research,** Vol. 36, pp. 4415-4427.

Healy, M., T.G. Ibrahim, G.J. Lanigan, A.J. Serrenho, O. Fenton (2012) 'Nitrate removal rare, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors' **Ecological Engineering,** Vol. 40, pp. 198-209.

Hekmatzadeh, A.A., A. Karimi-Jashani, N. Talebbeydokhti, B. Kløve (2012) 'Modeling of nitrate removal for ion exchange resin in batch and fixed bed experiments' **Desalination,** Vol. 284, pp.22-31.

ITRC (2002) 'A systematic approach to in situ bioremediation in groundwater: decision trees on in situ bioremediation for nitrates, Carbon Tetrachloride, and Perchlorate' ISB-8. Accessed at <http://www.itrcweb.org/user/isb-8r.pdf>. 10/3/2015.

Jing, L., H. Wang, T. Jiang, D. Li, H. Feng, M. Chen (2010) 'Denitrification of Groundwater Using Walnut Shell as Solid Carbon Source' **Conference on Environmental Pollution and Public Health,** pp. 1059-1063.

Knowles, R. (2005) 'Denitrifiers associated with methanotrophs and their potential impact on the nitrogen cycle' **Ecological Engineering,** Vol. 24 No. 5, pp. 441-446.

Liu S.J., Z.Y. Zhao, J. Li, J. Wang, Y. Qi. (2013) 'An anaerobic two layer permeable reactive biobarrier for the remediation of nitrate-contaminated groundwater' **Water Research,** Vol. 47, pp. 5977-5985.

Misiti, T.M., M.G. Hajaya, S.G. Pavlostathis (2011) 'Nitrate reduction in a simulated free -water surface wetland system' **Water Research,** Vol. 45, pp. 5587-5598.

Muñoz-leoz, B., I. Antigüedad, C. Garbisu, E. Ruiz-Romera (2011) 'Science of the Total Environment Nitrogen transformations and greenhouse gas emissions from a riparian wetland soil: An undisturbed soil column study' **Science of the Total Environment,** Vol.409, pp. 763-770.

Nancharaiah, Y.V., P.V. Venugopalan (2011) 'Denitrification of synthetic concentrated nitrate wastes by aerobic granular sludge under anoxic conditions' **Chemosphere**, Vol.85, pp. 683-688.

Omirou, M., P. Dalias, C. Costa, C. Papastefanou, A. Dados, C. Ehaliotis, D.G. Karpouzas (2012) 'Exploring the potential of biobeds for the depuration of pesticide-contaminated wastewaters from the citrus production chain: laboratory, column and field studies' **Environmental Pollution,** Vol. 166, pp. 31-39.

Patterson, B.M., Y. Ma, M.E. Grassi, B.S. Robertson, G.B. Davis, M. Lipman (2005) 'Assessment of different carbon sources and delivery techniques to promote an in situ reactive zone for bioprecipitation of metals in groundwater. Permeable Reactive Barriers' **Proc. Int. Symp. Belfast, Northern Ireland, March 2004**, IAHS Publ. 298, 2005

Patterson, B., J. Pearce, T. Spadek, K. Linge, F. Busetti, A. Furness, P. Blair, A. Heitz (2009) 'Fate of trace organics using laboratory column experiments' (www.clw.csiro.au/publications/.../wfhc-MAR-requirements-WA-Ch2.pdf) accessed 20/02/2015

Rivett, M., S. Buss, P. Morgan, J. Smith, C. Bemment (2008) 'Nitrate attenuation in groundwater: A review of biochemical controlling processes' **Water Research,** Vol. 42, pp. 4215-4232.

Robertson, W.D. (2010) 'Nitrate removal rates in woodchip media of varying age' **Ecological Engineering,** Vol. 36, pp. 1581-1587.

Robinson-Lora, M.A., R.A. Brennan (2009) 'The use of crab-shell chitin for biological denitrification: batch and column tests' **Bioresource Technology,** Vol. 100, pp. 534-541.

