Understanding the feasibility of growing reed canary grass on a historic mine site for phytostabilisation

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Declaration

I hereby declare that the work presented in this thesis has not been submitted for any other degree or professional qualification, and that it is the result of my own independent work.

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Abstract

Historic mining tailings and spoil are typically too physically, chemically and biologically deficient for spontaneous vegetation regrowth, allowing for the redistribution of contaminated material. The use of contaminated land, such as historic mines, to grow bioenergy crops could increase the sustainability of bioenergy resulting in an increase in land available for agriculture and the remediation of degraded soils. The aim of this study was to understand the feasibility of growing a novel energy crop grass species in mine soils amended with two organic waste soil amendments (OWSA) used to promote plant growth and decrease the bioavailability of potentially toxic elements (PTE) for the purposes of remediation. The organic waste amendments used in this study were green waste compost (GWC) and drinking water treatment residue (DWTR). Reed Canarygrass (RCG) (Phalaris arundinacea), a perennial grass species and novel energy crop able to rapidly colonize and establish on contaminated soils whilst not (usually) accumulating high levels of PTE, was the plant species used throughout this study.

The approach that was taken involved both field and pot trials with soils from a historic Pb-Zn mine site in the North East of England. Soils from this mine site were found to have very low levels of nutrients and very high levels of PTE (Pb and Zn >13000 mg/kg). The application of the BCR sequential extraction on the unamended pot trial mine soils found that many of the PTE analysed for were mostly in bioavailable fractions. The same analysis on the soils amended with the OWSA found a significant decrease in the bioavailability of Pb and Cu ($P < 0.05$) after plant growth. The field trial involved 648 pre-grown

RCG plants planted-out into mine soils amended with either one of the two OWSA or a combination of the two, all at a target rate of 30% (amendment weight/ soil weight). A quarter of the planted soils were left unamended as a control. Three cultivars of RCG, each bred for a different commercial use, were planted. Biomass production was assessed by sampling all above ground material and measuring dry weight. The mobility of PTE was assessed by biomass digestion and analysis (ICP-MS), and through the imaging of the biomass with x-ray computed tomography (XCT).

Results from field trial sampling showed plant survival rates of above 97%. Cultivars bred for greater biomass (SW RF5004 and SW RF5032) presented statistically significant increases ($P < 0.05$) in dry mass. Both amendments and the combination of the two had significant positive effects on biomass production compared to the control in the following order GWC, DWTR+GWC, DWTR ($P < 0.05$). Analysis of the biomass showed very high concentrations of PTE found within the biomass, particularly Pb (500 -7500 mg/kg). The application to soil of GWC was found to significantly lower Cu and Zn biomass concentrations ($P < 0.05$) however the effect of the two amendments on concentrations of PTE in biomass was generally ambiguous. High Pb concentrations found in biomass were investigated further using XCT and was found to be from a combination of particulate surficial contamination and plant uptake. The results suggest that RCG is a viable remediation option on even highly contaminated soils when grown with appropriate OWSA and that further work should be undertaken to gain a better understanding of the location and nature of PTE within the biomass.

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1 Introduction

1.1 Background

As a whole this study aims to explore whether historic metal mines can be remediated using plants and organic waste soil amendments. Historic metal mines have been identified as one of the most significant pollution threats in the UK affecting more than 5,000 km of rivers, as well as important drinking water supply aquifers (Johnston *et al.*, 2008). Historic metal mines tend to have several characteristics that make a greater impact on the local environment when compared to other contaminated land types. For instance; the contaminated material is normally finely crushed and left unvegetated and so is highly mobile (due to the ore treatment process) allowing for easy distribution. Local waterways are often impacted either by mine water or erosion of mine waste, the sites are normally very large combining point and diffuse sources of contamination and also are often open to the public (Davies & White, 1981; Gutiérrez, Mickus, & Mar, 2016; Johnston *et al*., 2008). The metals found within the soils at historic metal mine sites tend to be in very high concentrations and also are often chemically and physically bioavailable creating a risk to human and environmental health (Davies & White, 1981; Entwistle, Hursthouse, Marinho Reis, & Stewart, 2019; L. Rodríguez, Ruiz, Alonso-Azcárate, & Rincón, 2009). A key reason for the distribution of the contaminants associated with historic metal mines is their lack of vegetation, often due to the phytotoxicity of the soil. A relatively simple (when compared to alternative remediation options) and effective method of reducing the physical and chemical mobility of the contaminants is to revegetate the site. Traditional remediation methods, such as soil removal and capping, are cost prohibitive when applied to historic mining sites where contaminants are often heterogeneous and distributed over large areas (Gomes, 2012). In-situ biological and chemical immobilization of the contaminated soil is therefore becoming increasingly considered as the best option when managing the risks associated with historic mines (Bolan *et al*., 2014).

Not only are the mine soils at the sites contaminated by phytotoxic elements such as lead (Pb) but also there are often many other challenges to plant establishment. Most historic metal mines in the UK are in uplands and so can have weather of all extremes, they also tend to have very poor soil quality with little of the essentials for plant life (organic matter, nutrients etc.) and compact, inorganic soils consisting of rubble and finely crushed material. In order to improve these conditions, the soil can be amended with various organic materials (Brown & Chaney, 2016; Palansooriya *et al*., 2020; Vangronsveld *et al*., 2009).

A combination of more sustainable thinking and a rise in the cost of landfill disposal of wastes has led to an increasing interest in a 'circular economy' approach to soil amendment, using organic wastes for soil improvement (Maina *et al.*, 2017). Many organic waste soil amendments have been found to reduce the chemical mobility of contaminants as well as providing nutrients and improving soil quality at relatively low quantities (compared with top soil or clay capping), resulting in further interest and great potential for their use (Brown & Chaney, 2016).

Once the mine soil has been amended, plants must be selected that are able to tolerate the challenging conditions presented by historic metal mines. For this study suitable plant characteristics included a tolerance to biogeochemical site conditions typically found in historic mining areas of the UK and the ability to quickly establish a fine binding network of rootlets capable of stabilising gravelly/sandy soil. Reed Canary Grass (*Phalaris arundinacea*) (RCG), a perennial rhizomatous C3 (carbon fixation which involves a 3-carbon molecule) grass species native to the UK, has been shown to exhibit all of these traits (Jensen *et al*., 2018).

Once a site has been successfully revegetated further efforts can be brought forward to turn the site from one that was at best non economically rewarding to one that can be used to yield a product. One option for historic metal mine sites is the production of second generation biofuel energy crop such as RCG. Biofuels are any fuel derived from biomass and have been identified as having a key role in the road to net zero. For example by 2032 the UK government plans for 12.5% of transport fuels to be biofuels (UK Government, 2021). When combined with carbon capture and storage technology bioenergy could result in successful carbon negative energy systems (Yue *et al.*, 2014). In "Bioenergy to Save the World" Schroder *et al*. (2008) proposed the use of marginal lands, abandoned for reasons of degradation and/or contamination, for the production of energy crops (Schröder *et al.*, 2008). The use of this type of land to grow bioenergy crops could increase the sustainability of bioenergy resulting in an increase in land available for agriculture.

Interest in RCG as a perennial grass bioenergy crop has grown considerably in recent years, especially in Northern Europe (Strašil *et al.*, 2005; Wrobel *et al.*, 2009; Smith and Slater, 2010; Strašil, 2012; Lord, 2015a; Kołodziej *et al.*, 2016; Šiaudinis *et al.*, 2021). This interest is due to several characteristics of the species; it grows well on marginal lands, is tolerant to soil contaminants, highly persistent, produces a relatively high biomass yield, is cost competitive with similar crops, is carbon efficient, has both superior drought and water-logging tolerance and can adapt to a wide range of soil types, habitats, and management systems (Jensen *et al*., 2018). Despite these qualities RCG remains relatively underdeveloped for a commercial plant. For example, Lindvall evaluated 16 wild populations of reed canary grass growing in Sweden and found that many had yields greater than the standard commercially available varieties (Lindvall, 1997). The current commercially available cultivars of RCG remain those that were developed for traditional agriculture and also soil conservation (erosion control) applications (Casler, 2010). Whilst finding a plant that will grow reliably on historic mine soils is rare, finding a plant also capable of binding the soils whilst potentially producing a harvestable product is even more uncommon. It is clear that further research should be conducted on this species in order to develop its potential as a bioenergy crop and as a remediation option. Today, despite many of our historic mines being non-operational for over 50 years little has been done to remediate their impacts and potential risks. This study aims to discover whether the growth of RCG using organic wastes for soil amendment could offer the solution for this problem.

1.2 Aims and objectives of this research

The principle aim of this programme of research has been directed towards determining if a novel bioenergy crop can be successfully grown on a highly impacted mine soil and what the implications are to soil and plant following the application of two organic waste soil amendments. The principle objectives of this study are summarised as follows:

1. To review the previous work conducted on the key aspects of this study chiefly: potentially toxic elements, contaminated land, historic metals mines, organic waste soil amendments, reed canary grass, phytoremediation and biofuel crops.
2. To review and analyse the published data on concentrations of PTE found in RCG and other lignocellulosic biofuel crop species when grown on impacted soils in order to determine the suitability of RCG for phytoremediation and provide a comparison for the results produced by this study.
3. To investigate and characterise the materials used throughout this study through the analysis of the mine soils, and their impacts at Whiteheaps, and the organic waste amendments.
4. To evaluate the effect of the organic waste soil amendments had on the growth and PTE concentrations of reed canary grass through an assessment of the results of laboratory pot trials and analysis of the biomass produced in the pot trials.
5. An evaluation of the impact of the organic waste soil amendments and RCG growth on the bioavailability, and thus the inferred ecological risk of specific elements, found through the application of a sequential extraction procedure on the pot trial soils.
6. To investigate whether RCG can be used as a phytostabilising remediation option for historic mine soils through the evaluation of a 1.5-year long field trial on a real mine site involving organic soil amendments at different rates and different genetic varieties of RCG.
7. To summarize and draw conclusions from the findings of this study.

1.3 Outline of the thesis

This thesis consists of nine chapter as follows:

- **Chapter 1**

  A brief introduction to the areas covered in this thesis and the overalls aims and objectives of this study.

- **Chapter 2**

  A general literature review of contaminants in soils, potentially toxic elements, contaminated land, organic waste soil amendments, phytoremediation and some of the options for the analysis of soil and biomass samples

- **Chapter 3**

  A specific literature review of the botany, growth and development of reed canary grass. The use of reed canary grass as a biofuel is discussed. The results of comparisons between published data on reed canary grass and two other similar biofuel species in terms of their interaction with potentially toxic elements in soils is presented.

- **Chapter 4**

  An explanation of the general analytical methods used thorough this study. The specific methods and experimental designs of the individual experiments are outlined in their relevant chapters.

- **Chapter 5**

  A background to the materials used throughout this thesis, chiefly from the Whiteheaps mine site and the organic waste growth amendments. These materials are analysed and the results of the analysis are discussed. The results of a desk study of the mine site is included as is the initial sampling of the soils and sediments connected to the site which help guided the location and manner of the subsequent experiments.

- **Chapter 6**

  The results of a laboratory pot trial involving the growth of reed canary grass in the Whiteheaps mine soils amended with the organic waste soil amendments.

- **Chapter 7**

  The results of the application of a sequential extraction procedure used on the soils recovered from the pot trials in order to determine changes in bioavailability of potentially toxic elements following soil amendment.

- **Chapter 8**

  The results of a two-year field trial at the Whiteheaps mine site in which reed canary grass was grown in soils amended with the organic waste soil amendments. The results of the analysis of the harvested biomass from this field trial are discussed.

- **Chapter 9**

  A summary of the conclusions and recommendations relating to the present study

- **Appendix**

  Analytical ANOVA data, data from Northumbrian Water and images taken during initial site visit of Whiteheaps for investigating best field trial location.

2 General literature review

2.1 Potentially toxic elements in our environment

> ‘The progress of human civilization has been driven by revolutions in the exploitation of mineral resources’ (Zhang *et al.*, 2012 p1).

This exploitation has resulted in the development of agriculture, the powering of the industrial revolution, and recently the manufacture of modern technology; but it has also been the primary source and distribution mechanism of elements which can cause harm to human or environmental health. These elements can be grouped under the classification ‘potentially toxic elements’ (PTE) (Davidson *et al.*, 2006). Potentially toxic elements represent an imprecise group of inorganic chemical hazards with relatively high densities, atomic weights and atomic numbers. Unlike organic contaminants, which are oxidized to carbon (IV) oxide by microbial action, PTE do not undergo microbial or chemical degradation, resulting in their persistence within the environment (Wuana and Okieimen, 2011).

Although PTE do occur naturally in the soil environment through the weathering of parent material, these levels are regarded as ‘trace’ (below 10-100 mg/kg depending on the element) (Kabata-Pendias, 2000), anthropogenic releases of heavy metals into the environment are emitted through leaded petrol and paints, mines and mine tailings and the disposal of wastes containing metals (Khan *et al.*, 2008). These releases pose risks to human, animal and ecosystem health through processes such as poisoning through direct ingestion, bio accumulation through the food chain and the drinking of contaminated water (McLaughlin *et al.*, 2000). Economic losses can also be a consequence of heavy metal pollution via land tenure complications, food insecurity and reductions in land usability for agricultural production (Kabata-Pendias, 2000).

The most common PTE that result in environmental contamination through human activity are, in order of abundance, lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) (USEPA, 1997). The movement of each of these within the environment differs depending on their chemical form, which governs their ability for ion exchange, adsorption, biological mobilisation and bio availability (Wuana and Okieimen, 2011). They are all also able to activate, migrate and accumulate in various target media causing direct or indirect impacts to plants, animals and humans. The chemical speciation of PTE plays an important role in their solubility, potential toxicity and impact on the environment (Ure, 1991). This study will focus on the distribution, mobility and impacts of PTE connected with historic metal mining.

2.2 The processes and parameters governing the mobility of PTE

The mobility of PTE in soils is largely governed by the phenomena of sorption and desorption (Cerqueira *et al.*, 2011). Adsorption is the process whereby ions are removed from the solution and accumulated on the surface of the soil solid phase. This process chiefly controls the availability of ions in soil solution for uptake by plants and supply to the environment in general. There are various mechanisms involved in the adsorption of metal ions onto the soil particles. These different mechanisms lead to retention (sorption, precipitation, and complexation reactions) or loss (plant uptake and leaching) of metals (Seshadri *et al.*, 2015). They involve both physical and chemical processes and include: cation exchange (or non-specific adsorption), specific adsorption, co-precipitation and organic complexation (Seshadri *et al.*, 2015). The most important parameters controlling PTE adsorption and their distribution are soil type, metal speciation, metal concentration, soil pH, solid: solution mass ratio, and contact time. In general, greater metal retention and lower solubility occurs at high soil pH (Young, 2013). The main interfaces involved in PTE adsorption in soils are predominantly inorganic colloids such as clays and metal oxides and organic materials such as humic and fulvic acid (Bradl, 2004).

2.2.1 Organic complexation

Organic materials in soils such as humic acids, fulvic acids, lignin and other organic compounds with high molecular weight can adsorb metal ions from soil solution by forming chelate complexes (Gondar *et al.*, 2006). The complexation of metal ions by humic acid is extremely important in affecting the retention and mobility of metal contaminants in soils (Pandey *et al.*, 2000). The fulvic acid fraction of organic matter is assumed to form more soluble metal complexes because it is soluble in water, whereas humic acids tend to produce more insoluble metal complexes. The ability of humic substances to bind heavy metal ions can be attributed to their high content of oxygen-containing functional groups, including carboxyl, phenol, hydroxyl, enol and carbonyl structures of various types (MacCarthy *et al.*, 1990). Pandey *et al*. found that the stability of the chelates of common metal ions formed with humic substances went by the following order: Cu > Fe > Pb > Ni > Co > Ca > Cd > Zn > Mn > Mg (Pandey *et al.*, 2000).

Soluble complexes are also formed with low molecular weight organic ligands such as short chain organic acids, amino acids and other organic compounds, which prevents the precipitation and adsorption of the ions (Gondar *et al.*, 2006). Yang *et al*. point out that although the impact that humic acids have on the mobility of PTE and their availability to plants has been widely studied, contradictory results have been reported due to significant differences in soil types, plant species, and experimental formats (Yang *et al.*, 2013). However, Sparks suggests that a general rule for organic matter can be applied in this context, i.e. the non-mobile fractions of PTE in soils increase with enhancing concentrations of soil organic matter (Sparks, 2003a).

2.2.2 Cation exchange

Cation exchange involves exchange between ions in the soil solution and the counter-ions balancing the surface charges on the soil (Sparks, 2003b). Certain soil minerals, such as clay, particularly in combination with organic matter, possess a number of electrically charged sites which can attract and hold oppositely charged ions, preventing them from being leached down the soil profile. The cations held by the soil particles are called exchangeable cations, and are the most important source of immediately available plant nutrients (Mukhopadhyay *et al.*, 2019). On the other hand, negatively charged soil particles repel anions (negatively charged ions).

Concentrations of cations are expressed in centimoles of positive charge per kilogram of soil (cmol(+)/kg). Organic matter has the highest cation exchange capacity (CEC) (150 - 300 cmol/kg) due to its large surface area and the predominance of variable charges (Naidu *et al.*, 1997). The CEC of clay minerals can be compromised by impurities tightly held to the isomorphous substituted cations through hydrogen bonds. Generally, the CEC of soil increases as pH increases due to the development of more negative charges on the organic and clay minerals (Tan, 2010). A few studies have shown promising results regarding the retention of PTE in soils, partly attributed to increasing CEC through soil amendment (Beesley and Marmiroli, 2011; Karami *et al.*, 2011).

2.2.3 Clays

Clay, one of the most abundant materials on earth, is a natural material with a fine grain size, composed chiefly of silica, alumina, water and formed from weathered rock (Xu *et al.*, 2017). Clay has been described as a “scavenger” of pollutants as it uptakes cations and anions either through ion exchange or adsorption (Yuan *et al.*, 2013). Tiller (1996) showed that clay has stronger affinity for heavy metal ions than for alkali and alkaline-earth ions (Tiller, 1996). Clay minerals such as bentonite, sepiolite and palygorskite have been found to be an effective adsorbent of metal ions present in contaminated soils; as these are readily available materials they have been researched extensively (Uddin, 2017; Ugwu, 2019; Otunola and Ololade, 2020).