Rodríguez, D.C., N. Pino, G. Peñuela (2011) 'Monitoring the removal of nitrogen by applying a nitrification-denitrification process in a Sequencing Batch Reactor (SBR)' **Bioresource Technology,** Vol. 102, pp. 2316-2321.

Saeed T., G. Sun (2012) 'A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media' **Journal of Environmental Management,** Vol. 112, pp. 429-448.

Schipper, L.A., W.D. Robertson, A.J. Gold, D.B. Jaynes, S.D. Cameron (2010) 'Denitrifying bioreactors-An approach for reducing nitrate loads to receiving waters' **Ecological Engineering,** Vol. 36, pp. 1532-1543.

Su, S., R. Puls (2007) 'Removal of added nitrate in cotton burr compost, mulch compost, and peat: Mechanisms and potential use for groundwater nitrate remediation' **Chemosphere,** Vol. 66, pp. 91-98.
Tang, S., Q. Yang, H. Shang, T. Sun (2010) 'Removal of Nitrate by autosulfurotrophic denitrifying bacteria: optimization, kinetics and thermodynamics study' **FEB,** Vol. 19, No. 12b, pp. 3193-3198.
Van Rijn, J., Y. Tal, H. Schreier (2006) 'Denitrification in recirculating systems: theory and applications' **Aquaculture Engineering**, Vol. 34, pp. 364-376.
Xing, X., B.Y. Gao, Q.Q. Zhong, Q.Y. Yue, Q. Li (2011) 'Sorption of nitrate onto aminecross linked wheat straw: characteristics, column sorption and desorption properties' **Journal Hazardous Materials,** Vol. 186, pp. 206-211.
Zhou, W., Y. Sun, B. Wu, Y. Zhang, M. Huang, T. Miyanaga, Z. Zhang (2011) 'Autotrophic Denitrification for nitrate and nitrite removal using sulfur-limestone' **Journal of Environmental Science,** Vol. 23 No. 11, pp.1761-1769.

ΚΑΘΑΡΙΣΜΟΣ ΥΠΟΓΕΙΟΥ ΝΕΡΟΥ ΜΕΣΩ ΒΙΟΛΟΓΙΚΗΣ ΑΠΟΝΙΤΡΟΠΟΙΗΣΗΣ ΜΕ ΤΗΝ ΧΡΗΣΗ ΥΠΟΛΕΙΜΜΑΤΩΝ ΤΣΑΓΙΟΥ ΚΑΙ ΦΟΥΝΤΟΥΚΙΟΥ

Σ. Γκιουζέπας✉, C. Knapp, R. Kalin
University of Strathclyde, Department of Civil and Environmental Engineering, James Weir Building, Level 5, 75 Montrose Street, Glasgow, G1 1XJ, ✉ stylianos.gkiouzepas@strath.ac.uk

Περίληψη

Τα υψηλά επίπεδα του αζώτου στο νερό είναι ένα παγκόσμιο πρόβλημα λόγω των λιπασμάτων που χρησιμοποιούνται. Τα πειράματα εστιάζονται στην επαναχρησιμοποίηση υπόγειων υδάτων για γεωργικούς σκοπούς. Η διαδικασία της απονιτροποίησης ερευνάται με πειράματα στήλης όπου οι συνθήκες περιβάλλοντος ελέγχονται και μπορούν να προσομοιωσούν οποιοδήποτε φυσικό περιβάλλον. Τα υλικά υποστρώματος που χρησιμοποιήθηκαν ήταν υπολείματα από τσάι και από φλοιούς φουντουκιού. Το αρχικό ποσοστό του αζώτου παραμένει σταθερό (0.521 mmol/l), με την προσθήκη συγκεκριμένης ποσότητας νιτρικού καλίου (KNO_3). Οι στήλες χωρίστηκαν σε δύο κατηγορίες, μία με υψηλή και μία με χαμηλή ταχύτητα ροής. Ο σταθερός ρυθμός ροής εξασφαλίζεται με τη χρήση περισταλτικής αντλίας. Οι στήλες περιείχαν σε ποσοστά 40% και 60% w/w τα υλικά υποστρώματος σε συνδυασμό με άμμο. Μείωση παρατηρήθηκε στο ολικό άζωτο (97%) σε όλες τις περιπτώσεις. Το πρόβλημα που παρουσιάστηκε ήταν τα υψηλά επίπεδα φωσφόρου και αντιμετωπίστηκε με τη χρήση περλίτη που έχει την ιδιότητα να απορροφά τον φώσφορο και μειώνει ταυτόχρονα το άζωτο. Η μέθοδος αυτή ελέγχθηκε επιτυχώς σε βραχυπρόθεσμα και μακροπρόθεσμα πειράματα.

Λέξεις κλειδιά: Απονιτροποίηση, Τσάι, Φλοιούς φουντουκιού, Περλίτη, Μείωση φωσφόρου

DENITRIFICATION PROCESS TO CLEAN GROUNDWATER WITH TEA WASTE MATERIALS AND HAZELNUT HUSK WASTES

S.Gkiouzepas✉, C. Knapp, R. Kalin
University of Strathclyde, Department of Civil and Environmental Engineering, James Weir Building, Level 5, 75 Montrose Street, Glasgow, G1 1XJ, ✉ stylianos.gkiouzepas@strath.ac.uk

Abstract

The high nitrogen levels in the water, is a worldwide problem due to high levels of fertilizers that used in agriculture. The experiments focus on the reuse of groundwater for agriculture. The denitrification process is under investigation in column studies that the environment is controlled. The substrate materials that used were tea waste materials and hazelnut husk wastes. The initial amount of nitrogen levels remains stable by adding specific amount of potassium nitrate to receive initial amount of nitrate levels 0.521 mmol/l. The columns were separated in 2 categories, one with high flow rate and one with low flow rate. The flow rate is ensured by the use of peristaltic pump. The columns had 40% and 60% w/w substrate materials combined with sand. The reduction that received in columns was more than 97% at all cases. A concerning issue noticed in the phosphate levels and faced with the use of perlite which absorbs phosphate and nitrogen compounds. The method was checked successfully for short and long term experiments.

Key words: Denitrification, Tea and Nut waste materials, Perlite, Phosphate removal

1. INTRODUCTION

Nitrate is worldwide the most common groundwater contaminant; with primary source all agricultural activities. The main sources of groundwater contamination includes point sources such as animal feeds, waste lagoons and septic tanks and non-point sources like applied chemical fertilizers(nitrogen and phosphorus based) and manure(Su and Puls, 2007; Robinson-Lora et al., 2009).

Several industrial activities like combustion of fossil fuels, fertilizers production, explosives, glass, plastics and cured meat can also result to nitrate pollution (Misiti et al., 2011). Groundwater contains nitrate levels less than 3 mg/l as N. However, in contaminated areas, nitrate concentrations can exceed 200 mg/l as N. The Water Framework Directive (2000/60/EC) limits nitrate levels from agricultural sources at 50 mg/l NO_3-N and 10 mg/l NO_3-N in drinking water (Blowes et al., 1994).

Nitrate and nitrite concentrations higher than limits create health problems methemoglobinemia (blue baby syndrome at infants), oesophagus problems, and cancers of the digestion system (Hekmatzadeh et al., 2012).

High nitrate levels cause several problems in nature. The main problem on rivers and lakes is eutrophication which followed by hypoxia and fish-kill, acidification and other environmental effects (Carrera et al., 2004).

In nature the incomplete denitrification processes, the gas nitrous oxide (N_2O) can be transformed, and it is responsible for global warming (Bothe et al., 2007).

Several techniques have been used for nitrate reduction such as biological denitrification, reverse osmosis, electro dialysis, ion exchange, adsorption, chemical reduction using zero valent iron, and catalytic reduction (Della Rocca et al., 2007; Zhou et al., 2011).