2.2.4 Mineral (hydr)oxides

Aluminium (Al), Iron (Fe), and Manganese (Mn) are the common mineral (hydr)oxides found in soil, and are secondary minerals formed through the weathering of rocks containing Al, Fe and Mn (Stanjek, 2003). These mineral (hydroxides) are ubiquitous and usually found in small amounts in highly weathered soils. Mineral (hydr)oxides play a significant role in the sorption of PTE in soils due to their large surface area (a particle size range which is usually ≤ 0.002 mm) and the reactivity of their surface sites which strongly bind oxyanions and metal cations (Hillel and Hatfield, 2005; Bolan *et al.*, 2014). Mn oxides have a strong oxidizing power and may degrade organic pesticides, decrease or increase the toxicity of PTE, and contribute to the formation of soil organic matter (Hillel and Hatfield, 2005). In soil environments, Fe and Al may coprecipitate, forming mixed Fe–Al precipitates which may also include other PTE. Colombo and Violante found that the fate of the initially formed precipitates is very dependent on other soil factors such as pH, the presence of clay minerals and organic ligands (Colombo and Violante, 1996). Naturally occurring oxides, synthesized oxide particles and industrial by-products have been increasingly studied for use in soil remediation over the past two decades, due to their ability to reduce metal mobility. Komárek *et al*. reviewed the use of Mineral (hydr)oxides in contaminated land remediation and conclude that “despite the partial successes in the field, recent knowledge highlights the importance of long-term and large-scale field studies evaluating the stability of the oxide-based amendments in the treated soils and their efficiency in the long-term (Komárek *et al.*, 2013)”

2.2.5 Root exudates

The roots of plants can also effect the mobility of PTE through the release of root exudates, several substances that are secreted passively by plants chiefly to aid the adsorption of nutrients (Koo *et al.*, 2005). For example, organic ligands released by plant roots have an important role in the sorption of PTE as they are specifically adsorbed on metal oxides forming stable inner-sphere complexes (Palansooriya *et al.*, 2020). Organic ligands have been found to both hinder and promote the sorption of heavy metals as the ligand can form complexes with the metals which may be more or less strongly bound than the free cations (Jackson, 1998; Palansooriya *et al.*, 2020).

2.3 Bioavailability of PTE

When assessing the risk of PTE in the environment, it is becoming more common to determine the fraction of the element that is available to life and could potentially cause harm (Petruzzelli *et al.*, 2019). This is known as the ‘bioavailability’ of the element. The term bioavailability is often misused in the field of soil science as it has different meanings in different disciplines (Davidson, 2013). In the case of this study and the field of contaminated land in general, the term ‘bioavailable’ refers to the fraction of an element that is able to enter and effect the biological processes of an organism and not the total metal content of an element (Petruzzelli *et al.*, 2019). Davidson (2013) states that when a risk assessment is conducted concerning bioavailability, workers “should always make clear the target organism(s), chemical species and exposure route(s) being considered (Davidson, 2013 pp111)”.

2.3.1 Evaluating mobility and bioavailability of PTE in the environment

Currently, lacking one universal procedure that is considered the standard procedure to determine the mobility of an element within a sample, each investigator uses the method that they consider best fits the goals of the study (Rodríguez *et al.*, 2009; Houben *et al.*, 2013). For example, Houben *et al*. determined environmental mobility as the amount of Cd, Pb and Zn extracted by a $CaCl_2$ solution, and compared these values to the amount of metal taken up by plants in a pot trial experiment (Houben *et al.*, 2013). Many studies have taken place in order to discover which chemical extractants (complexing agents, dilute acids and salt solutions etc.) are best at separating the plant-available fraction of PTE from a soil sample (Steinnes, 2013). A critical review of available literature on extractants for Cd, Cu, Ni, Pb and Zn concluded that there is generally no correlation between the phytoavailable fraction and the total soil concentrations of PTE (Menzies *et al.*, 2007). The review concluded that neutral salt extractants (such as 0.01 M $CaCl_2$ and 0.1 M $NaNO_3$) provide the most useful indication of phytoavailability for the PTE in question (Menzies *et al.*, 2007).

2.4 Lead

Lead (Pb) is a metallic element, found in Group 14 of the periodic table. It has an atomic number 82 and high density, 11.3 g cm^{-3}, due to its high relative atomic mass and its face centred cubic structure (Jank and Hafner, 1990). Pb has been mined by humans since prehistoric times as a result of its versatility (i.e. the high malleability of the element), its wide distribution and its geological relationship with other metals such as silver, copper and zinc (Tong *et al.*, 2000). The ore that is most commonly mined is Galena PbS, the natural mineral form of lead (II) sulphide (Lar *et al*., 2013). Other Pb ores include cerussite and anglesite (Pulford and Flowers, 2007; Alloway, 2012). Both anglesite and cerussite are formed by the weathering of galena. Lead has four naturally occurring stable isotopes, ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb (Skerfving and Bergdahl, 2015).

The mean content of Pb in soils that have not been impacted by industry worldwide has been estimated to be around 17 mg/kg (Chen *et al.*, 1991). Although Pb is naturally dispersed in trace amounts throughout the environment from the weathering of parent material, there is evidence that much of the observed soil Pb has originated from anthropogenic sources (Steinnes *et al.*, 1989; Csavina *et al.*, 2012). Approximately 98% of atmospheric Pb has also been found to originate from human activities (Tiwari and Tripathi, 2012). It is estimated that as a result of total human processing of lead ore, 300 million tons of Pb have been released into the environment (Tong *et al.*, 2000).

The key anthropogenic sources of Pb are connected to mining and smelting, paint containing Pb for pigmentation, leaded petrol (containing tetraethyl lead) and sport shooting/military activity (lead-based munitions) (Palansooriya *et al.*, 2020). The ubiquity of anthropogenically derived Pb is related to the volatility of several compounds of the element at high temperatures, which are released as aerosols in processes such as smelting or burning leaded petrol (Steinnes, 2013). As Pb has a tendency to be concentrated on the smaller particle fractions of these emissions it is thus available for long-distance atmospheric transport (Davies and White, 1981b; Csavina *et al.*, 2012; Gil-Loaiza *et al.*, 2018). Anthropogenically derived Pb has been found in the ice of both Greenland and Antarctica, confirming its global distribution (Murozumi *et al.*, 1969).

Pb in soil occurs predominantly in the +2 oxidation state. The mobility of Pb chiefly depends on the organic matter content and pH of a soil. Pb is generally more strongly sorbed than other PTE such as Cd, Ni and Zn. A study of soils at historic mining sites determined that most of the lead present within the soil was not in a readily mobile form (Maskall *et al.*, 1995). Cerqueira *et al*. found that Pb^{2+} sorbed and retained to a greater extent than Cu^{2+} when they were both introduced to natural soils (Cerqueira *et al.*, 2011).

The main forms that lead is found in within mining soils (PbS, $PbSO_4$ and $PbCO_3$) have low solubility in water. A decrease in pH has been found to increase the solubility of most lead compounds. Some soils, even with high Pb contamination, do not appear to have an effect on plants, animals or humans; this is thought to be because the Pb is in an insoluble form. Ruby *et al*. found when comparing Pb derived from mine soils with that derived from urban environments, that lead from mine soils had a lower bioavailability in the following order; mine soil < $PbSO_4$ <

$Pb(C_2H_3O_2)_2$ (Ruby *et al.*, 1992). This result was attributed to the relatively lower solubility of the mine soil's Pb-bearing phases in their respective mineral assemblages (Ruby *et al.*, 1992).

With increasing pH, Pb typically becomes less soluble due to complexation with organic matter, sorption on oxide and silicate clay minerals or precipitation in the form of a carbonate, sulfate or phosphate. Solubility has, however, been found to increase in some alkaline soils due to the formation of soluble Pb-organic and Pb-hydroxy complexes (McBride, 1994).

At pH 4 and above, Pb is strongly adsorbed onto humic matter (Kerndorff and Schnitzer, 1980; Senesi and Loffredo, 2005). The affinity that Pb has for humic matter is shared only with Cu and Hg when common PTE are considered (Steinnes, 2013). Clemente and Bernal found that the addition of humic acid (which was isolated from a mature compost) to a Pb-Zn mine soil (pH 3.4) diminished the carbonate associated Pb by 19% and increased the organic matter complexed and residual Pb by 34.6% and 76.9% respectively (Clemente and Bernal, 2006).

In the absence of humic matter Pb can be strongly adsorbed onto clay minerals. Helios *et al*. found that on illite, beidellite and montmorillonite, Pb sorbed onto the clays at a rate of 80% of the total concentration, this was a greater rate than for the other PTE tested (Helios Rybicka *et al.*, 1995). Wan *et al*., found that the incorporation of rice straw on Pb impacted soils reduced the mobility of Pb by promoting its binding to iron oxides (Wan *et al.*, 2018).

Uptake from contaminated soils and deposition of contaminants from the atmosphere onto plant surfaces are sources of elevated levels of Pb in terrestrial plants grown in mining/smelting areas (Dudka and Adriano, 1997). Koeppe found that the translocation of Pb in plants is dependent on environmental conditions surrounding the plants, as well as the physiological condition of the plants (Koeppe, 1977). As Pb serves no biological function to plants it does not degrade following uptake (Mitchell, 2009).

The effects of Pb toxicity on plants can cause negative physiological and biochemical effects (Sharma and Dubey, 2005). These include rapid inhibition of root growth, stunted growth, chlorosis, enzyme activity inhibition, disturbed mineral nutrition and changes in hormonal status (Sharma and Dubey, 2005). Van Assche and Clijsters found that at high concentrations, Pb phytotoxicity can eventually lead to cellular death though the inhibition of enzymes containing sulfydryl (-SH) groups, which are necessary for cell activity (Van Assche and Clijsters, 1990). Increased concentrations of soluble Pb has also been shown to cause a decrease in the germination rate of rice seeds (Sharma and Dubey, 2005).

A study by Dudka *et al.*, found that agricultural crops had elevated concentrations of Pb when grown in areas connected to historic and active Pb mining processes, particularly smelting (Dudka and Miller, 1999). Areas of extensive Pb contamination have been found to remain barren of vegetation long after mining processes cease (Gunn *et al.*, 1995). This effect often leads to highly contaminated areas remaining unvegetated, allowing for the further distribution of contaminated soils through the movement of wind and water (Csavina *et al.*, 2012).

Antonovics *et al*. reported that the uptake of Pb by a plant is generally consistent with increasing soil Pb levels, until a threshold is reached where uptake becomes unrestricted and rises exponentially (Antonovics *et al.*, 1971). Concentrations of Pb in plants grown on historic mine tailings have been found to regularly be in excess of 8000 mg/kg, with levels as high as 21,000 mg/kg not uncommon (Reeves and Brooks, 1983; Baker *et al.*, 1994; Reeves *et al.*, 2018). However, Van der Ent *et al*. propose that Pb biomass concentrations found above 1000 mg/kg maybe inaccurate and that contamination by wind-blown or rain-splashed dusts and soil, or even (in the case of smelters) from vapour-phase deposition may result in inaccuracies (van der Ent *et al.*, 2013). Van der Ent *et al*. suggest a rigorous and standardised biomass washing method for contaminated biomass (using a non-polar wax-dissolving agent such as hexane in an ultrasonic bath), and the implementation of laboratory greenhouse trials where greater control can allow for interferences such as aerial deposition to be entirely precluded (van der Ent *et al.*, 2013).

2.5 Copper

Copper (Cu) is a metallic element with an atomic number of 29, and is the first of the IB group in the periodic table. Copper has many uses for human civilisation due to its soft, malleable, and ductile nature and its very high thermal and electrical conductivity. Copper is also an essential trace element which is vital for the health of all living organisms because of its role in several metabolic processes. The recommended daily intake of Cu for humans is 1-2 mg a day; as little as 10 mg of copper can have a toxic effect (National Research Council (US), 1989).

Although the bulk of global demand for Cu is connected to electrical applications, the metal has also been widely used in transport, coinage and as biocide (Epstein and Bassein, 2001; Oorts, 2013). Chalcopyrite ($CuFeS_2$) is the most abundant and economically viable Cu mineral. Other Cu minerals include malachite ($Cu_2(OH)_2(CO_3)_2$), bornite (Cu_5FeS_4), chalcocite (Cu_2S), covellite (CuS) and as native metallic copper (Pulford and Flowers, 2007; Oorts, 2013; Entwistle *et al.*, 2019). Average global soil concentrations of Cu are typically in the range of 2 mg/kg – 100 mg/kg with a mean of 30 mg/kg (Pulford and Flowers, 2007; Rankin, 2009).

Copper was used by some of the earliest civilisations and its smelting appears to have been independently discovered in societies across the globe as early as 10,000 BC (Oorts, 2013). The long history of human usage of Cu and ways in which the metal was processed and utilised (e.g. in agriculture and by smelting) has led to global environmental enrichment. The ubiquity of Cu within the environment makes distinguishing 'natural background' levels from anthropogenic sources unfeasible in many parts of the world (Oorts, 2013). The term 'ambient background' levels is used instead to describe the unmeasurably impacted 'natural background' (Reimann and Garrett, 2005). The average ambient copper concentrations found by Heijerick *et al*. for European agricultural soils were 31.1 mg/kg (Heijerick *et al.*, 2006).

Copper is a transition metal and so exhibits variable oxidation states, Cu^+ (cuprous) and Cu^{2+} (cupric) are the most common forms. Cuprous Cu, when not bound to insoluble compounds or stabilised in complexed forms, is unstable in aqueous media and readily converts to Cu^{2+} (Pulford and Flowers, 2007; Oorts, 2013). In soil,

Cu^{2+} preferentially binds to dissolved humified organic matter. Cuprous ions typically bind to organic and inorganic components in the general order of Mn oxides > organic matter > Fe oxides > clay minerals. In general, organic matter is the dominant Cu adsorption matrix in soils and is generally responsible for retaining adsorbed Cu. The soil pH plays an important role in the binding affinity of Cu with inorganic and organic matter. For example, Degryse *et al*. collated data from studies concerning the measuring of the solid–liquid partitioning of metals in aerobic soils and found that the proportion of free Cu^{2+} ions in solution generally decreased with increasing levels of soil pH (Degryse *et al.*, 2009).

Although essential for plant growth, and involved in many enzymatic activities as well as for chlorophyll and seed production, Cu can have a toxic effect at high concentrations in plant tissue (Alvarenga *et al.*, 2008). Phytotoxic concentrations of Cu in plants range from 4 – 40 mg/kg dry biomass (Davis and Beckett, 1978). Plants tend to exhibit a strong translocation barrier to Cu and roots tend to have a greater critical range for phytotoxicity, typically 100 – 400 mg/kg (Degryse *et al.*, 2008). The growth of plants can have an effect on the bioavailability of Cu due to the effect of root exudates on lowering the soil pH local in the rhizome and as plant growth results in an increase in soil organic matter. Although this effect results in either the increase or decrease in Cu availability depending on soil pH, free Cu^{2+} activity in the soil solution typically decreases in the presence of plant growth due to complexation with organic materials (Römkens *et al.*, 1999).

2.6 Zinc

Zinc (Zn) is a metallic element with an atomic number of 30 and is found in group 12 of the periodic table. The metal is slightly brittle at room temperature and has a grey and silver appearance once oxidation is removed; the normal oxidation state is Zn^{2+}. Zinc (Zn) is the 24th most abundant element globally and is typically found in soils at concentrations ranging from 10 - 100 mg/kg. The most common Zn ore is sphalerite which is a zinc sulphide mineral, but other Zn minerals such as carbonates (smithsonite) and silicates (hemimorphite) are also mined. The main workable lodes of Zn are currently found in the USA, China, Peru, Iran and Australia (Smolders and Mertens, 2013a). Zinc is both an essential micronutrient for plant growth and development and is a crucial element for most organisms, including humans (Gupta *et al.*, 2016).

Although Zn is ubiquitously geochemically distributed, there are many localised areas with very high soil concentrations due to high Zn in weathered parent material, the application of sewage sludge onto land and the disposal of mine waste material, or atmospheric deposition, connected to Zn mining and smelting. Although Zn alloys were used in the classical period and metallic Zn was produced in the 12 Century, the industrial mining of Zn did not really begin until the eighteenth century, following the discovery of its galvanisation ability. Since then the production of Zn has continued to increase and now fifty percent of its use is for galvanisation, with the remaining fraction used for rubber, cosmetics and Zn based alloys (predominantly brass) (Smolders and Mertens, 2013a).

In soils, Zn is present in its +2 oxidation state. The concentration of Zn in soils is largely affected by pH and the presence of clay minerals, organic matter and iron species within the soil (Barrow, 1993). At a low pH the solubility of Zn increases, and therefore it is liable to leach or be taken up by plants. Soils with a high clay content are typically found to contain more Zn than sandy soils due to the higher capacity of clay to adsorb and retain Zn. Zinc is an essential micronutrient for plant growth and development, and is mainly used by plants to produce chlorophyll (Brown *et al.*, 1993). Although Zn typically occurs on the surfaces of clays, organic matter and hydrous oxides (iron), it must pass through to the solution phase before it can be taken up by plants via the roots (Marschner, 1993). It has been estimated that about 30% of agricultural soils are deficient in Zn and that subsequently about one in three people suffer a Zn deficiency (Sillinpaa, 1982). This is not simply the result of a low soil concentration of Zn, but is also attributed to the low bioavailability of the element, normally as a result of high organic matter content and neutral/high pH (Alloway, 2008).

2.7 Arsenic

Arsenic (As) is the twentieth most abundant element in the earth’s crust, has an atomic number of 33 and is classified as a metalloid rather than a metal, because it has some properties similar to a metal and others of a non-metal (Wenzel, 2013). Globally arsenic soil concentrations range from 0.1 – 55 mg/kg, although concentrations above 10,000 mg/kg have been reported at sites impacted by geogenic activity that has led to As distribution, or anthropogenic activity such as mining and smelting (Wenzel, 2013).

Arsenic sits in the 5th main group of the periodic table just below Phosphorus, and shares chemical similarities with this element which allow it to act as an analogue for it in various biological processes. This is a key cause of its toxicity in the environment, and to humans (Ghosh and Sil, 2015). Arsenate (pentavalent) and arsenite (trivalent) are the most common toxic inorganic oxidation states of Arsenic (Hughes, 2002). Arsenate can substitute for inorganic phosphate in cellular respiration pathways resulting in a breakdown in mitochondria processes. Arsenite (trivalent arsenic) is considered to be the more toxic inorganic form compared to arsenate (pentavalent arsenic). Arsenite reacts with thiol and sulfhydryl groups, which are major organic components to multiple proteins and enzymes found throughout the body. These reactions cause the dysregulation and inhibition of the proteins and enzymes involved (Chen *et al.*, 2009b).