The process of denitrification is the reduction of nitrate to nitrite and subsequently the reduction of nitrite to nitric oxide (NO), then to nitrous oxide (N_2O) and finally to molecular nitrogen (N_2), which is released into the atmosphere. Denitrification is mainly carried out by two routes: heterotrophic denitrification which requires an environment that is rich at organic carbon and autotrophic denitrification where autotrophs organisms are able to synthesize organic matter from minerals (Liu et al., 2013; Schipper et al., 2010).

The research reports the results of column experiments using tea wastes materials and hazelnut husk wastes as substrate materials to reduce the nitrogen compounds to acceptable limits under anoxic conditions, without any light connection and any other carbon source. These materials are from east Mediterranean area and they are used either as fertilizers in agricultural fields or as fuel in local production areas. The research took place in University of Strathclyde, Glasgow, UK. Column experiments were carried out to determine the ability of wastes to provide dissolved organic carbon and stimulate the activity of denitrifying bacteria and the effectiveness in short and long term time periods.

2. MATERIALS AND METHODS

2.1. Substrate materials

The substrate materials that used were tea waste materials and hazelnut husk wastes. Tea waste materials received from the east Black Sea region in Turkey, from the province Caykur which is famous for the tea production (*Camelia sinensis*). Tea wastes received from tea waste factories and all wastes air dried and screen to obtain particles less than 4mm (Mahavi et al., 2005).

Hazelnut husk wastes received from the middle Black Sea region from the province Ordu. The hazelnut husk waste came from the turkeys hazel plant (*Corylus colurna*). The husks were cleaned and dried at 105 °C for 3 hours (Jing et al., 2010).

In the columns saturated waste materials added to avoid the presence of trapped oxygen. The saturation of the samples became before the application in columns. Duran bottles 1000 ml filled in with waste materials and tap water until the bottles were totally full. The bottles remained in fridge temperatures below 4 °C for more than 1 month before the application in the columns.

2.2. Columns design

A lab scale barrier system consisting of eight PVC columns was set up. The dimensions of the columns were 50 cm length and 5 cm internal diameter. There were four columns that filled in with 40% (w/w) organic substrate materials and 60% (w/w) sand, two columns with 60% (w/w) organic substrate material and 40% (w/w) sand) and two only with sand (100%) (w/w). All the substrate materials were saturated to avoid the presence of oxygen. The porosity of the each columns measure from the volume of displaced water. The solution that used in the experiments was groundwater from an agriculture area outside Glasgow, Largs, UK. The groundwater spiked with specific amount of KNO_3 to receive initial concentration (32.2 mg/l /0.526 mmol/l). The groundwater had up flow mode (Gilbert et al., 2008; Omirou et al., 2012).

The experiment separated in two flow rates. The stable speed insured by the use of two multichannel peristaltic pumps. The hydrodynamic characteristics, the flow rate and the hydraulics retention time of columns recognised by tracer test. In the test 1000 mg/l of chloride solution were used. The hydraulic retention times in the columns were 3.25 days for the fast flow rates and 6.10 days for the slow flow rates.

All columns were covered with aluminium folio to avoid light penetration and sealed to obtain anoxic conditions. Experiments took place in stable room temperature (20±2 °C) and sample solutions received five times per week and analysed.

The experiments separated in two parts. The first part only with these columns and in the second part there was an addition of one more column at the end of each one. The dimensions of the new columns were 20cm length and 5 cm diameter and filled in with perlite. The flow rate remained the same at all parts of experiments. The duration of first part was 32 days and in the second part 98 days.

2.3. Methodology

Water samples received from the output of each column five times per week and filtered through 0.45 μm cellulose membrane filter. At all samples received direct and indirect measurements. In direct measurement exactly after samples analysed for pH, oxidation reduction potential, conductivity levels (Seven Mutlimeter Metter Toledo). Dissolved oxygen tested in the initial solution with DO meter (Model 9143, Hanna Italy). In the indirect methods were the analyses of anions and cations with ion chromatography (Metrhom 850 Professional), to monitor NO3-N, NO2-N, NH4-N and PO4-P concentrations. Also total organic carbon analysed with TOC analyser (Apollo 9000, Teledyne, Tekmar). All measurement became according to standard methods (APHA, 1999). Total Nitrogen (TN) concentrations were determined by summing the concentrations of all nitrogen compounds.

3. RESULTS

The experiment was separated in two phases. The first one was the phase with groundwater and without the addition of perlite columns. That part of experiment had duration 32 days.

The two sand columns used as control columns the one in low flow rate (Sand-L) and one in high flow rate (Sand-H). In low flow rate there are two columns with 40 and 60% respectively substrate material of tea and nut (T-40-L, T-60-L, N-40-L, N-60-L) and in high flow rate only with 40% substrate materials (T-40-H, N-40-H).

3.1. Results part one without perlite columns

The groundwater that used as solution in the first part of the experiment received from the suburban area outside of Glasgow, UK where a lot of farms and heavy industries are located in the close area. The collection of groundwater received at the middle of autumn where the water levels expect from the groundwater are affected by the rain volumes.

The duration of the experiment was 32 days. The systems remain the same and the calibration of the lab barrier became for 10 days before the application of potassium nitrate. The groundwater analysed before the application to find out the initial concentrations in anions and cations. The groundwater spiked with potassium nitrate to receive the initial quantity of nitrate levels. The groundwater from the collection day till the time of application remain in temperature less than 4°C to keep alive all the microcosm of the ecosystem. All the results are given in Table 1.

Table 1. Groundwater solution results without perlite columns

		Initial	SandL	SandH	T40L	T60L	T40H	N40L	N60L	N40H
pH	mean	7.52	7.92	7.63	7.70	7.64	7.58	7.86	7.71	7.60
	st dev	0.08	0.13	0.11	0.16	0.23	0.11	0.16	0.16	0.08
	% change		5.36	1.46	2.51	1.60	0.82	4.57	2.63	1.10
Conductivity µS/cm	mean	399.41	499.69	500.18	658.81	483.53	439.44	485.03	496.95	451.58
	st dev	21.06	84.18	20.00	20.09	16.88	34.92	16.47	20.95	23.83
	% change		25.11	25.23	59.94	21.06	10.02	21.44	24.42	13.06
Redox mV	mean	146.95	149.65	151.08	145.01	141.48	146.98	151.69	148.48	147.93
	st dev	17.84	16.33	18.01	21.76	19.80	19.42	17.08	16.39	17.33
	% change		1.84	2.81	-1.32	-3.72	0.02	3.09	1.04	0.66
TOC ppm	mean	20.35	12.15	14.83	77.22	44.26	21.53	17.64	22.26	15.94
	st dev	1.68	2.63	4.40	25.27	9.10	2.99	3.24	3.24	2.98
	% change		-40.30	-27.13	279.32	117.50	5.82	-13.31	9.39	-21.67
NO3 mmol/l 10^{-3}	mean	516.32	287.28	388.29	0.00	0.15	1.65	0.38	0.48	12.83
	st dev	18.23	104.59	63.74	0.00	0.29	3.00	0.61	0.56	18.31
	% change		-44.36	-24.80	-100.00	-99.97	-99.68	-99.93	-99.91	-97.52
NO2 mmol/l 10^{-3}	mean	2.78	3.35	143.71	0.00	1.55	2.49	0.66	1.00	1.59
	st dev	0.54	0.82	170.01	0.00	1.33	7.20	1.15	1.29	1.25
	% change		20.31	3062.16	-100.00	-44.33	-10.46	-76.39	-64.10	-43.03
NH4 mmol/l 10^{-3}	mean	1.18	1.46	1.39	0.23	10.17	1.37	0.46	0.35	0.24
	st dev	1.26	3.13	2.24	0.74	19.70	2.33	0.75	0.52	0.57
	% change		24.19	17.69	-80.38	762.01	15.94	-61.39	-79.20	-79.88
PO4 mg/l	mean	3.33	11.63	6.89	11.37	12.89	12.77	7.64	11.13	10.89
	st dev	0.67	11.17	6.41	12.16	17.65	15.85	4.84	7.50	11.62
	% change		229.56	94.93	227.33	264.78	261.46	116.11	214.61	208.07
TN mmol/l 10^{-3}	mean	320.28	292.10	333.38	0.23	11.86	3.51	1.49	1.73	14.69
	st dev	18.88	103.00	120.94	0.74	19.30	9.26	2.91	2.34	18.87
	% change		-43.86	2.52	-99.96	-97.72	-98.94	-99.71	-99.67	-97.16