The toxicity of As to insects, bacteria and fungi led to its global use in wood preservatives and insecticides from the beginning of the 1900s. Today about 70% of the world production of As is used in timber treatment, 22% in agricultural chemicals, and the remainder in glass, pharmaceuticals and metallic alloys. From the 1930s to 2004 a wood preservative known as chromated copper arsenate (CCA) or ‘Tanalith’ was the most extensive industrial application of As, leading to its global distribution; this was the principle use of trivalent arsenic in the USA at the time (Chen and Olsen, 2016).

Although banned by the EU in 2004, the management of the disposal of this treated wood in the EU has created problems. The majority of waste wood products were burned in incinerators thus creating further distribution via emissions, apart from in

Denmark where treated wood is landfilled, resulting in contaminated leachate (Humar *et al.*, 2006; Baastrup *et al.*, 2008). Many of the treated products also remain in circulation as they were used on relatively expensive items such as outdoor tables that are likely to be used well, and resold (Humar *et al.*, 2006). The use of CCA wood preservatives has been linked to skin cancer from dermatological contact, as well as to other cancers arising from the inhalation of Arsenic and Chromium particulates in smoke when the wood products are burnt in poorly ventilated environments (Rahman *et al.*, 2004). This particularly affects people who use waste wood as their primary cooking fuel, and is a human health concern in South Africa (Niyobuhungiro *et al.*, 2013).

2.8 Cadmium

Cadmium (Cd) has an atomic number of 48 and exists in the environment in one oxidation state (+2); it does not undergo oxidation-reduction reactions (Smolders and Mertens, 2013b). Anthropogenic sources of Cd include metal mining, smelting, P fertilizer, the ash from fossil fuel combustion, and waste incineration. Global soil concentrations typically range from 0.1 – 1 mg/kg; the majority of soils have been impacted by the atmospheric deposition of Cd (both from geogenic sources through volcanic eruptions and anthropogenic sources), and following the use of P fertilisers (Loganathan *et al.*, 1996; Smolders and Mertens, 2013b). Although Cd is normally found in concentrations so small it cannot effect chemical environmental soil processes, its pronounced toxicity can have an effect on human and environmental health (Smolders and Mertens, 2013b). As Cd is absorbed by plants relatively easily when grown on contaminated soil, high concentrations of Cd can be found in edible crops (Liu *et al.*, 2005; Clemens *et al.*, 2013; Clemens and Ma, 2016). Following oral exposure, Cd targets the kidneys, prostate and liver and is a carcinogen (Waalkes, 2000; Satarug *et al.*, 2010; Kasprzak and Waalkes, 2011).

2.9 Chromium

Chromium (Cr) has an atomic number of 24, is abundant in the earth's crust and is commonly associated with iron oxides, such as chromite, in the soil (Gonnelli and Renella, 2013). Due to its high corrosive resistance Cr is mostly used in the productions of paints, in the tanning process, in electroplating and for wood preservation. Although Cr is found in a relatively consistent concentration (approximately 100 mg/kg) within the earth's crust globally, its concentrations in soil range from 1 – 3000 mg/kg (Kotaś and Stasicka, 2000). High geogenic concentrations of Cr in soil are associated with the weathering of mafic/ ultra-mafic and volcanic rocks. Anthropogenic sources, such as wood treatment, of Cr have been reported to impact soils to concentrations ranging from 1500 – 4000 mg/kg (Kumpiene *et al.*, 2006; Mench and Bes, 2009). The most common oxidation states of Cr are III and VI. Whereas Cr (III) is essential for humans in small doses, its deficiency resulting in poor metabolism of glucose and lipids, Cr (VI) is highly toxic to all life, and can result in death at even small doses (MERTZ, 1993; Kimbrough *et al.*, 1999; Shanker *et al.*, 2005; Zhitkovich, 2011). Chromium (III) can also be toxic and has been associated with lung cancer, although at far higher doses when compared to Cr (VI) toxicity (Barceloux, 1999).

2.10 Nickel

Nickel (Ni) is abundant in the earth's crust (80 mg/kg) and has been distributed globally through the weathering of igneous rocks and volcanic eruptions (Gonnelli and Renella, 2013). Nickel exhibits a very diverse range of geochemical behaviour as it can be either siderophilic (an element with little affinity for oxygen and sulphur, and so often found in its metallic phase bound with Iron) and lithophilic (preference for silicate bonding) (Griffin and Chassé, 2016). Whilst Ni can exist in various oxidation states, Ni(II) is the prevalent oxidation state under normal environmental conditions (Iyaka, 2011). Typically, Ni is found in background concentrations of 3 - 48 mg/kg with the greater concentrations found in soils originating from volcanic rocks (Adriano, 2001). A key anthropogenic source of Ni is the burning of fossil fuels as the element is found in considerable concentrations in coal and oil; other sources include mining, smelting and the application of sewage sludge and phosphate fertilizer onto agricultural soils (Gonnelli and Renella, 2013). Both inhalation and oral exposure to Ni have been found to have carcinogenic and a variety of other pathologic effects on humans, such as skin allergies and lung fibrosis (Kasprzak *et al.*, 2003).

2.11 Source-pathway-receptor concept for understanding contaminated land

In the UK, in order for land to be declared ‘contaminated’, a valid contaminant ‘source – pathway – receptor’ linkage must be present, as defined in the Model Procedures for the Management of Contaminated Land - CLR11(Environment Agency, 2004). The CLR11 has now been officially withdrawn and replaced with the Land contamination risk management website (Environment Agency, 2020) The concept of contaminant linkages is used to create a Conceptual Site Model (CSM), an example of which is shown in Figure 1.

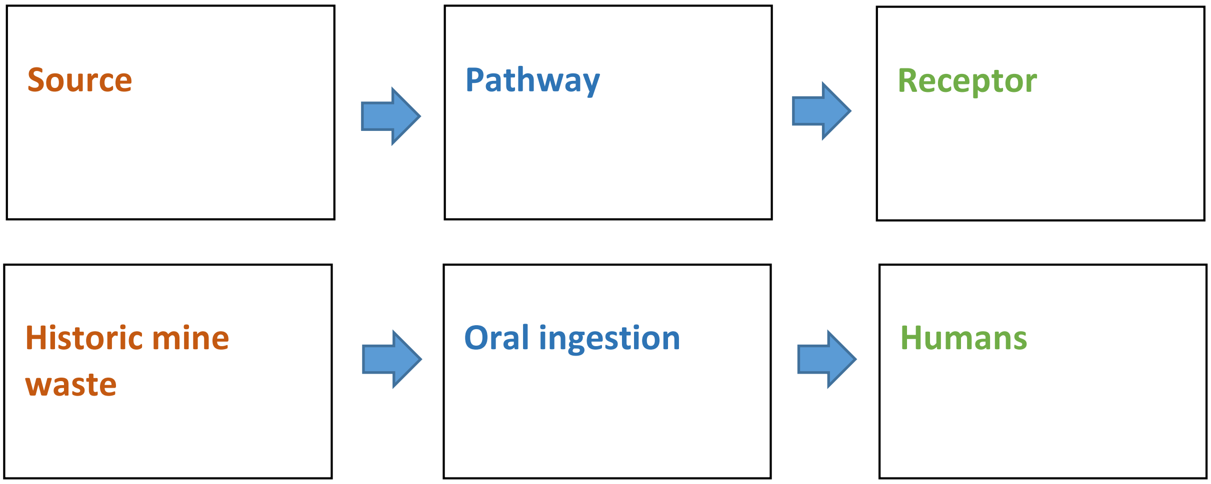


Figure 1 The pollutant linkage (including source, pathway and receptor) concept applied when considering the risk of a contaminated site and an example of its application in a Conceptual Site Model (CSM).

In the CLR11 model a contaminant is defined as “a substance which is in, on or under the land, and which has the potential to cause harm or to cause pollution of controlled waters”. A pathway is defined as a “route or means by which a receptor can be exposed to, or affected by, a contaminant”, and a receptor is defined as "something that could be adversely affected by a contaminant, such as people, an ecological system, property, or a water body" (Environment Agency, 2004). Without any one of the contaminant linkage elements, there is no contaminant linkage and thus no resulting risk, i.e. the presence of a contaminant is not, in itself, sufficient to be considered as a risk. A CSM should also consider all relevant exposure pathways as there may be more than one pathway of exposure. Part IIA of the Model Procedures for the Management of Contaminated Land - CLR11 states that the contaminant concentration must be sufficient to pose significant harm to the receptor, or that there is a significant possibility of significant harm being caused to the receptor, in order to establish a contaminant linkage and thus define the land as 'contaminated land' (Environment Agency, 2004). Once a contaminant linkage is established, a risk assessment is required. The risk assessment may be either qualitative (based on the CSM), or quantitative, then using either generic assessment criteria values (Privett *et al.*, 2009) or a site-specific detailed quantitative risk assessment (DQRA).

2.12 Contaminated land risk assessment guideline values

The Department of the Environment and Rural Affairs (DEFRA) and the Environmental Agency have produced a human toxicological risk assessment method to guide the human health risk assessment for contaminated land (Environment Agency, 2009). In this method, soil guideline values (SGV) are applied to ‘standard’ land use classes characterised by generic exposure assumptions. The standard land use classes are ‘residential’, ‘allotment’ and ‘commercial’ (Environment Agency, 2009). The SGV are contaminant-specific ‘trigger values’ used in assessing the risk to human health from chronic exposure to soil contamination. If soil concentrations are found to be above a given soil guideline value then the contaminants could pose a risk to human health, and further investigation or remediation should be considered (Environment Agency, 2009).

In addition to the SGV, DEFRA’s research project ‘SP1010’ led to the development of provisional ‘Category 4 Screening Levels (C4SLs)’ for a number of soil contaminants, including As, Cd, Cr and Pb (CL:AIRE, 2014). The C4SLs introduce a four-category system for identifying and managing contaminated land, ranging from Category 4 to Category 1 (DEFRA, 2012). Category 4 refers to situations in which there is no risk that the land poses a significant possibility of significant harm (SPOSH) or where the level of risk is low, whereas Category 1 refers to situations in which the risk that the land poses a SPOSH is unacceptably high. The C4SLs are used in a similar manner to SGVs. However, C4SLs are less stringent than the SGVs (CL:AIRE, 2014).

In 2015 the Suitable 4 Use Levels (S4ULs) were published by the Land Quality Management and the Chartered Institute for Environmental Health (LQM/CIEH).

This assessment criterion is based on a Health Criteria Value (HCV), representing the tolerable levels of risks for 89 substances, including metals and hydrocarbon fractions, pesticides and explosives. Similar to the C4SLs, the S4ULs were calculated using the CLEA Model for six standard land uses, including residential with/without home-grown produce, allotments, commercial and two categories for public open spaces near residential housing or parks.

Table 1 shows the SGV, S4ULs and C4SLs for commercial land; this category of land has been selected as this study focuses on land with an industrial past, that does not align with the other possible standard land use classes. Due to a lack of guidelines for Cu and Zn, the Code of Good Agricultural Practice (CoGAP) regulations for the use of potentially contaminated agricultural manures have also been included (CoGAP, 2009). These statutory guidelines can be assessed against the background levels of PTE found within UK soils. Lastly, the UK Soil and Herbage Pollutant Survey provides a comprehensive evaluation of baseline soil levels of common PTE found in the UK (Environment Agency, 2007). The UKSHS results for English ‘rural’ soils were selected as the most relevant to this study.

Table 1 Statutory soil guidelines used to assess the risk associated with land contaminated by potentially toxic elements, and mean average PTE concentrations in rural soils for England found in the UK Soil and Herbage Pollutant Survey (UKSHS) (n=183). All values are given in mg/kg

Element	Soil guideline value - Commercial/ Industrial land (ENVIRONMENT AGENCY, 2009).	Category 4 Screening Levels - Commercial land (CL:AIRE, 2014)	Suitable 4 Use Levels- Commercial land (LQM/CIEH S4ULs, 2015)	CoGAP soil guideline values (CoGAP, 2009)	UKSHS 'rural' soils (Environment Agency, 2007)
As	640	640	640	Not available	13.9
Cd	230	220	190	Not available	0.43
Cr	Not available	Not available	8600 (trivalent) 33 (hexavalent)	Not available	33.4
Cu	Not available	Not available	68,000	80 at pH >5.0 100 at pH >5.5	19.8
Ni	1,800 (Report withdrawn)	Not available	980	Not available	19.5
Pb	2300	2330	Not available	Not available	62.0
Zn	Not available	Not available	730,000	200 at pH > 5.0	94.8

2.13 Historic mining

This study will concentrate on the concentrations of PTE in soils and plants impacted by historic metalliferous mining. Section **Error! Reference source not found.** of this study provides an introduction and literature review of historic metal mining in the UK as well as the mine site involved in this study, a historic Pb mine. In the UK the most extensive lead mining period occurred between 1750 and 1900, a time in which mining methods were relatively inefficient, resulting in a legacy of environmental contamination (Morrison, 1998; Jarvis *et al.*, 2007; Mitchell, 2009). These mines have typically been abandoned following their closure, and therefore continue to impact the local environment.

2.14 Human health impacts from PTE connected to historic mining

In areas where historic mining has left a significant legacy of exposed PTE contaminated mine waste, the release of metalliferous dusts and contamination of water and sediments continue to cause harm to human health (Csavina *et al.*, 2012; Entwistle *et al.*, 2019). Many PTE have no useful role in the human physiology and can be toxic at low levels of exposure (Järup, 2003; Wuana and Okieimen, 2014; Kumar *et al.*, 2018). Whilst several PTE connected with metal mine waste are essential for life (such as Zn), this study only considers toxic levels of exposure. Once absorbed by the body, some PTE continue to accumulate in vital organs like the brain, liver, bones, and kidneys, potentially resulting in serious health consequences. For example Baldwin and Marshall found that children exposed to Pb in historic mine dusts can show signs of impaired development, exhibited in a lower IQ, hyperactivity and a shortened attention span (Baldwin and Marshall, 1999). A study conducted in 1989 on the residents of Wanlockhead (a village surrounded by historic Pb mine sites in the Scottish southern uplands) found that human blood Pb concentrations were found to be between 1.45 and 1.7 times control levels found in a village 25 miles away (Moffat, 1989). This study indicated that possible routes of exposure developed from the spread of airborne contaminants and concluded that the Wanlockhead area had a generally increased human Pb exposure. Children were found to have the greatest susceptibility to Pb exposure, potentially as a result of dirt ingestion (Moffat, 1989). Table 2 provides a concise guide to the potential impact of selected PTE exposure on human health.

Table 2 Toxicology of common PTE found in metalliferous mine dust, and related human health effects

PTE	**Exposure Route of interest to this study**	**Affected Organ**	**Health Effect**	**Reference**
As	Inhalation, ingestion, dermal exposure	Digestive, liver, nervous, respiratory systems and skin.	Interference with normal foetal development. Known carcinogen (liver, bladder, lungs and skin)	(Klevay, 1976; Squibb and Fowler, 1983; Abernathy *et al.*, 2001; Csavina *et al.*, 2012)
Cd	Adsorption through inhalation more damaging than ingestion.	Digestive, kidney liver, nervous, reproductive systems	Carcinogen. Irritant to stomach causing vomiting and diarrhoea. Damage to lungs through inhalation. Can cause damage to kidneys even at low concentrations	(WHO, 1992; Järup *et al.*, 1998; Waalkes, 2000; Manahan, 2010; Satarug *et al.*, 2010; Kasprzak and Waalkes, 2011)
Cr	Inhalation, ingestion, dermal exposure	Cr (III) respiratory system Cr (VI) respiratory system – nasal and sinus system	Carcinogen. Can cause asthma. Severe eczematous dermatitis with skin ulcers	(MERTZ, 1993; Kimbrough *et al.*, 1999; Shanker *et al.*, 2005; Zhitkovich, 2011)
Cu	Inhalation, ingestion, dermal exposure	Lungs, gastrointestinal tract, kidneys, liver, blood, skin, eye.	High liver and brain concentrations of copper results in Wilson's disease, which is often fatal. Inhalation causes irritation and ingestion nausea. Skin contact can lead to burns	(National Research Council (US), 2000; Mulligan and Bronstein, 2020)
Pb	Ingestion and inhalation both of which can lead to poisoning, known as 'plumbism'	Gastrointestinal tract, kidneys, and central nervous system	Loss of memory, decreased reaction time, insomnia and weakness of joints. Can damage the nervous, skeletal, circulatory, enzymatic, endocrine, and immune systems	(WHO, 1995; Wuana and Okieimen, 2014)
Ni	Inhalation and ingestion	Respiratory system, skin and alteration in gene expression	Interference with the metabolism of essential metals	(Kasprzak *et al.*, 2003)
Zn	Inhalation, ingestion, dermal exposure	Respiratory system, gastrointestinal tract and haematological system	Chronic ingestion may lead to anaemia. Low toxicity and pathway risk to humans. Can cause respiratory problems	(ATSDR, 2005; Kasprzak and Waalkes, 2011; Smolders and Mertens, 2013a; Entwistle *et al.*, 2019)

2.15 Ecological impact of PTE in soils

The impact of PTE contamination on land based ecology such as vegetation has been shown in many studies (Van Assche and Clijsters, 1990; Dudka and Adriano, 1997; Sharma and Dubey, 2005). Dukda and Adriano found that uptake from contaminated soils and deposition of contaminants from the atmosphere onto plant surfaces are sources of elevated levels of PTE in terrestrial plants grown in historic mining areas (Dudka and Adriano, 1997). Plants can be used as ‘bioassays’ for soil contamination, as measurement of changes in their physiological parameters (chlorophyll concentrations, stomatal conductance or fluorescence characteristics) can provide a clearer understanding of the impact a PTE is having on ecology than chemical analysis (Gyuricza *et al.*, 2010).