The pH levels in Tea and Nut columns remain in neutral levels with slightly higher levels in Tea columns. There is a small increase from the initial solution.

The conductivity levels increase at both substrate materials columns. The increase in Tea columns was higher than Nut columns.

The redox potential in both substrate materials remained in close levels. There was a reduction in Tea columns from the initial solution till 3.7% and in Nut column an increase till 3.09%.

The TOC levels in Tea columns increased especially in low flow rate more than two times. In Nut columns there was decrease in 40% substrate materials even in low (-13%) even in high (-21%) flow rate and increase in 60% column.

In nitrogen compounds the reduction was more than promising and not expected. The reduction at nitrate and TN levels was more than 97% at all columns with the best results in T-40-L and N-40-L columns.

Finally phosphate levels were the concerning point of research. The highest levels noticed in Tea columns with levels between 11-13 mg/l and the highest levels in T-60-L column. In Nut columns phosphate levels were lower between 7-12 mg/l. The lowest levels noticed in N-40-L column.

3.2. Results part two with perlite column

The groundwater that used as solution in the second part of experiment received from the same place in spring. In the meantime between two experiments, columns spiked with clear groundwater without the addition of KNO3. The HRT at the second experiment remain the same as the first. All the results are given in Table 2.

Table 2. Groundwater solution results with perlite column

		Initial	TeaL	TeaH	TeOxL	TeOxH	NutL	NuOxL	NuOxH
pH	mean	7.71	7.56	7.44	7.87	7.41	8.06	8.07	7.97	7.69
	st dev	0.3	0.22	0.13	0.19	0.19	0.26	0.32	0.23	0.22
	% change		-1.99	-3.57	2.06	-3.89	4.43	4.63	3.35	2.28
Conductivity µS/cm	mean	276.98	347.1	328.5	273.87	250.33	247.24	293.33	242.08	325.24
	st dev	68.86	72.34	59.43	36.04	53.93	50.9	68.46	55.48	83.87
	% change		25.31	18.6	-1.12	-9.61	-10.74	5.91	-12.6	17.42
Redox mV	mean	168.47	171.82	171.75	169.38	171.83	177.85	168.64	172.33	170.44
	st dev	30.41	30.64	32.64	34.18	36.92	27.21	39.12	34.94	39.99
	% change		1.99	1.94	0.54	1.99	5.57	0.1	2.3	1.17
TOC ppm	mean	6.63	6.26	7.13	8.92	11.84	8.22	6.56	7.05	7.68
	st dev	3.99	4.22	3.22	5.07	5.45	4.74	3.57	3.4	3.39
	% change		▪5.52	7.57	34.61	78.64	24.04	▪1.07	6.41	15.9
NO3 mmol/l 10^-3	mean	514.79	390.45	358.48	0.28	0.93	1.63	3.71	0.11	194.82
	st dev	19.98	54.74	25.2	0.33	2.16	5.89	7.9	0.29	44.74
	% change		-24.15	-22.59	-99.94	-99.82	-99.68	-99.28	-99.98	-62.16
NO2 mmol/l 10^-3	mean	1.28	2.94	0.43	1.24	0.33	0.51	0.32	1.71	13.67
	st dev	1.37	2.38	0.38	2.99	0.28	1.44	0.8	10.33	22.76
	% change		98.21	▪63.03	▪5.04	▪80.19	▪60.19	▪75.19	33.58	968.65
NH4 mmol/l 10^-3	mean	8.33	3.26	3.75	6.45	9.11	12.04	20.3	1.71	10.17
	st dev	21.61	4.5	5.5	6.93	12.12	20.67	23.1	3.92	17.77
	% change		▪60.89	▪54.98	▪22.6	9.36	44.41	143.55	▪79.45	22.07
PO4 mg/l	mean	10.97	1.29	1.34	0.51	0.16	0.97	1.5	0.6	1.99
	st dev	1.91	1.51	1.1	0.55	0.22	0.99	1.17	1.29	2.03
	% change		▪88.13	▪87.65	▪97.14	▪98.51	▪91.11	▪86.17	▪94.51	▪81.71
TN mmol/l 10^-3	mean	524.4	396.25	402.67	7.98	10.28	14.17	24.32	3.54	218.66
	st dev	20.17	53.93	26.85	9.08	12.41	24.68	29.49	10.76	65.94
	% change		-24.44	-23.21	-98.48	-98.04	-97.3	-95.36	-99.33	-58.3

The pH levels in the second part of experiment were higher than the first part except from T-60-L column. There were more basic results but the best range for denitrification process with higher levels in Nut columns with pH=7.8-8.1 (Rivett et al., 2008).

The conductivity levels were much lower than part one even in Tea even in Nut columns. The highest levels were in Nut columns in contrast to part one (242-325 µS/cm).

The TOC levels were much lower than part one, but again much higher than the initial levels. The TOC levels are higher in Tea columns with levels between 8-12 ppm.

In nitrogen compounds the results as in part one are very promising. At all columns there is reduction of nitrate and TN levels more than 95%. The only columns that the reduction was not achieved the same levels was N-40-H column with reduction only 60%.

Finally, phosphate levels that was the problem in part one, with the use of perlite column received reduction more than 80% at all columns. The best reduction received in Tea columns with levels 94-98%. The best reduction noticed in T-60-L column.

4. DISCUSSION

At the both parts of experiment, denitrification process takes place and it is visible in detail at all the results. The reduction is achieved in the first day at both experiments. This phase is the initial face and the microbial activity follows the first order kinetics. The second phase is the stable phase. The degradation rates in the stable phase follow the zero order kinetics. Retention time and degradation rates were changing along the experiment. The environmental conditions and carbon source which were the substrate materials that used are connected with denitrification process (Tang et al., 2010).

At both parts of experiment the reduction of nitrogen levels was more than 95%. In part on the best reduction levels noticed in T-40-L column (-99.96%). In the second part the best reduction noticed in N-60-L column (-99.33%).

The concerning point was the phosphate levels. In part on the phosphate levels increased at all column with the highest levels in Tea columns. In part two with perlite columns there is reduction of phosphate levels at all columns more than 82%. The best reduction received in Tea columns (between 91-99%).

The denitrification process noticed and the bacteria colonies that noticed in columns were the genes denitrifiers. The identification became with q-PCR and that was the evidence that there were denitrifiers bacteria (*Thiobacillus denitrificans* and *Pseudomonas*) as it is noticed at several researchers in denitrification process (Munoz-leoz et al., 2011).

5. CONCLUSION

Denitrification process investigation in columns studies with tea waste materials and hazelnut husk wastes was successful. The HRT was 3.25 for fast flow rate and 6.1 days for slow flow rate. The nitrogen removal at all cases was more than 98% even with groundwater as solution. The concerning point is the high levels of phosphates that are visible in first part experiment. In part two with perlite, phosphate levels reduced more than 80% at all columns. In all cases the reduction that achieved in slow flow rate and in Tea columns is more successful than in fast flow rate.

REFERENCES

APHA., 1999. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. APHA, AWWA & WWF, Washington, D.C.