Lead (Pb) toxicity in plants can cause negative physiological, biochemical and ultrastructional effects (Sharma and Dubey, 2005). These include rapid inhibition of root growth, stunted growth, chlorosis, enzyme activity inhibition, disturbed mineral nutrition and changes in hormonal status (Sharma and Dubey, 2005). At high concentrations, Pb phytotoxicity has been found to eventually lead to cellular death though the inhibition of enzymes containing sulphydryle (-SH) groups, which are necessary for cell activity (van Assche and Clijsters, 1990). For example, studies have found that increased concentrations of soluble Pb caused a decrease in the germination rate of rice seeds (Verma and Dubey, 2003). A study conducted in southern Poland found that crops had elevated concentrations of Pb when grown in areas connected to historic and active Pb mining processes, particularly smelting (Dudka and Miller, 1999). Areas of extensive Pb contamination have been found to remain barren of vegetation long after mining processes cease (Gunn *et al.*, 1995). A number of studies have found that PTE contamination from historic mining can have negative impacts on species which inhabit local waterways (Clegg, 1974; Gandy *et al.*, 2007; Martínez-Ruiz *et al.*, 2007; Mitchell, 2009). The scale of this impact is currently unknown as it has been predicted that initial estimates (made by the national rivers authority in 1994) of over 200 km of streams and rivers in the UK could be significantly lower than real levels (Jarvis *et al.*, 2007).

A study of the legacy of historic metal mines in Cumbria found that impacts can include changes in benthic macroinvertebrate communities such as the exhibition of biochemical effects from PTE exposure on riparian plants, aquatic invertebrates, and fish (Mitchell, 2009). This study concluded that the diversity of macroinvertebrate species significantly decreased as a direct result of the increased presence of Pb and Zn (Mitchell, 2009). Once this impact is confirmed it is suspected that other effects at higher trophic levels are taking place within the wider environment (Martinez-Ruiz, *et al*, 2007). In 1984 a river water regulator in Scotland revealed concentrations of Pb and Cd in large fish swimming in waterways close to historic mining sites were far greater than those measured in fish collected from a reference site (Clyde River Purification Board, 1984). These concentrations were observed to be causing the blackening of tails as well as spinal abnormalities in brown trout (*Salmo trutta*), characteristics of chronic lead exposure (Clyde River Purification Board, 1984).

2.16 Typical rural plant PTE concentrations

When attempting to understand the concentrations PTE found within plant biomass, it is important to provide comparisons with other reported concentrations. This is because found concentrations of PTE in plant biomass may only represent ‘background’ levels. The most comprehensive resource for UK plant PTE concentrations is the UK Soil and Herbage Pollutant Survey (UKSHS) (Environment Agency, 2007). The UKSHS provided the first coherent picture of baseline concentrations of PTE in both soils and plants and was intended to supply a “reliable baseline against which intensive local surveys and future national surveys can be assessed” (Environment Agency, 2007). In this survey, samples of soils and plants were taken from 22 rural, 28 urban, and 50 industrial locations and analysed for concentrations of common PTE and organic contaminants. The results of the survey which are relevant to this study are shown in Table 3 and are used for comparisons with found plant concentrations throughout this study.

Table 3 PTE concentrations in rural plants for England, found in the UK Soil and Herbage Pollutant Survey (UKSHS) (all values in mg/kg n=183) **(Environment Agency, 2007)**

	Range	Mean	Median	SD
Cd	0.10–1.43	0.15	0.10	0.15
Cr	1.0–13.0	1.62	1.00	1.58
Cu	3.56–14.6	7.22	7.03	2.28
Ni	1.00–8.97	1.72	1.19	1.12
Pb	1.0–19.1	1.87	1.00	2.11
Zn	14.7–102	33.6	32.0	11.8

2.17 Phyto-Remediation

Historic mining tailings are typically too physically, chemically and biologically deficient to allow for natural vegetation regrowth, resulting in the continued distribution of PTE contaminated sediment not stabilized by a rhizosphere or sheltered by the above ground portions of plants (Davies and White, 1981b; Gil-Loaiza *et al.*, 2018). The Environmental Protection Act (1990) regulates the management and remediation of contaminated land in the UK (HMSO, 1990). Traditional remediation methods, such as soil removal and capping, are cost prohibitive, involve technically complicated procedures, rely on intensive labour use and can cause irreversible changes in soil properties and the disturbance of native soil microflora (Gomes, 2012).

Traditional remediation methods are also difficult to apply to historic mining sites where the PTE are often heterogeneous and distributed over large areas with multiple point sources (Gomes, 2012). One of the examples of traditional remediation is ‘capping’, which is an in-situ remediation option that involves the application of a ‘cap’ to a contaminated soil such as topsoil, concrete, geo textiles or clay, covering the contaminated area in order to decrease the mobility of contaminant (USEPA, 1997; Martin and Ruby, 2004). The surface of the contaminated soil is typically covered to a depth of 30-50 cm (USEPA, 1997). The advantages of this technique are that it is simple and can be rapidly applied to a contaminated area; it does not produce secondary waste and has little impact on surrounding area that is unaffected by the contamination (Wuana and Okieimen, 2011). However, capping is only a temporary solution that does not remove contamination. It requires permanent monitoring and there is also the potential for the physicochemical properties of the contaminated soil to change, resulting in the remobilisation of contaminant (Martin and Ruby, 2004) It is estimated that the average remediation cost of in-situ soil capping is €139 per cubic meter within the EU (Summersgill, 2006).

An alternative to conventional options for the remediation of contaminated soils is phytoremediation. Phytoremediation has been defined as “the use of green plants to remove pollutants from the environment or to render them harmless” (Salt *et al.*, 1998a). Despite its relatively recent acceptance as an appropriate remediation strategy, the technique is becoming increasingly popular as it is not only viewed as an “eco-friendly” option but also a cost effective one (LeDuc and Terry, 2005). For example, it has been estimated that the average remediation cost of a phytoremediation strategy is €15 per cubic meter within the EU (Summersgill, 2006).

There are five main subgroups of phytoremediation that have been identified and researched, predominantly over the past 30 years (Salt *et al.*, 1998b):

- Phytoextraction: the uptake of PTE from the soil into the above-ground or ‘harvestable’ parts of the plant (Vysloužilová *et al.*, 2003; Meers *et al.*, 2007; Melo *et al.*, 2009; Kocoń and Jurga, 2017).

- Rhizodegradation: the degradation of organic pollutants by microbial activity, which is enhanced by the presence of the root zone of plants (Kaimi *et al.*, 2007; Wenzel, 2009; de-Bashan *et al.*, 2012; Memarian and Ramamurthy, 2012; Allamin *et al.*, 2020).

- Phytovolatilization: the volatilisation of pollutants into the atmosphere via plants. Primarily the uptake of volatile organic compounds in water which pass through the plant and are evaporated/vaporized, resulting in their substantial dilution and photochemical decay in the atmosphere (Seeger *et al.*, 2011; Sovova *et al.*, 2012; Limmer and Burken, 2016; Guarino *et al.*, 2020; Lee *et al.*, 2020).

- Rhizofiltration: the remediation of water (ground water, surface water or waste water) contaminated with a range of potential substances (organics, non-organics, pathogens and excess nutrients) via filtration through the root mass of plants (Odinga *et al.*, 2019; Tiwari *et al.*, 2019; Sikhosana *et al.*, 2020; Castro-Castellon *et al.*, 2021; Han *et al.*, 2021; Kristanti *et al.*, 2021; Woraharn *et al.*, 2021).

- Phytostabilisation: the reduction of the mobility and bioavailability of pollutants in the environment (soil, air water) either by physical immobilisation or by prevention of chemical migration (such as reduction in PTE bioavailability) by plants (Stoltz and Greger, 2002; Mench *et al.*, 2003; French *et al.*, 2006; Brunner *et al.*, 2008; Vamerali *et al.*, 2009; Vangronsveld *et al.*, 2009; Dary *et al.*, 2010).

These five main phytoremediation techniques can be applied appropriately in different circumstances depending on the desired outcome. For example, if the priority is to address the contaminant source then a phytoextraction, phytovolatilization or rhizodegradation strategy would be selected. However, if the priority is to address the contaminant pathway to a receptor then a phytostabilisation or rhizofiltration strategy would be implemented. Individual techniques can also be used in conjunction with the others in certain circumstances.

2.17.1 Hyperaccumulators

A key question when selecting a phytoremediation technique and therefore plant species, is whether the accumulation of the contaminant within the above ground biomass is desirable or not. If the accumulation of contaminants in the above ground (harvestable) portion of the plant followed by the ‘contaminated’ plants removal (phyto extraction) is the desired outcome, then a ‘hyperaccumulator’ species should be used. Hyperaccumulator plants are plants that have an ability to accumulate greater than normal concentrations of metals/metalloids in their tissue by a factor of up to 1000 times when compared to non-accumulators (McGrath *et al.*, 2002). Plants have been found to be able to accumulate a wide variety of elements including As, Cd, Cu, Cobalt (Co), Mn, Ni, Pb, Thallium (TI), Selenium (SI), some rare earth elements and Zn (Baker *et al.*, 1994, 2000; Reeves, 2003; van der Ent *et al.*, 2013; Reeves *et al.*, 2018). Hyperaccumulator species have evolved efficient biomolecular mechanisms for the uptake of metals/metalloids in their root systems, which is followed by the translocation to and sequestration within the above ground parts of the plant (Baker, 1981). The reasons why hyperaccumulator species evolved in this way is an active field of enquiry, with research focusing on biotic interactions through the production of allelochemicals (bio chemicals that influence the germination, growth, survival, and reproduction of other organisms) by hyperaccumulators, and anti-herbivore defence mechanisms allowing for improved competition with other species (Boyd, 2013; Fones *et al.*, 2013). For example, Fones *et al*., found that the species *Noccaea caerulescens* is protected from disease by the accumulation of high concentrations of Zn in its above ground tissues, which are toxic to many pathogens (Fones *et al.*, 2013). Hyperaccumulators are not only used for phytoextraction but also phytomining and in applied biotechnologies (Zhang *et al.*, 2014; Wiche *et al.*, 2017).

A need to collate the otherwise widely diffuse reporting of information regarding hyperaccumulator species led to the creation of the Global Hyperaccumulator Database (www.hyperaccumulators.org) in 2015 which is shown in Table 4.

This database shows the wide range of hyperaccumulator species and also provides a background, threshold and reported record for use when evaluating found concentrations of PTE in biomass analysis.

Table 4 Hyperaccumulator species in the Global Database (as of September 2017) with the global records of the highest concentrations reported to date. This table was derived from data reported in Reeves et al., 2018 as the database is currently offlin*e*** (Reeves *et al.*, 2018).

Element	**Threshold (mg/kg)**	**Plant Families**	**Genus**	**Species**	**Global records (element % of mass)**	**Reference**
Arsenic (As)	> 1000	1	2	5	*Pteris vittata (2.3%)*	(Ma *et al.*, 2001)
Cadmium (Cd)	> 100	6	7	7	*Arabidopsis halleri (0.36%)*	(Stein *et al.*, 2017)
Copper (Cu)	> 300	20	43	53	*Aeolanthus biformifolius (1.4%)*	(Malaisse *et al.*, 1978)
Cobalt (Co)	> 300	18	34	42	*Haumaniastrum robertii (1%)*	(Brooks, 1977)
Manganese (Mn)	> 10,000	16	24	42	*Virotia neurophylla (5.5%)*	(Jaffré, 1979)
Nickel (Ni)	> 1000	52	130	532	*Berkheya coddii (7.6%)*	(Mesjasz-Przybyłowicz *et al.*, 2004)
Lead (Pb)	> 1000	6	8	8	*Noccaea rotondifolia subsp. cepaeifolia (0.8%)*	(Reeves and Brooks, 1983)
Rare earth elements (lanthanum, (La); cerium, (Ce))	> 1000	2	2	2	*Dicranopteris linearis (0.7%)*	(Shan *et al.*, 2003)
Selenium (Se)	> 100	7	15	41	*Astragalus bisulcatus (1.5%)*	(Galeas *et al.*, 2007)
Thallium (Tl)	> 100	1	2	2	*Biscutella laevigata (1.9%)*	(LaCoste *et al.*, 1999)
Zinc (Zn)	> 3000	9	12	20	*Noccaea caerulescens (5.4%)*	(Reeves *et al.*, 2001)

2.17.2 Phyto-removers

Many hyperaccumulators are however not suitable for growth on contaminated sites and may not be practically applicable for a phytoextraction strategy. For example, Ebbs *et al*. found that whilst *Thlaspi caerulescens* was able to hyperaccumulate Cd it was not suitable for phytoextraction, as the plants were too small and grew too slowly, making them difficult to harvest mechanically (Ebbs *et al.*, 1997).

An alternative to using hyperaccumulator species on contaminated land is to grow plants with high biomass production rates and low contaminant uptake ability. This is because these plants can then be used in industries such as the biofuel and bio-based chemical industry, providing a slower remediation option but a financial return in the process. Olvivares *et al*. successfully grew *Ricinus communis* L. (castor oil plant) on mine tailings containing high concentrations of Cu, Zn, Mn, Pb and Cd, demonstrating a new option for adding value to suitable metal-polluted areas (Ruiz Olivares *et al.*, 2013). Van Slycken *et al*. grew several different clones of short rotation coppice willow (*Salix sp.*) on agricultural soil contaminated with Cd and Zn; the study found that certain clones exhibited acceptable productivity levels, and thus a phytoextraction potential greater than energy maize or rapeseed grown on the same soil (Van Slycken *et al.*, 2013). They also found that phyto extraction of Cd and Zn can be increased by 40% if the leaves are harvested.

2.18 Phytostabilisation

Phytostabilisation involves the use of plants *in-situ* to reduce mobility and migration of contaminants and thus their bioavailability (Singh, 2012). The phytostabilisation technique aims to contain contaminants within the vadose zone (the part of the earth which extends from the top of the ground surface to the water table) through accumulation by roots, precipitation within the rhizosphere and the establishment of a vegetative cover (Bolan *et al.*, 2011). This containment prevents further soil contamination and direct contact with humans or animals (Vangronsveld *et al.*, 2009; Bolan *et al.*, 2011). Phytostabilisation has become a popular option for remediation recently as it has become widely recognised that it is not the presence of a contaminant in soil that creates an environmental risk but its negative interaction with a receptor (Bolan *et al.,* 2011). Therefore, it may be wiser to limit the mobility and migration of a contaminant rather than attempt to remove it as is done with phytoextraction.

The establishment of a phytostabilisation remediation strategy has been shown to decrease the vertical and horizontal leaching of PTE available for transportation via wind or groundwater (Vangronsveld *et al.*, 2009). The vegetative cover reduces water flux through the soil profile, mechanically stabilizes the soil through root growth, prevents soil erosion, protects soil from rain splash, reduces wind velocity and slows the movement of surface runoff, thereby increasing soil infiltration i.e. the soil's ability to allow water movement into and through the soil profile (Bolan *et al.*, 2011). Each of these effects reduces the mobilisation potential of PTE. The effectiveness of phytostabilisation in PTE containment depends on the type of vegetation and the extent of cover (Bolan *et al.,* 2011). For example, Simon and Collison found that the use of large vascular plants with wider root systems and canopy resulted in a greater increase in soil structural integrity and interception of rainfall, thus preventing soil erosion and further PTE mobilisation (Simon and Collison, 2002). Plants which support ground vegetation and earthworm (*Lumbricid sp.*) populations are also more effective, as these factors increase the water infiltration capacity of the soil (Bolan *et al.,* 2011). A summary of the benefits of the phytostabilisation remediation option can be found in Figure 2.

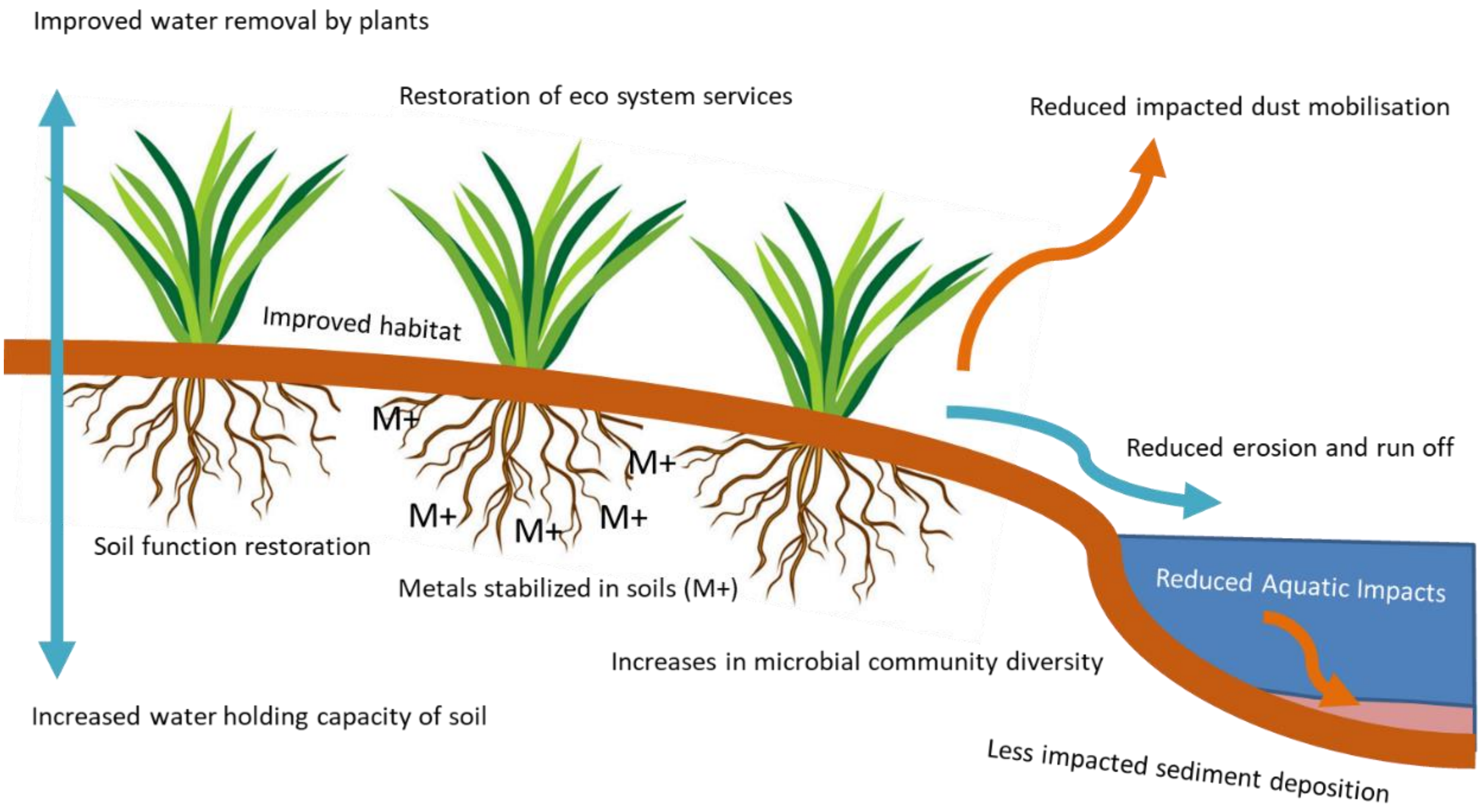


Figure 2 A graphical summary of the benefits of Phytostabilisation informed by a review of relevant literature (Simon and Collison, 2002; Mendez *et al.*, 2007; Mendez and Maier, 2008; Bolan *et al.*, 2011; Jensen, 2019; Palansooriya *et al.*, 2020)

2.18.1 Implementation of Phytostabilisation

Several authors have highlighted that existing phytostabilisation studies are limited in scope and have not addressed several important factors (Mendez and Maier, 2008; Brown *et al.*, 2016). An example of this is that the accumulation of PTE by plants has not been documented in the majority of field studies (Johansson *et al.* 2005). This is an important factor in determining the long-term fate of PTE and thus the efficacy of phytostabilisation in permanently reducing their mobilisation (Mendez and Maier, 2008). Mendex and Maier also point out that little is known on the ranges of metal tolerance in species suitable for growth on mine tailings, and the minimum amounts of organic amendments (materials applied to the topsoil to change soil properties and improve plant growth) required for successful plant establishment. It is therefore still necessary to carry out a pilot study or pot trial for each specific historic mine site to increase the chances of successful phytostabilisation. Mendex and Maier suggest that long term success of phytostabilisation strategies on historic mine sites are evaluated by the criteria shown in *Table 5*.