Blowes, D., W. Robertson, C. Ptacek, C. Merkley, 1994. *Removal of agricultural nitrate from tile-drainage effluent water using in-line bioreactors.* Journal of Contaminant Hydrology, 15, 207-211.

Bothe, H., S.J. Ferguson, W.E. Newton, 2007. *Biology of the nitrogen cycle.* Primera edición. Elsevier.

Carrera, J., J.A. Baeza, T. Vicent, J. Lafuente, 2004. *Biological nitrogen removal of high-strength ammonium industrial wastewater with two-sludge system.* Water Research, 37(17), 4211-4221.

Della Rocca, C., V. Belgiorno, S. Meric, 2007. *Overview of in-situ applicable nitrate removal processes*. Desalination, 204, 46-62.

Gibert, O., S. Pomierny, I. Rowe, R. Kalin, 2008. *Selection of organic substrate as potential reactive materials for use in a Denitrification permeable reactive barrier (PRB)*. Biosource Technology, 99, 7587-7596.

Hekmatzadeh, A.A., A. Karimi-Jashani, N. Talebbeydokhti, B. Kløve, 2012. *Modeling of nitrate removal for ion exchange resin in batch and fixed bed experiments*. Desalination, 284, 22-31.

Jing, L., H. Wang, T. Jiang, D. Li, H. Feng, M. Chen, 2010. *Denitrification of Groundwater Using Walnut Shell as Solid Carbon Source*. Conference on Environmental Pollution and Public Health, 1059-1063.

Mahavi, A.H., Naghipour, D., Vaezi, F., Nazmara, S., 2005. *Tea waste as an adsorbent for heavy metal removal from industrial wastewaters*. American J. App. Sci. 2(1), 372-375.

Misiti, T.M., M.G. Hajaya, S.G. Pavlostathis, 2011. *Nitrate reduction in a simulated free-water surface wetland system*. Water Research, 45, 5587-5598.

Muñoz-leoz, B., I. Antigüedad, C. Garbisu, E. Ruiz-Romera, 2011. *Science of the Total Environment Nitrogen transformations and greenhouse gas emissions from a riparian wetland soil: An undisturbed soil column study*. Science of the Total Environment, 409, 763–770.

Omirou, M., P. Dalias, C. Costa, C. Papastefanou, A. Dados, C. Ehaliotis, D.G. Karpouzas, 2012. *Exploring the potential of biobeds for the depuration of pesticide-contaminated wastewaters from the citrus production chain: laboratory, column and field studies*. Environmental Pollution, 166, 31-39.

Rivett, M., S. Buss, P. Morgan, J. Smith, C. Bemment, 2008. *Nitrate attenuation in groundwater: A review of biochemical controlling processes*. Water Research, 42, 4215-4232.

Robinson-Lora, M.A., R.A. Brennan, 2009. *The use of crab-shell chitin for biological denitrification: batch and column tests*. Bioresource Technology, 100, 534-541.

Schipper, L.A., W.D. Robertson, A.J. Gold, D.B. Jaynes, S.D. Cameron, 2010. *Denitrifying bioreactors-An approach for reducing nitrate loads to receiving waters*. Ecological Engineering, 36, 1532-1543.

Su, S., R. Puls, 2007. *Removal of added nitrate in cotton burr compost, mulch compost, and peat: Mechanisms and potential use for groundwater nitrate remediation*. Chemosphere, 66, 91-98.

Tang, S., Q. Yang, H. Shang, T. Sun, 2010. *Removal of Nitrate by autosulfurotrophic denitrifying bacteria: optimization, kinetics and thermodynamics study*. FEB, 19(12b), 3193-3198.

Zhou, W., Y. Sun, B. Wu, Y. Zhang, M. Huang, T. Miyanaga, Z. Zhang, 2011. *Autotrophic Denitrification for nitrate and nitrite removal using sulfur-limestone*. Journal of Environmental Science, 23(11), 1761-1769.