Table 5 Criteria for evaluating successful revegetation of mine tailings (Mendez and Maier, 2008).

Plant criteria	• Biomass and percent cover comparable or exceeding growth in uncontaminated soil • Self-propagation of introduced plants • Establishment of native colonizers • Shoot metal concentrations not exceeding the domestic animal toxicity limits • Plant survival and productivity maintained for > 10–20 years
Soil criteria	• Soil aggregation improved • Erosion and runoff reduced • Metal bioavailability and mobility decreased
Microbial criteria	• Heterotrophic bacterial and fungal counts increased • Autotrophic iron- and sulfur-oxidizing bacterial counts decreased

A review of the tools available to evaluate the efficiency of an amendment-based phytostabilisation strategy for historic mine site remediation confirmed the findings of Mendex and Maier (2008) and added that ecosystem function as whole should also be measured (Brown *et al.*, 2016). Brown *et al*. suggested adding long-term microbial function and diversity, phytoavailability of PTE, and earthworm/small mammal assays to the previously mentioned criteria (Brown *et al.*, 2016). Their study concluded that whilst amendment-based phytostabilisation is currently transitioning from the laboratory scale to field trial and full-scale use, and a range of tools have been developed in parallel to measure the success of this remediation strategy, an understanding of the benefits of such efforts is yet to realised (Brown *et al.*, 2016).

2.18.2 Limitations of a Phytostabilisation approach

Despite its relatively low cost when compared with traditional remediation approaches, a phytostabilisation approach does have considerable set-up and ongoing costs (Bolan *et al*., 2011: Miller and Miller, 2007). Phytostabilisation also requires significant levels of site assessment so that the correct plant species and (if required) soil amendments are chosen (Bolan *et al*., 2011). There are also ongoing monitoring costs as the contaminant is not removed from the soil (Pulford and Watson, 2006). However, Bolan *et al* suggest that there are many ways to counter these costs through income generation involving tourism, timber product generation such as oils or phytochemicals, and through carbon fixation schemes (Bolan *et al*, 2011).

Another concern with the use of the phytostabilisation approach is that restoring ecological function on a highly contaminated site will result in creating an exposure pathway for the PTE to enter the food chain that did not previously exist (Bolan *et al*, 2011). Brown and Chaney describe this phenomenon as creating an 'attractive nuisance' and report that following the restoration of a vegetative cover on an historic mine site many small mammals began to inhabit the site (Brown and Chaney, 2016). For example, the use of white poplar (*Populus alba*) on a contaminated ex-mining site in Guadimar was found to expose herbivores to high concentrations of Cd (Dominguez *et al*, 2010).

2.19 Organic waste soil amendments

The chemical immobilization of PTE can be achieved through the use of organic waste soil amendments capable of adsorption, precipitation and complexation reactions, resulting in the redistribution of contaminants from solution phase to solid phase, thereby reducing their bioavailability and mobilisation potential within the environment (Palansooriya *et al.*, 2020). The use of amendments in contaminated soil remediation is increasingly seen as a competitive and sustainable option (Johnson *et al.*, 2018) and compares well to more cost and energy intensive techniques such as capping or soil washing (Gomes, 2012). Mendez and Maier point out that it is the extent of mine sites that prevents the application of an ideal strategy for remediation such as the addition of topsoil amended with organic matter and nutrients as this extent renders such strategies uneconomical (Mendez and Maier, 2008).

Despite recent interest, there have been few field trials that demonstrate the value of amendment-based PTE immobilisation (Bolan *et al.*, 2014). This has led to a lack of evidence on the impact of the application of soil amendments on full-scale historic mine sites, such as the effect on co-contaminants (Bolan *et al.*, 2014). Kennan and Kirkwood (2017) warn that it is important to ensure that the addition of amendments does not cause PTE to become more water soluble and thus able to leach into groundwater and migrate (Kennen and Kirkwood, 2017). Their study also highlighted the potential for the increased mobilisation of PTE during the addition of amendments by causing additional wind or water erosion events (Kennen and Kirkwood, 2017). Bolan *et al.*, shared similar concerns, and hypothesised that the addition of P compounds to a soil contaminated with both As and Pb could both facilitate the immobilization of Pb whilst resulting in the release of As (Bolan *et al*., 2014).

In one recent amendment PTE immobilisation trial example, Rodríguez *et al*. grew white lupin (*Lupinus albus*) in soils taken from a historic Pb/Zn mine amended with inorganic sugar production waste, sludge from a drinking water treatment residue, organic waste from olive mill waste and paper mill sludge. They found that all the amendments were capable of significantly decreasing extractable Pb, Zn and Cu concentrations and the bioavailability of Pb and Zn to the lupine plants, whilst also providing necessary growth conditions such as organic matter (OM), pH, cation exchange capacity (CEC) and plant nutrient requirements, leading to improved plant biomass (Rodríguez *et al*., 2016). Table 6 gives further examples of the effects of different organic waste amendments applied to historic mine sites. Descriptions on the observed effect of the amendments on the mobility of PTE are also included in the table.

Table 6 Selected references on the effects of various soil amendments on PTE mobility in soils from historic mines following a review of relevant literature.

Amendment	**Site/type of growing media/contamination source**	**Concentration of PTE**	**Amendment application rate**	**Observations**	**Source**
Green waste compost	Tyndrum, Scotland / Mine soil / Pb⁄Zn mining operations	Pb 2280 mg⁄kg Cu 131 mg⁄kg Zn 1820 mg⁄kg	10 and 20% w/w	Decrease in soil extractable PTE following application of amendment	(Nwachukwu and Pulford, 2009) –
Chicken fertilizer	Cyprus / Cu Mine	Cu 787 mg/kg	Chicken fertilizer and mine waste soils mixed at a ratio of 1:1	Green house trial – Biomass did not increase	(Johansson *et al.* 2005) -
Municipal solid waste compost and Green waste compost	Portugal / Pyrite mine	Cu 387 mg/kg Pb 4350 mg/kg Zn 245 mg/kg	25,50 and 100 t/ha	All lead to a decrease in the mobile fractions of Cu, Pb and Zn	(Alvarenga *et al.*, 2008) -
Fly ash and Sewage sludge (Iron sulphate as precipitation agent)	Gillervattnet mine Boliden, Sweden / sulfidic mine tailings / mining operations	Cu – 669 - 1310 mg/kg Zn 503 – 6560 mg/kg	Sewage sludge - 100 g Fly ash - 40 g Mine tailings - 70 g	Ash and Sludge both decreased soil leaching and plant uptake of PTE	(Neuschütz and Greger, 2010a) -) -
Biochar (Sugar cane straw derived)	Vazant, Brazil / Former open cast Zn mine / mining operations	Pb 2330 - 3690 mg/kg Zn 1065 - 2027 mg/kg	Biochar applied at 1.5,3 and 5% w/w.	Application of amendment decreased PTE availability by approximately 50%	(Puga *et al.*, 2015) –

2.19.1 Drinking water treatment residue

Drinking water treatment residue (DWTR) is a by-product of the coagulation-flocculation process used in the treatment of drinking water. $Al(OH)_3$ or $Fe(OH)_3$ metal hydroxide compounds are used in this process to precipitate clay, colloidal particles, algae and humic substances from water resource (McCann *et al.*, 2015). The precipitates and metal compounds are then bound and removed as waste 'sludge'. Several recent studies have shown that there is great potential for DWTR to be used in PTE remediation as the large, active surface areas and amphoteric nature (ability to react as an acid or base) of metal hydroxides make them suitable for sorption and immobilization of a wide range of soil contaminants (Bolan *et al.*, 2014). This is of particular interest as DWTR currently has no other use and is typically put into landfill leading to financial and environmental costs (Hidalgo *et al.*, 2016). The 1986 Sewage Sludge Directive 86/278/EEC is the EU directive which defines the rules on how sewage sludge, as a fertiliser, can be used in order to prevent it harming the environment and human health (ECC, 1986). ANNEX IB of the directive sets out the limit values for the concentrations of PTE in sludge and is an applicable regulatory standard for DWTR when used as a soil amendment.

Table 7 shows the permissible PTE limits of biosolids under the Sewage Sludge Directive 86/278/EEC.

Table 7 PTE limits of biosolids under the Sewage Sludge Directive 86/278/EEC (ECC, 1986)

Element	Cu	Zn	Ni	Cd	Pb
Regulation limit value(mg/kg)	1000 - 1750	2500 - 4000	300 - 400	20 - 40	750 - 1200

A study conducted in Egypt found that DWTR (using $Fe_2(SO_4)_3$ coagulant) is an efficient adsorbent for Cd^{2+} and Ni^{2+} and has an extremely high adsorption efficiency towards Pb^{2+} (Abo-El-Enein *et al.*, 2017). The study also concluded that when using DWTR as an adsorbent the contact time, pH, dose and initial metal ion concentration all affected the adsorption process (Abo-El-Enein *et al.*, 2017). Hidalgo *et al*. suggested that the reason why DWTR is not more widely used in remediation projects or as a building material is that it is often wrongly associated with sludge from wastewater treatment plants and that a change in perception of the waste product would allow greater use (Hidalgo *et al.*, 2016).

Johnson *et al.* investigated the Pb adsorption capacity of DWTR using a 10 month lysimeter trial (Johnson *et al.*, 2018). The study involved the amendment of a historically Pb contaminated soil with 10% DWTR by weight. Two spectroscopic techniques were used (electron probe microanalysis and X-ray photoelectron spectroscopy) to show Pb immobilisation through sorption to Mn Oxides (MnOx) within the DWTR. Sorption of Pb to MnO_x has been shown in previous studies to be irreversible. This is due to strong inner and outer sphere complexes on octahedral vacancy sites in the malleable crystal structure of MnOx minerals (McKenzie, 1980). Although the study by Johnson *et al*. was not the first to notice this chemical process, it is reportedly the first time that using the naturally occurring MnOx within DWTR has been considered for the remediation of Pb contaminated soils. Table 8 shows the ranges of PTE found in studies on DWTR globally using both $Al(OH)_3$ or $Fe(OH)_3$ metal hydroxide compounds.

Table 8 Typical concentrations of PTE in DWTR reported in the literature

Al or Fe based WTR	**Cu (mg/kg)**	**Zn (mg/kg)**	**Ni (mg/kg)**	**Cd (mg/kg)**	**Cr (mg/kg)**	**Pb (mg/kg)**	**Country**	**Reference**
Al	22 (13-33)	56 (28-82)	17.3 (10-24)	0.26 (0.2-0.3)	14.7 (6.1-28)	12 (4-17)	UK	(Finlay, 2015)
Fe	19.4 (7.9-36)	265 (40-1100)	53.8 (22-120)	1.0 (0.3-3.6)	30 (15-39)	60 (5-190)	UK	(Finlay, 2015)
Al	41	46	18.9	1.04	41	114	Czech Republic	(Kyncl, 2008)
Fe	31	3	12	0.83	37	24	Czech Republic	(Kyncl, 2008)
Al	2.4	52.5	-	0.4	-	37.3	Spain	(Rodríguez *et al.*, 2018)
Al	36	33	6	0.1	8	<0.05	USA	(Ippolito *et al.*, 2009)
Al	-	<0.05	<1.6	<0.17	<0.17	<1.02	USA	(Sarkar *et al.*, 2007)
Fe	-	<0.05	<1.6	<0.17	<0.17	<1.02	USA	(Sarkar *et al.*, 2007)
Al	171 (135-230)	527 (195-815)	44 (26-65)	1.6 (1-2)	50 (40-60)	204 (47-439)	USA	(Elliott *et al.*, 1990)
Fe	272 (135-485)	575 (215-865)	136 (33-218)	<1 (<0.1 -2)	269 (62-513)	245 (18-840)	USA	(Elliott *et al.*, 1990)

Note: where a range of values have been reported, they are presented in parenthesis

2.19.2 Green Waste Compost

Green waste compost (GWC) is the product of composting operations that degrade waste from gardens such as grass clippings, leaves or other plant material. This material is typically collected by local authorities and composted using the open windrow method, followed by the maturation and screening of the material. In the UK the production of GWC has considerably increased over the last 20 years, making GWC a widely available waste product (Karami *et al.*, 2011; Eades *et al.*, 2020). The GWC used throughout this study conforms to the PAS 100 specifications.

The BSI PAS 100 is a non-statutory document that states the minimum quality standards and minimum safety parameters for compost (BSI, 2018). Specified requirements include the composting process, analysis of the compost, input materials and traceability of composted products. The composting process is monitored following a hazard analysis and critical control point plan which includes the following requirements:

- The measurement of temperature and moisture during the process,
- a temperature greater than 65 °C and a moisture content above 50 % (mass/mass) should be maintained for 7 days to eradicate pathogens during the sanitization stage,
- the material should be mixed at least twice in this period, and
- the input materials for composting must be source segregated and separated from non-biodegradables.

In order for GWC to be BSI PAS 100 certified, a number of parameters and limits focusing on concentrations of PTE, pathogens, the physical characteristics of the material, stone content, stability/maturity, plant response, weed seeds and propagules must be met. These parameters are shown in Table 9. If a compost meets the criteraset out by the PAS 100 Quality Protocol then it can be defined as a product rather than a waste, meaning that no waste regulatory controls are applicable (Environment Agency & WRAP, 2012).

Table 9 Limits of PTE relevant to this study that composted material must pass to become BSI PAS 100 certified (BSI, 2018)**.**

Parameter	**Upper limit**
Cadmium (Cd)	1.5
Chromium (Cr)	100
Copper (Cu)	200
Lead (Pb)	200
Nickel (Ni)	50
Zinc (Zn)	400

GWC is a rich source of organic matter and therefore humic acids, which behave as complexing agents, ion-exchangers and surfactants. These qualities have been found to give GWC a high capacity for metal binding (Montoneri *et al.*, 2009)**.** Some of the PTE frequently encountered on historic mining sites, such as Zn, show varying degrees of affinity for organic matter (Brown *et al.*, 2003). Other studies have found that the fulvic acids within composts can mobilize PTE in amended soils, potentially leading to increases in the bioavailability of PTE (Kulikowska *et al.*, 2015). This is possible as fulvic acids are able to form more soluble, mobile and bioavailable metal complexes than humic acids are. A glasshouse pot trial conducted by Badmos *et al*. studied the addition of 90 and 180 t ha^{-1} (wet weight) GWC to contaminated soil obtained from a former industrial site with PTE and hydrocarbon contamination (Badmos *et al.,* 2015). The addition of GWC was found to increase soil nutrient status (organic matter, total N, total P and soil nitrate) of the contaminated soil and the dry biomass of plants grown after a 30 day period by 120-337% when compared to an unamended control (Badmos *et al.,* 2015).

Increasing interest in integrating remediation with the provision of ecosystem services, such as carbon sequestration in soils, has made the addition of soil amendments such as GWC an attractive option (Lord and Sakrabani 2019). Lord and Sakrabani analysed soils from a brownfield site that had been amended with GWC 10 years previously at application rates of 250, 500 and 750 t ha^{-1} (Lord and Sakrabani 2019). Increases in soil organic carbon and total nutrients N and P were recorded in the higher rates of amendment application, showing that large single applications of GWC can result in long term carbon sequestration whilst also providing fertility benefits (Lord and Sakrabani 2019). Brown *et al*. amended PTE contaminated soils on a historic mine site named Bunker Hill in Idaho with GWC at a rate of 44-66 t ha^{-1}. Contaminated soil PTE concentrations were relatively high compared to the majority of similar studies, with total Cd, Pb and Zn ranging from 9 to 28, 2100 to 27,000 and 6000 to 14,700 mg kg^{-1}, respectively (Brown *et al.*, 2003). The GWC amendments were combined with wood ash (220 t ha^{-1}) and resulted in a significant decrease in subsoil extractable metals and increases in plant cover following a 2-year period. The study also concluded that the use of conventional metal mine remediation amendments such as limestone were not sufficient to support plant growth when compared to GWC (Brown *et al.*, 2003).

2.20 Extraction of PTE from environmental solid samples

Acid digestion (the decomposition of a sample in strong acids) of a sample is required to extract the analytes into a solution before analysis. This is necessary for most methods of instrumental analysis and is common due to the cost and reliability of other methods. For example, an alternative to digestion and instrumental analysis is X-ray fluorescence (XRF) which reads emissions from a material that has been excited by being bombarded with high-energy X-rays. Although a clear benefit of this technique is that it is non-destructive, particularly when compared to the acid digestion of a sample, it can be cost-prohibitive, less accurate, suffer from strong matrix effects and radiation exposure concerns for operators.

The type of extractant used in digestions to release metals from a matrix is of vital importance as different reagents liberate metals from different soil components and therefore provide different information. For example, the total digestion of a sample typically requires hydrofluoric acid (HF) to dissolve silicates and therefore solubilise silicate bound metals. This type of digestion is rarely performed outside of geochemical surveys, as studies which aim to investigate anthropogenic sources of PTE often do not need to consider metals from geogenic sources. Due to its highly corrosive nature HF also requires prohibitive levels of personal protective equipment (PPE) and risk assessment (RA).

For studies concerning anthropogenic sources of PTE, the key areas of interest are typically the mobility of the element within its environment, it's bioavailability and impact on biological receptors (Davidson, 2013). Digestion of samples therefore focuses on dissolving the more weakly bound elements in solution in stages, the collective analysis of which should equate to the 'pseudo total' (PT) of elements in a sample.

Typically, the regents used in a PT digestion are nitric acid (HNO_3), hydrochloric acid (HCL), a 1:3 combinations of the two known as 'aqua regia' (AQ), or a mixture of HNO_3 and HCL the above with hydrogen peroxide (H_2O_2). Recently, as microwave assisted digestion has replaced hot plate assisted digestion, the use of a 'reverse' aqua regia mix has become more common. Siaka *et al*. found that when using a microwave to assist digestion, reverse AQ was the most effective acid mix and was able to recover metals more reliably than standard AQ (Siaka *et al.*, 1998). Gaudino *et al*. trialled several soil sample digestion methods including combinations of HCl, HNO_3, H_2O_2 and HF and concluded that due to differences in recoveries, the digestion procedure is an essential part of the definition of the measurement procedure in environmental analysis (Gaudino *et al.*, 2007). Wilson *et al*. measured the elemental recoveries in digested soil and biomass samples taken from historic metalliferous working sites with 4 difference reagent mixes and found differences in the efficiency of extraction, concluding that standard AQ produced the most accurate, efficient and relocatable results (Wilson *et al.*, 2005).

2.21 Analysis of extracts for PTE concentrations.

After the digestion of a solid environmental sample, elemental analysis can take place using atomic spectrometry instruments capable of identifying the liberated elements. Atomic spectrometry involves the measurement of electromagnetic radiation emitted or absorbed by free atoms when their valence electrons undergo transitions between atomic energy levels. Energy is transferred from a photon to an atom, causing an electron to be promoted to an excited state. As the electrons de-excite, their excess energy is emitted as photons. Since each element has a different set of energy levels, each absorbs or emits light at different wavelengths, and has a characteristic atomic absorption or emission spectrum (Davidson, 2013). The percentage of atoms in the excited state is controlled by factors such as temperature, available orbitals, and the energy of the transition. The theoretical ratio or distribution of atoms is calculated by the Boltzmann distribution shown in Equation 1.

$$\frac{n1}{no} = \left(\frac{P1}{Po}\right) e - \Delta \in / kT$$

Equation 1 Boltzmann distrubtion equation

Where:

n1 = number of atoms in the excited state
no = number of atoms in the ground state
P1 = statistical factor (related to the number orbitals) for electrons in the excited state
Po = statistical factor (related to the number orbitals) for electrons in the ground state
ΔE = energy transition
k = Boltzmann's constant
T = temperature (K) (Boltzmann, 1868)

There are several different instrumental analytical techniques that are based on atomic spectrometry, the most common are: atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES). Regardless of which atomic spectrometry technique is used, energy has to be supplied to generate free analyte atoms from the sample, i.e. to vaporise the sample solution, to dissociate molecules and so forth. This is achieved in the 'atom cell' which is any environment where the analyte is observed in a spatially confined arrangement such as a flame, plasma or a heated graphite tube. Atomic absorption spectrometry typically consists of a light source to provide photons, an atom cell to create free (vaporised) analyte atoms, a monochrometor to isolate the wavelengths of interest and a photo-multiplier tube detector. In this technique, light of the same wavelength as an absorption line of the element of interest is shone through its atomic vapour. Some of this light is absorbed by the atoms of the element, and the amount absorbed is directly proportional to the number of atoms present in the sample. This can then be used to determine the concentration of element in the sample. Flame atomic adoption spectrometry (FAAS) is a typical analytical instrument associated with this technique (Davidson, 2013).

Atomic emission spectrometry involves the digestate being subjected to a high temperature, enough not only to cause dissociation into atoms but also cause the excitation of the atoms. The electrons in the excited atoms, on returning to a lower energy level, emit radiation of a specific wavelength, which is measured and used to determine the concentration of the elements of interest in the sample. The AES method uses the intensity of light emitted from a flame, plasma, electric arc discharge, or spark at a particular wavelength to determine the quantity of an element in a sample. The wavelength of the atomic spectral line in the emission spectrum gives the identity of the element while the intensity of the emitted light is proportional to the number of atoms of the element. The sample may be excited by various methods with the most common today using Inductively coupled plasma (ICP) to atomise the sample and also excite the analyte atoms. The two types atomic spectrometry instruments used in this study for the measurement of PTE in solid samples are Inductively coupled plasma atomic emission spectroscopy (ICP-AES) and Inductively coupled plasma mass spectrometry (ICP-MS).

2.21.1 Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

The heating source for ICP-AES is plasma which is a body of gas which has a high degree of ionisation for atoms and molecules (<0.1 – 10 % of the atoms present) and there is a high electron concentration. A schematic diagram of a typical set up for ICP-AES is shown in Figure 3. Most commercial ICP-AES instruments use argon plasma with a temperature in the range of 7,000 - 10,000 K. The argon plasma is formed in a torch made of three concentric quartz tubes known as the outer, intermediate and inner gas tubes. Around the top of the torch is a water-cooled induction copper coil which is powered by a radio-frequency (RF) generator producing typically 700 - 1500 W of energy, at a frequency of 27 or 40 MHz. This sets up a high frequency oscillation of electric and magnetic fields around the top of the torch. The ionisation of the flowing argon is initiated by a spark from a Tesla coil, accelerating seed electrons which then collide with argon atoms, producing a self-sustaining plasma. Digests and extracts are then transported to the plasma by means of a nebuliser which breaks the sample liquid into aerosols. The most commonly employed are pneumatic nebulisers, which use high speed argon gas to break the liquids into the aerosols. On entering the plasma, the aerosol is dissolved, vaporised, atomized and excited. As the atoms de-excite, a high resolution monochromator (most commonly an Echelle spectrometer) is used to separate, isolate and measure the radiation corresponding to the wavelengths of interest allowing for multi element detection. A photomultiplier tube (PMT) is made of a cathode followed by several dynodes to amplify the signal. This is one of the advantages of ICP-AES over traditional forms of AAS such as FAAS, which can typically only measure one analyte at a time. When compared to FAAS, ICP- AES also provides higher selectivity between elements, higher sensitivity, a large dynamic range, lower detection limits and fewer matrix interferences. Most of these improvements are associated with the far higher temperatures involved in plasma use, compared to the relatively low temperatures (~2000 - 2500 C) of a flame-based system, in which not all of the atoms or elements present in the sample are excited.

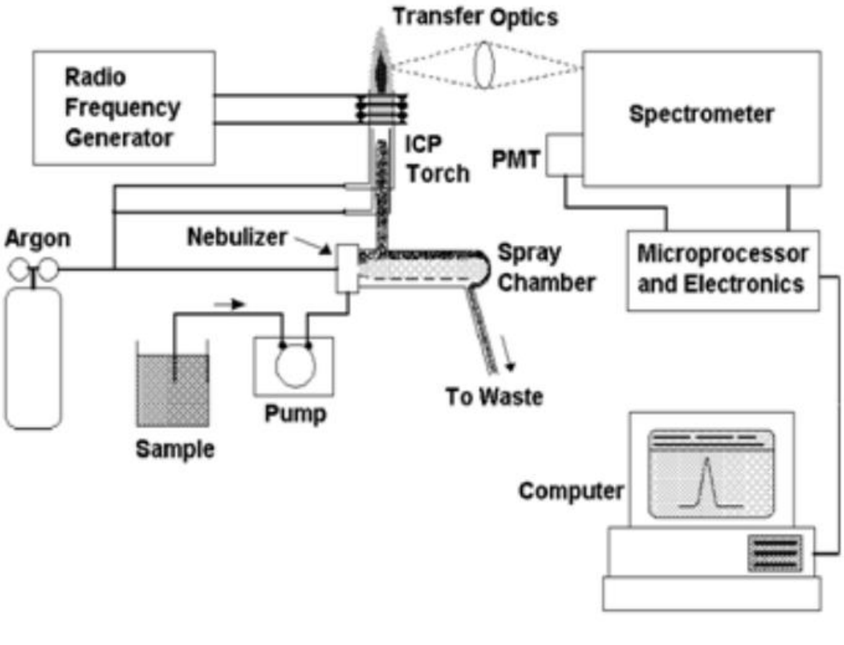


Figure 3 Schematic diagram describing ICP-OES instrument(Kiran and Raja, 2017)

2.21.2 Inductively coupled plasma mass spectrometry (ICP MS)

ICP-MS has been developed more recently than ICP-AES and has been widely applied to both soil and plant analysis (Gaudino *et al.*, 2007; Butler *et al.*, 2017). Whilst ICP-MS is similar to ICP-AES in that involves the use of an ICP, it does not measure the wavelengths of light emitted but the ions generated in the plasma. This is carried out through the use of a quadrupole mass spectrometer (QMS) which is shown in the schematic diagram in Figure 4. The QMS separates ions on the basis of their mass-to-charge ratio, and a detector receives an ion signal proportional to the concentration. The elemental concentrations of a sample are then determined through calibration with certified reference materials and elemental standards. Whilst ICP-MS shares the benefits of ICP-AES over AAS, the use of the QMS allows for far lower limits of detection (LOD). While ICP-AES allows for both trace and major concentrations across a wide range of elements down to part per billion (ppb), ICP-MS provides a lower detection limit down to part per trillion (ppt). The high sensitivity or low detection limits of ICP-MS make it more suitable for measuring samples in which concentrations of elements are expected to be low, for example PTE in plants (Koelmel and Amarasiriwardena, 2012). ICP-AES is mainly used for samples with high total dissolved solids (TDS) or suspended solids and is, therefore, more robust for analysing ground water, wastewater, soil, and solid waste. ICP-MS has a far lower tolerance for TDS (about 0.2%), meaning that digestates with high TDS must be diluted, potentially resulting in a reduction in accuracy.

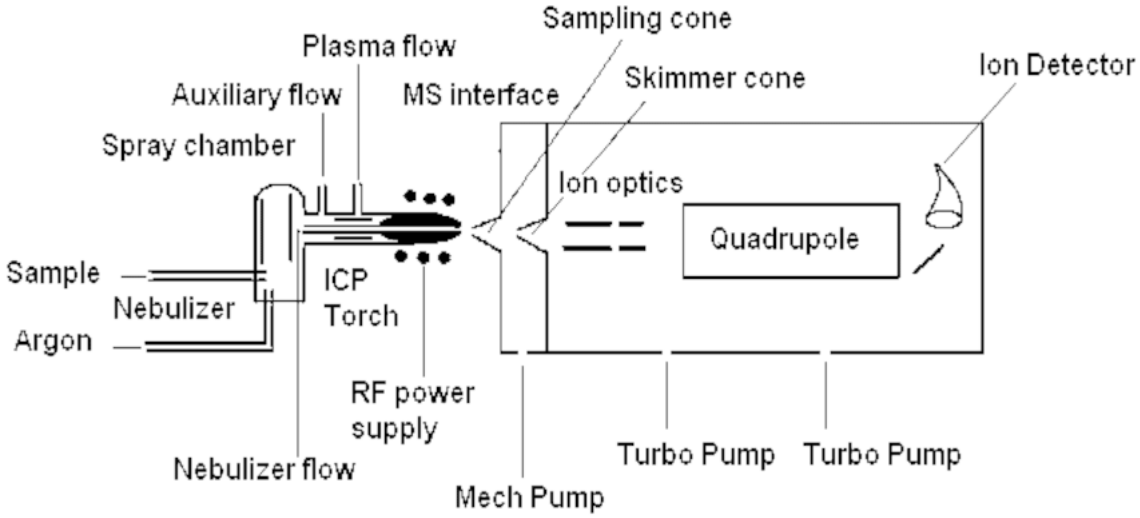


Figure 4 Schematic diagram describing ICP-MS instrument (Brima *et al.*, 2006)

2.22 X-ray computed tomography (XCT)

XCT is a frequently used non-destructive 3D imaging and analysis technique where X-rays are used to create a radiographic image of the scanned component (Cnudde and Boone, 2013). The techniques utilise X-rays in a similar manner to conventional X-ray radiography, generating a set of radiographs of the object which reveal hidden, internal structures. The object is placed between an X-ray source and a detector, after which a beam of x-rays is fed through the object. The amount of the x-rays attenuated by the object (a value that is related to absorptive properties of the material with regard to x-rays) is then recorded by the detector plate as shown in Figure 5(Sun *et al.*, 2012). The object is then rotated by a small increment and the process is repeated, until X-ray projections of the object from all angles through 360º are collected. This process requires minimal or no sample preparation (Staedler *et al.*, 2013). The X-ray projections are then converted into a three-dimensional volume through a process known as reconstruction, which combines the projections together to create a greyscale volume consisting of voxels. A Voxel is a unit of graphic information that defines a point in three-dimensional space, since a pixel defines a point in two dimensional space with its X and Y coordinates, a third Z coordinate is needed. These values are representative of the relative attenuation of the x-rays and can be used to discover a number of properties of the material, such as its density, chemical composition and thickness. Brighter values indicating highly attenuating material and darker values a less attenuating material. The use of this technique for 3D imaging of plant root and soil systems and in plant structures is increasing (Korte and Porembski, 2012; Staedler *et al.*, 2013; Scotson *et al.*, 2021).

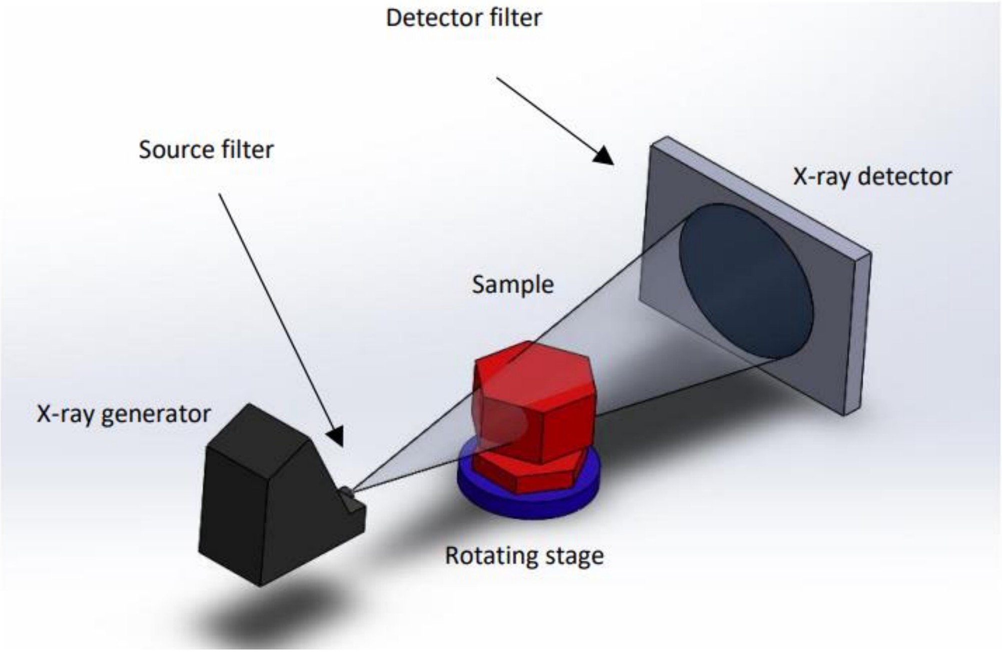


Figure 5 Schematic illustration of XCT (Tawfik *et al.*, 2018)

3 Literature associated with Reed Canary Grass

3.1 Aims and Objectives

As reed canary grass (RCG) (*Phalaris arundinacea*) is the plant used throughout this study this chapter intends to provide a comprehensive review of literature associated with the plant species. This is followed by an evaluation of its interaction with PTE in soils contrasted with two lignocellulosic plant species that are used in the biofuel industry. The aim of this chapter was to gain an understanding of the interaction between RCG and PTE in soils. This chapter also aims to discover how RCG compares to other plants, in terms of interaction with soil PTE, which are used for the same purpose i.e. ligno cellulosic bio energy crops.

Specific objectives were:

- A comprehensive literature review of the reported concentrations of PTE found in RCG biomass grown on or with industrially impacted land.
- A comparison between soil concentrations and RCG biomass concentrations of PTE found in review of literature
- A selective literature review of the reported concentrations of PTE found in SRC Willow and Miscanthus biomass and the impacted soils the plants were grown in
- A comparison between the results found for the three plants in order to gain an understanding of whether RCG is a more or less suitable option for phytoremediation when compared to alternatives.

3.2 Reed canary grass

3.2.1 Botany

Reed canary grass is a temperate perennial C3 grass species with a distribution throughout most of the northern hemisphere (Casler, 2010). As the grass species is broadly adapted to many stresses such as flooding, drought, freezing, and grazing, it can be found in a wide array of habitats which include wetlands, riparian zones, stream banks, irrigation channels, roadsides, forest margins, pastures, and disturbed areas (Lavergne and Molofsky, 2004; Casler *et al.*, 2009).

Phalaris arundinacea is one of 21 species in the *Phalaris* genus, is the most cultivated and the most suited from cooler wetter climates. Reed canary grass is wind pollinated and is also unable to self-pollinate due to a self-incompatibility system promoting cross-pollination between individual populations (Carlson *et al.*, 1996). This affinity for cross-pollination has resulted in a high genotypic and phenotypic variability between individual populations (Casler *et al.*, 2009; Jakubowski *et al.*, 2014). Jakubowski *et al.*, carried out DNA marker analysis on 23 cultivars and 83 wild accessions of RCG collected throughout Eurasia and found seven subpopulations were present in Europe with a high level of admixture between the populations (Jakubowski *et al.*, 2011). In Northern Europe RCG is considered to have poor establishment from seed dispersal despite each inflorescence (the complete flower/seed head of a plant including stems, stalks, bracts, and flowers/seeds) of the plant producing approximately 600 seeds, due to competition from dense foliage. Therefore, the majority of establishment is through rhizomes and rhizome fragments for which the most prominent vector of transportation is in water (Anderson *et al.*, 2008). Both the rhizomes and the seeds have dormancy functions which enable them to live for an extended period of time (Anderson *et al.*, 2008).

3.2.2 Description and growth

Reed canary grass is a stout, perennial reed which can reach heights of 0.6 – 2 meters with a far reaching rhizome (Fernald, 1950). The leaves, which are flat and smooth and tapered, grow 10-35cm long and 5-25mm wide and so are approximately 20 times long as they are wide (Welsh *et al.*, 1987). Leaves are alternate and evenly spaced along the stem, they turn a bleached tan colour and persist through winter. The stem of the plant is erect, non-branching with a diameter of up to 13 mm and some reddish coloration at the top. Stem sheaths are hairless with thin with translucent edging (Waggy, 2010). The ligule (the membrane where the leaf joins the sheath) is approximately four to nine mm long and often folded over (Fernald, 1950). The collar (the outer junction between the blade and sheath) is prominent and yellowish. The entire upper vegetative portion of this RCG fades to a pale colour during the dormant winter season (Arny *et al.*, 1930). The panicle is lobed with a narrow oval shape that tapers to a point at each end approx. 7-40cm long and 1-4cm wide with branches that spread during flowering (McAnnich, 2000; Lavergne and Molofsky, 2004). Apfelbaum and Sams (1987), state that RCG is a highly variable species and that different individual populations can vary in height, size, colour, and the shape of the flower head (Apfelbaum and Sams, 1987). The discussed parts of a RCG plant are shown in Figure 6.

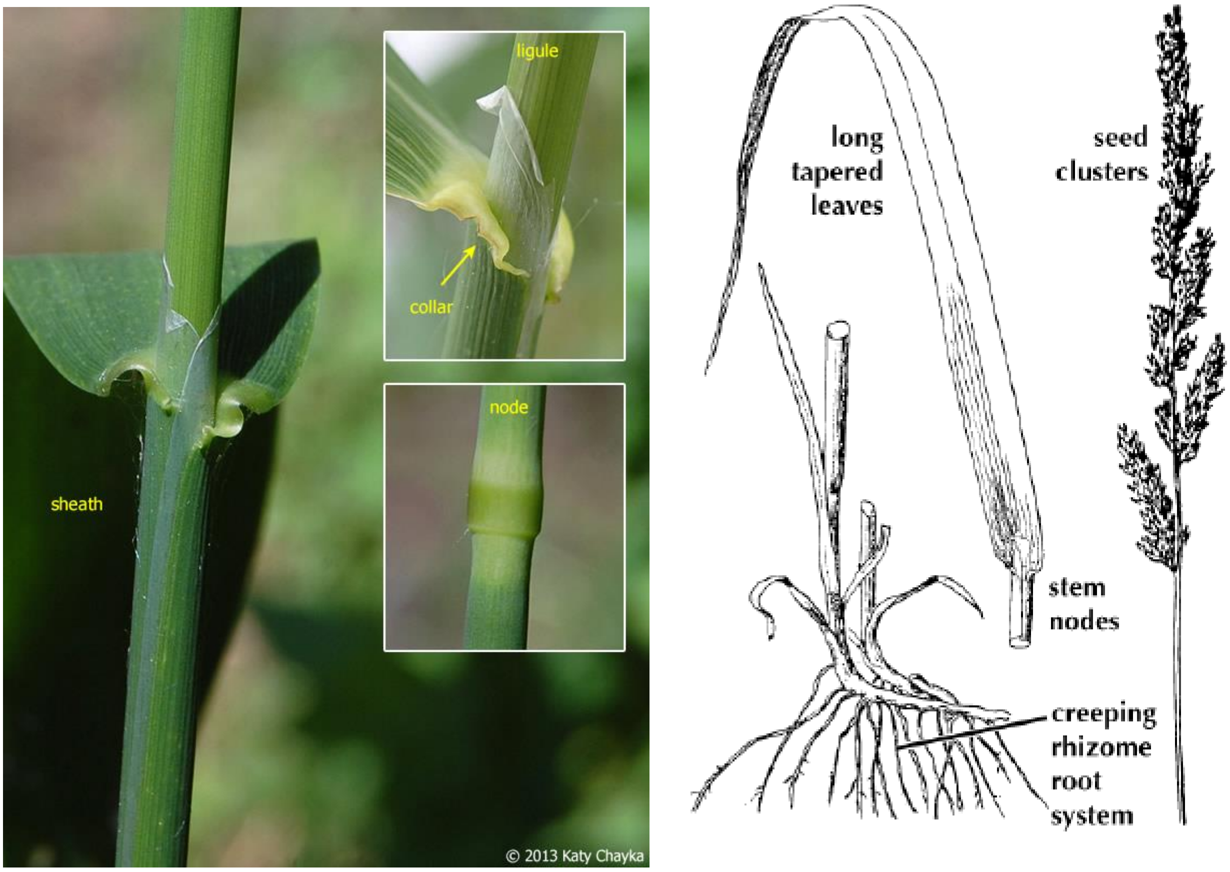
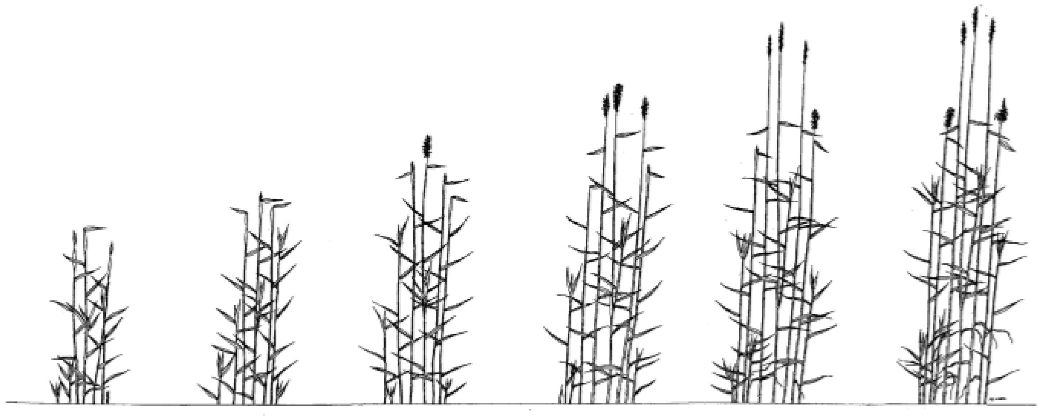


Figure 6 Annotated diagram and image showing botanical aspects of reed canary grass (King Country Council, 2021; MinnesotaWildflowers, 2021)

Initial plant growth for RCG occurs in the spring (Hutchison, 1992). Following over ground shoot development, a seed head grows vertically, maturing in summer (Hutchison, 1992). The flowers form in dense clumps and are initially a green/purple colour changing to a beige. Heide (1994), found that flowering requires exposure to shorter day-length conditions (spring) for primary floral induction and long day-length conditions (13-15 h summer) for secondary induction (Heide, 1994) . When seed heads are fully ripened, they shatter, dispersing small, flat and shiny seeds with an approximate individual weight of a 0.001g (Lewandowski *et al.*, 2003). Figure 7 shows six developmental stages for RCG as determined by a Finish study which aimed to describe variation in RCG development, height and stem elongation among local and commercial populations (Sahramaa and Jauhiainen, 2003).



Code	1	2	3	4	5	6
Description	Flag leaf emerged	Inflorescence visible	Inflorescence emerged	Beginning of anthesis	Completed anthesis	Seed ripened
Remarks	Flag leaf ligule visible	Upper 1 to 2 cm of inflorescence visible	Stem below inflorescence visible	Branches of the inflorescence open (from top to bottom), anthers visible	Branches of the inflorescence closed along the rachis	Inflorescence and stem below turning yellow, seeds fully matured, possible shattering started

Figure 7 Description of the stages of development of reed canary grass (Sahramaa and Jauhiainen, 2003)

The high productivity periods of growth for RCG occur in the late spring and late summer. The spring growth concerns the development of the leaves and inflorescence and the summer growth period involves the growth of the rhizomes and stem. Anthesis (the flowering period of a plant) tends to take place at mid-summer (June-July) in Northern Europe and is followed by seed ripening and then shattering (dispersing) as is shown in stages four to six in fig 4 (Sahramaa and Jauhiainen, 2003). Shoots that develop in the spring die in within that year but the shoots that develop in the Autumn have been found to persist throughout the winter senescence period (Clapham *et al.*, 1990). The rhizome of RCG grows vigorously up to 3m from the centre producing shoots from terminal buds allowing for the spread of the plant

(Lewandowski *et al.*, 2003). During plant senescence upright hollow stems remain which are typically 2 m high and 5 mm in diameter (Jensen *et al.*, 2018a).

3.2.3 Establishment

3.2.3.1 Natural

Reed canary grass has the ability to establish and grow naturally within a large hydrological range but generally favours moist to inundated soils and has been used as an indicator of soil moisture (van Strien and Melman, 1987). Although predominantly present in wetlands, RCG has been found to be resistant to drought conditions establishing itself in dryer, low grade soils which contain less nutrients such as mountainous regions (Strašil, 2012). RCG can withstand flooding for long periods of time even at large pH range (4.9 - 8.2) (Carlson et al., 1996). Bankhead et al., found that RCG was able to withstand a 100-year flood discharge event showing uprooting resistance to both bending and flowing water following just one year of growth (Bankhead et al., 2017). Analysis of the roots from Bankhead et al., study found that the RCG rhizome consisted of a fibrous root structure (shown inFigure 5) which was predominantly contained within the first 0.3m of the soil.

Figure 8 Images of the rhizome structure of reed canarygrass plants, showing the thick mat of roots growing >30 cm below the surface (Arny *et al.*, 1930; Citizens for Conservation, 2011; Bankhead *et al.*, 2017)**.**

3.2.3.2 Agricultural

Historically, RCG was grown as a forage crop in Europe but poor palatability due to the presence of alkaloids led to low intake by ruminants (Marten, 1973; Marum *et al.*, 1979). RCG was still considered a crop worth developing due to its vigorous growth and ability to establish a monoculture, in a four-year experiment in the 1930s, RCG produced 30% more hay than all other grasses tested (Wilkins and Hughes, 1932). As a crop, RCG can be planted in either spring or late summer. Seed can be either drilled, broadcast or the establishment can be achieved using no-till techniques (Leep *et al.*, 2003; Lewandowski *et al.*, 2003).

During an experiment to compare six perennial forage crops Buxton and Wedin (1970) found that RCG seedlings grew very slowly and that the establishment rate was a long, slow process (Buxton and Wedin, 1970). The work of Casler *et al*., confirmed this, they found that even increased seeding rates have little effect on short-term establishment of RCG under competitive conditions with annual weeds (Casler *et al*., 1999). Sheaffer and Marten found that the biggest impediment to the successful establishment of an RCG is competition from weeds (Sheaffer and Marten, 1995). Due to the slow establishment rates, maximum production of RCG is often not achieved until the second or third year of production with spring seeding being the more effective option for reducing the yield production lag (Sheaffer and Marten, 1995).

Seeding rates for RCG differ depending on the type of crop with 6-9 kg/ha (Bittman *et al.*, 1988) reported for forage production and 11-20 kg/ha for bioenergy production (Lewandowski *et al.*, 2003). Sowing is typically followed by rolling to conserve moisture in the seedbed and encourage soil/seed contact, a limiting factor to germination for RCG (Lewandowski *et al.*, 2003). Light quantity and quality has also been found to be a limiting factor to RCG seed germination(Hoffman *et al.*, 1980). A study on the effect of light by Lindig-Cisneros and Zerlder (2001) aimed to clarify the role of light on RCG development. They found a 1.22% germination rate for RCG seed when kept in the dark and a 42% rate for seeds exposed to a variety of other light wavelengths (Lindig-Cisneros and Zedler, 2001). White light was found to be the most proficient for seed germination and the researchers linked this with the plants ability to rapidly colonize following vegetation disturbance and thus inability to compete well with weeds, which requires photosynthesis with red light (Lindig-Cisneros and Zedler, 2001). This is due to the plants C3 photosynthetic pathway (Kephart and Buxton, 1993; Carlson *et al.*, 1996). Waggy (2010), which suggest that seed germination rates may improve after undergoing a period of dormancy, at least through the winter (Waggy, 2010)

3.2.4 Breeding and selection history

As RCG has been predominantly used in traditional agriculture as a feed and fodder stock the breeding objectives associated with the species have focused on seedling vigour and establishment capacity and seed retention (Casler, 2010). Reed canary grass has also been selectively bred over the past 50 years for a reduction in alkaloid content. Alkaloids function as a defence against insect predation but were found to be suppressing palatability, intake, and live weight gains of ruminant livestock (Marten, 1973). The alkaloids associated with RCG (tryptamines, β-carbolines, and gramine) have since been mostly eliminated from modern varieties of RCG and cultivars with low-alkaloid content have become so dominant in the marketplace that the alkaloid containing cultivars can only be found in gene banks (Casler, 2010). There is still a lot of scope for further development of RCG (Lindvall, 1997; Brummer *et al.*, 2000). For example, Lindvall evaluated 16 wild populations of reed canary grass growing in Sweden and found that many had yields greater than the standard commercially available varieties.(Lindvall, 1997). Brummer *et al*., evaluated seven cultivars of RCG (Palaton, Venture, Vantage, PSC1142, Rival, Bellevue, and Common) and found that as dry matter biomass yields ranged considerably, higher yields were potentially possible than those found in current commercially available cultivars (Brummer *et al.*, 2000). The current commercially available cultivars of RCG remain those that were developed for traditional agriculture and also soil conservation (erosion control) applications (Casler, 2010; Jensen *et al.*, 2018a).

3.2.5 RCG and phytostabilisation

Plant characteristics ideal for phytostabilisation include a tolerance to biogeochemical site conditions, a tolerance for PTE in soils, the ability to not translocate those PTE and to quickly establish a fine binding network of rootlets capable of stabilising gravelly/sandy soil. Reed canary grass has been shown to exhibit all of these traits (Evanylo *et al.*, 2005; Neuschütz and Greger, 2010b; Polechońska and Klink, 2014; Jensen *et al.*, 2018a). Further information on the phytostabilisation remediation technique can be found in the previous chapter.

A study in Poland showed that RCG was both tolerant to PTE and tended to not translocate contaminants to the above ground parts of the plant thus not creating a further mobilisation mechanism (Polechońska and Klink, 2014). Evanylo *et al.,* found that RCG is capable of forming a dense rhizome that can stretch over 3 m making it an ideal plant for a phytostabilisation remediation approach (Evanylo *et al.*, 2005). Neuschütz and Greger (2010b) applied RCG to sulfidic mine tailing as a phytostabilising remediation option and found that the presence of RCG in combination with a sludge and ash layer reduced the amount of leachate, potentially stabilising PTE and nutrient run off.

Two studies conducted by MSc students at the University of Strathclyde have focused on RCG grown on contaminated soils. Wishart (2019) analysed the above ground parts and roots of RCG growing naturally in a brownfield site with Pb concentrations of 269 – 827 mg/kg (Wishart, 2019). Wishart found that RCG exhibited high levels of resistance to the accumulation of Pb and other PTE (As, Cr, Cu and Ni) in biomass and a low translocation from roots to above ground biomass (Wishart, 2019). The results of a similar study conducted by Eccles supported these findings with Pb levels in above ground portions of RCG biomass below the limit of detection of the analytical technique used (ICP-OES) (Eccles, 2018). Both studies found greater levels of PTE in mature growth and relatively high biomass concentrations of Zn, suggesting that RCG has potential use as a Zn phytoextractor (Eccles, 2018; Wishart, 2019). Table 10 provides further evidence of RCG growth in environments impacted by PTE.

Table 10 Reed canary grass growth in PTE contaminated soils

Site/type of growing media/contamination source	**PTE concentration range of soils that have been used in RCG growth experiments (Pb and Zn)**	**Source**
Not disclosed UK / Soil/ Former industrial site impacted by PTE.	Pb 69.2± 38.3 mg/kg^{-1} Zn 82.8 ± 2.8 mg/kg^{-1}	(Badmos *et al.*, 2015)
Gillervattnet mine Boliden, Sweden/sulfidic mine tailings/ mining operations	Pb – N/A Zn 503 – 6560 mg/kg^{-1}	(Neuschütz and Greger, 2010a)
Bystrzyca River, Poland/ Sediments/Anthropegenic sources of PTE	RCG found growing in wild Pb 7.09 mg/kg^{-1} Zn 18.4 mg/kg^{-1}	(Polechońska and Klink, 2014)
Krakow/Municipal sewage sludge/Urban PTE wastewater contamination	Pb 13.63 – 42.9 mg/kg^{-1} Zn 31.00- 1005 mg/kg^{-1}	(Antonkiewicz *et al.*, 2016)

3.2.6 Biofuel

Biofuels from biomass has been identified as having a significant role in the decarbonisation of the transport industry both in the UK and globally in the short to medium term. The term biomass can refer to any solid or nonsolid biological energy source. Bioenergy has considerable sustainability challenges to overcome including the risk of diverting farmland for biofuel production to the detriment of food supply (Woods, 2008). This is known as indirect land use change (ILUC) and is critical due to lack of high quality agricultural land and increasing world population. In “Bioenergy to Save the World” Schroder *et al*. (2008) proposed the use of marginal lands, abandoned for reasons of degradation and/or pollution, for the production of energy crops (Schröder *et al.*, 2008). The use of these lands to grow bioenergy crops could increase the sustainability of bioenergy resulting in an increase in land available for agriculture and also remediation of degraded soils (Schröder *et al.*, 2018).

First-generation biofuels are used to make ethanol and biodiesel and are directly related to a biomass that has been grown on agriculture land. First generation bioenergy feed stocks include sugarcane, corn and sugar beet (Lee and Lavoie, 2013). As this study is focused on RCG, a second generation energy crop, no further attention will be given to first generation crops. Second generation energy crops include ligno cellulosic biomass (woody crops) and agricultural residues or wastes. Common ligno cellulosic energy crops include perennial grasses such as Miscanthus, *Arundo donax* and *Panicum virgatum* and short rotation coppice poplar and willow (Nanda *et al.*, 2018). Following pre-treatment to form cellulose these feedstock crops are all processed by either fermentation, gasification or pyrolysis to make biofuels such as bio methane, syngas or pyro-gas (Nanda *et al.*, 2018). This study has selected Miscanthus and SRC Willow as relevant ligno cellulosic energy crops to compare with RCG given their similar processing requirement and current usage within the UK (DEFRA, 2001, 2004)

3.2.7 Reed canary grass and biofuel

Interest in reed canary grass as a perennial grass bioenergy crop has grown considerably in recent years, especially in Northern Europe (Strašil *et al.*, 2005; Wrobel *et al.*, 2009; Smith and Slater, 2010; Strašil, 2012; Lord, 2015a; Kołodziej *et al.*, 2016; Šiaudinis *et al.*, 2021). This interest is due to several characteristics of the species; it grows well on marginal land reducing competition with food and feed crops, it is tolerant to PTE, highly persistent, produces a relatively high biomass yield, is cost competitive with similar crops e.g. can be grown from seed unlike Miscanthus, is carbon efficient, has both superior drought and water-logging tolerance and can adapt to a wide range of soil types, habitats, and management systems (Lewandowski and Schmidt, 2006; Pahkala *et al.*, 2008; Wrobel *et al.*, 2009; Neuschütz and Greger, 2010b; Lord, 2015a; Kołodziej *et al.*, 2016; Mårtensson *et al.*, 2017a; Meehan *et al.*, 2017; Jensen *et al.*, 2018a; Krol *et al.*, 2019; Usťak *et al.*, 2019).

Lord found, in a field-scale trial on five brown field sites RCG outperformed both SRC willow and *Miscanthus x giganteus* in establishment, tolerance, biomass yield, persistence and had lower biomass concentrations of Cd, Cu, Ni and Zn (Lord, 2015a). Krol *et al*., found during a field trial concerning land use change from grassland to bioenergy crops and N_2O emissions, that whilst the RCG crop did result in an initial rise in N_2O the emissions were offset by the future reduced fertilisation, carbon sequestration and the use of the produced bioenergy feedstock (Krol *et al.*, 2019). The study also found that RCG produced seven times less N_2O emissions than Miscanthus. Meehan *et al*., (2017) grew RCG on three marginal land sites (very wet site, very dry site, site prone to flooding) and a control site with a highly productive tillage soil. They found that the biomass harvests from the marginal lands were similar to that of the control with the very wet site producing the lowest yield (15% lower yield than the control) (Meehan *et al.*, 2017). Koloziej *et al*., (2016) harvested RCG with three different management systems: a one-harvest management system (in autumn), a two-cut regime (in spring and autumn), and a three-cut regime (in spring, summer, and autumn) in order to determine which option provided the best yielding potential. They found that single harvesting run in late autumn provided the highest yields followed by the two-cut system (Kołodziej *et al.*, 2016). These findings are complementary to those of Burvall (1997) who found that delaying harvesting of RCG as long as possible improves the fuel quality for both combustion and gasification (Burvall, 1997). This suggests delaying harvesting provides a better bioenergy crop and a greater yield. Burvall recommends harvesting at the end of winter when the critical elements that cause fouling and corrosion problems in biofuel boilers during combustion (e.g. chlorine) are reduced by a factor of two to six.

Reed canary grass crops have been found to be suitable for; combustion, anaerobic digestion, pyrolysis, gasification, and cellulosic ethanol production which provides a potential for RCG bioplastic production (Burvall, 1997; Kacprzak *et al.*, 2012; Oleszek *et al.*, 2014)

Oleszek *et al*., (2014) analysed wild and cultivated RCG for their efficiency in biogas production in the methane fermentation process of silages. The cultivated RCG was found to produce almost four times greater biogas yield as the uncultivated and this was attributed to the higher content of indigestible crude fibre fractions and ash content of the uncultivated plants. (Oleszek *et al.*, 2014). It is clear that RCG is likely to play an important role in the future of bioenergy crops and thus there need for further research and accession development.

3.2.8 Breeding RCG for biofuels

Cultivation for energy purposes has now become the focus of breeding selecction for reed canary grass. Existing cultivars of RCG have been described as “suboptimal for the development and production of dedicated bioenergy feedstock’s” (Jensen, 2019). Many of the ‘wild’ accessions obtained by recent exploration trips in Canada, the United States, and northern Europe, demonstrated superiority over existing cultivars with relatively little effort from breeding and selection (Sachs and Coulman, 1983; Lindvall, 1997; Olsson, 1999; Sahramaa, 2003, 2004; Casler *et al.*, 2009).
Ironically some of the cultivar selection has involved looking for conflicting traits to those initially selected for agriculture uses (Casler, 2010). For example, as alkaloids function to protect plants from insect predation and alkaloids are not an issue for biomass conversion, the use of genotypes with high alkaloid profiles could be a necessity for developing dedicated bioenergy feedstock’s naturally resistant to pests (Casler, 2010). Oleszek *et al*., found that cultivated varieties of RCG produced greater biogas yields than ‘wild’ varieties due to a lower indigestible fraction of crude fibre which can reduce biogas quantity and quality, suggesting the potential for further development of RCG cultivars for biogas (Oleszek *et al.*, 2014).

A recent EU funded Bioenergy project named "Optimisation of Reed Canary Grass as a Native European Energy Crop" (ORNATE) involved a collaboration between the United Kingdom and Sweden to investigate different cultivars and wild accessions of RCG. The project looked at over 60 accessions and varieties of RCG and found significant differences in dry weight yield which are shown in Figure 9. Both Swedish (SWRF) and English varieties (BS) bred for use in the biofuel industry outcompeted previously developed varieties (e.g. BAMSE) (ORNATE, 2017).

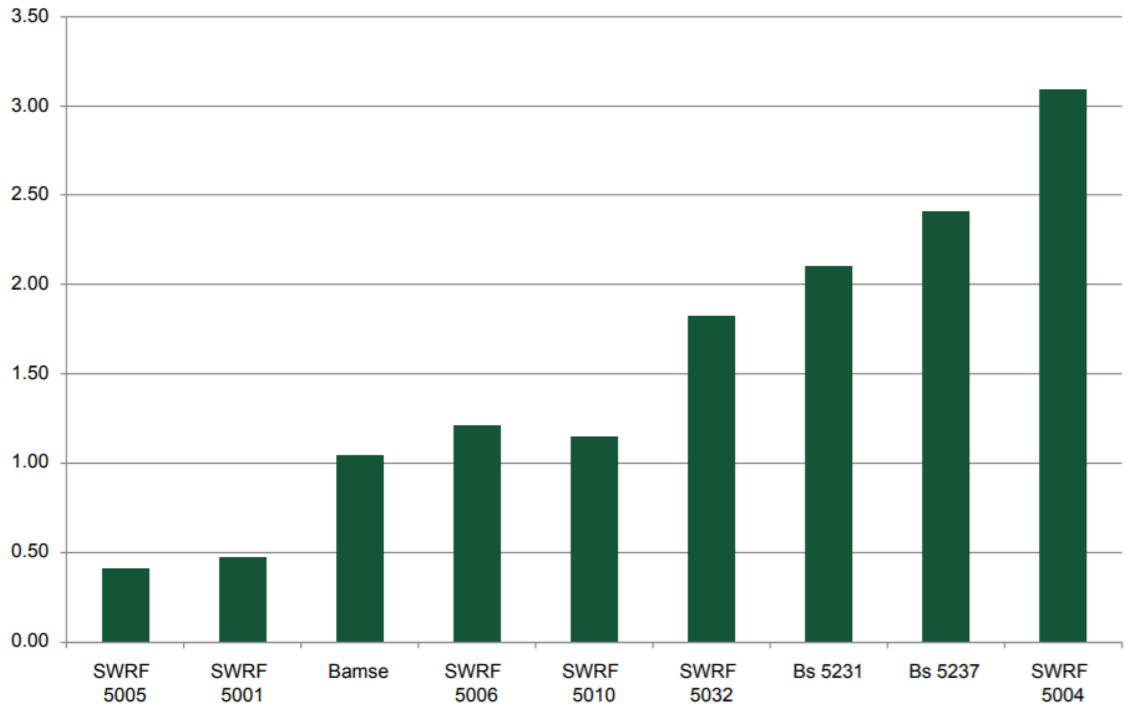


Figure 9 Dry weight yields (t/ha) from green harvest of RCG varieties grown during the ORNATE project **(ORNATE, 2017)**

Future breeding objectives for RCG as a bio energy crop are likely to focus on reduced lignification, reduced halogen (Cl) ash (Si and K) concentrations, lodging resistance, taller plants, reduced leaf area, high straw fraction and greater biomass yield (Lindvall, 1997; Sahramaa, 2003; Boateng *et al.*, 2008).

3.2.9 Miscanthus

Miscanthus species are a tall woody, high-yielding, non-food perennial grasses, capable of fast growth (reaching heights of 2.5–3.5 m in a single year) which are considered a promising biomass crop for energy and bio-based products (Lewandowski *et al.*, 2000; DEFRA, 2001; Rowe *et al.*, 2009; Nsanganwimana *et al.*, 2014). Although *Miscanthus sp.* origins are in East Asia, a natural hybrid, named *Miscanthus × giganteus (*a natural hybrid of *Miscanthus sinensis* and *M. sacchariflorus*) is generating enthusiasm in Europe as a biomass crop for its high yields and cold tolerance (Varnero *et al.*, 2018). As *Miscanthus × giganteus* is a sterile hybrid it does not produce viable seed and therefore is propagated through rhizome division or micropropagation, with rhizome division being the favoured method (DEFRA, 2001; Varnero *et al.*, 2018). *Miscanthus × giganteus* is capable of growing in various climates and can adapt to a wide range of soil conditions including marginal lands. Miscanthus is a C4 grass species, meaning it prefers warmer and dryer conditions when compared to a C3 species such as RCG.
As a bioenergy crop, *Miscanthus × giganteus* has been received well by agriculturalists in the UK as it has been found to thrive on poor soils, require few farming operations during growth, does not require specialised machinery and has a high water and nutrient efficiency when compared with other arable crops (McCalmont *et al.*, 2017; Shepherd *et al.*, 2020). Rhizomes are planted at a density of around 20,000 plants per hectare and harvested annually in late winter following senescence, individual plantations remain viable for at least 15 years (DEFRA, 2001). Yields from experimental plots within the UK have exceeded 13 dry tonnes ha/yr with the biomass being utilized in either dedicated biomass plants or for co-firing with coal (DEFRA, 2001; Rowe *et al.*, 2009).

McCalmont *et al*., (2017) found that the organic N in harvested material taken from a Miscanthus harvest of 10-15 t DM/ha would range from 49 to 73.5 kg N/ha, which is not in excess of the atmospheric N deposition rate of 35 – 50 kg ha/year found by Goulding *et al* (Goulding *et al.*, 1998; McCalmont *et al.*, 2017). Therefore Miscanthus is unlikely to benefit from high inputs of N fertilizer, resulting in far lower N_2O emissions when compared to annual crops and intensive pasture farming (Shepherd *et al.*, 2020). Recent modelling suggests that *Miscanthus x giganteus* could provide approximately 5% of global bioenergy requirement by 2090 (Shepherd *et al.*, 2020) Korzeniowska and Stanislawska-Glubiak (2015) grew Miscanthus in soils spiked with Cu, Ni and Zn in the following doses in a year-long pot trial: 100 200 400 Cu mg/kg, 60 100 240 Ni mg/kg and 300 600 1200 Zn mg/kg (Korzeniowska and Stanislawska-Glubiak, 2015). They found that whilst the grass species would not translocate considerable concentrations of Cu or Ni, Zn was absorbed into the above ground biomass particularly in the first of two harvests.

Leung *et al*., grew Miscanthus at a historic mine site in China as part of an experiment looking at arbuscular mycorrhiza fungi (Leung *et al.*, 2007). They found that the plant was sensitive to the physiological effects of metal toxicity absorbing relatively high concentrations of As, Pb and Zn when compared to soil concentrations. Wang et at., also carried out a field trial in China using Miscanthus and found very similar results regarding soil/biomass concentrations of Pb and Zn (Wang *et al.*, 2012b). The results of a field trial in Romania do not support these findings however, as Barbu *et al* found only 11.4 mg/kg of Pb in Miscanthus biomass grown at a smelter site with soil concentrations of 803 mg/kg Pb (Barbu *et al.*, 2013). A study in France found similar findings at a smelter site field trial where the Miscanthus biomass was found to have low concentrations of PTE (Cu 8.2, Ni 2.6, Pb 12.7 and Zn 37.6 mg/kg) whilst the soil concentrations were relatively high (Cu 870, Ni 71, Pb 2200 and Zn 1700 mg/kg). Similarly conflicting results were reported in two field trials, both at smelter sites in Poland. Kacprzak *et al* . (2014), found that Miscanthus biomass concentrations of Cd, Cr, Cu, Ni, Pb, Zn were similar to soil concentrations suggesting the absorption of PTE where as Krzyzak (2017) found that Miscanthus did not translocate high concentrations of PTE relative to soil concentrations (Kacprzak *et al.*, 2014; Krzyżak *et al.*, 2017). At a field trial on a historic steel mill site in Poland Fijalkowshi *et al*., found very low uptake of PTE in Miscanthus biomass when compared to soil concentration (Fijalkowski *et al.*, 2018)

3.2.10 Short rotation coppice willow

Willows are part of the Salicaceae tree family, which undergo C3 photosynthesis and are characterised by high growth rates, CO_2 exchange rates and light use efficiency (Karp and Shield, 2008). Short-rotation coppice (SRC) willow refers to a coppicing cycle of 2-5 year intervals which takes place in winter following leaf fall (Dou *et al.*, 2017). In SRC single stems are cut back to ground level to encourage the production of multiple stems, resulting in the development of dense plantations of multi-stemmed willow stools (DEFRA, 2004; Rowe *et al.*, 2009). This method has been found to reinvigorate the plant, accelerating growth resulting in greater yields (Sennerby-Forsse, 1995). This is due to the established root system of the plant and the nutrients stored in the roots and stumps which guarantee vigorous growth for the shoots in the spring (Dou *et al.*, 2017).

SRC willow is a well-established biofuel with solid biomass currently being used in applications such as district heating and electric power generating stations. Typically, the SRC willow species chosen are varieties of *Salix viminalis* which can be planted at a sampling density of over 15, 000 per hectare (DEFRA, 2004). SRC willow has been found to have many economically favourable characteristics when grown as a biofuel crop such as low maintenance requirement, a propagation technique that requires only unrooted stem cuttings and a tolerance to a wide range of soil types from heavy clay to sand including land reclaimed from gravel extraction and colliery spoil (Sennerby-Forsse, 1995; DEFRA, 2004; Dou *et al.*, 2017). Plantations of SRC willow have been found to yield 7 -18 tonnes of dry biomass ha/yr depending on site and efficiency of establishment and can be harvested for up to 20 years before requiring replanting (DEFRA, 2004; Dou *et al.*, 2017). Harvested material is chipped and dried for use in either dedicated biomass burners or for co-firing with coal (Rowe *et al.*, 2009). Some of the constraints of SRC willow biofuel production are that the crop does require specialised planting and harvesting machinery, has a higher water consumption than agricultural crops and does not grow well on compacted soils due to poor root development. (DEFRA, 2004; Rowe *et al.*, 2009).

Meers *et al*., (2007) conducted a pot trial using multiple salix cultivars (*Salix purpurea × Salix daphnoides, Salix schwerinii Salix dasyclados Salix triandra and Salix fragilis)* in soils collected from a historic smelter site. They found that all willow cultivars were capable of translocating Cu, Ni and Zn into the above ground biomass and excluding Pb. Greater concentrations of Zn were found in the willow biomass (1000 - 2900 mg/kg) than in the soil (275 mg/kg) suggesting that the willow plants are capable of bio accumulating the element. High concentrations of Zn and the exclusion of Pb in willow biomass have been reported elsewhere. For example Jensen *et al*., found greater concentrations of Zn were found in willow (*Salix viminalis*) leaf biomass (3500 mg/kg) than in the soil (3000 mg/kg) whilst also no Pb was extracted at a field trial in soils impacted by a recycling centre in Denmark (Jensen *et al.*, 2009). The stem of the plant in this experiment was found to contain 1000 mg/kg Zn.

Greater concentrations in willow leaves rather than woody parts of the plant have been reported elsewhere. For example, Vamerali *et al*., (2009) conducted a pot trial using soils from a pyrite extraction site in Italy with a concentrations of 886 mg/kg As, 1735 mg/kg Cu, 493 mg/kg Pb and 2404 mg/kg Zn (Vamerali *et al.*, 2009). The study found that whilst the willow species S*alix alba* excluded As, Cu and Pb, concentrations of 78 mg/kg Zn were found in the woody biomass and 510 mg/kg Zn in the leaves (Vamerali *et al.*, 2009). Similar findings, regarding leaf and wood biomass Zn concentrations, were reported by Vervaeke *et al*,. following a field trial using dredged sediments and *Salix viminalis* (Vervaeke *et al.*, 2003). A field trial conducted in the Czech republic using seven different cultivars of willow grown in flood plain impacted soils also found far greater concentrations of Zn in leaf material than in the woody biomass (Vysloužilová *et al.*, 2003), as did a similar study in Germany (Baum *et al.*, 2006)

One of the most comprehensive studies of different Willow cultivars grown in soils impacted by PTE was undertaken by Pulford *et al*., (Pulford *et al.*, 2002). This study grew twenty different varieties of willow over 2 years on soils impacted due to long-term sewage sludge disposal and showed that different cultivars of willow could have significant variation in survival, biomass production and the uptake of PTE. For example, some of the cultivars were found to have low Ni and Cu in the bark and high

Cd and Zn in the wood, with a good survival and biomass production rate. Some other cultivars were found to have relatively high Ni and Cu in their bark and low Cd and Zn in the wood. These cultivars performed badly and had lower survival and biomass production rates. Two of the cultivars did not survive the first harvest (Pulford *et al.*, 2002). This work highlights the variance within a species when it comes to reaction with PTE in the soil and suggests that much more research is required into cultivar selection or development for use on the great range of industrially impacted soils.

3.3 Experimental

In order to guarantee the review of published literature regarding concentrations of PTE found within RCG biomass was comprehensive three search engines were used. The search engines used were Web of science (www.webofscience.com) and Google scholar (www.scholar.google.com). In each search engine both 'Reed canary grass' or '*Phalaris arundinacea'* were searched for and the results were refined with each of the following key words in turn; Potentially toxic elements, PTE, heavy metals, contamination, brownfield land, phytoremediation, biofuels, phyto extraction, phyto stabilisation, mining, lead, zinc, copper, nickel. From these results, papers were selected if they contained both the corresponding soil and biomass concentrations of PTE.

An excel (Microsoft) spreadsheet was used to record the data found within the published studies found. The spreadsheet was used to record several data topics and included; the greatest and lowest concentrations of PTE reported in biomass, complimentary concentrations of PTE reported in soils or growing media, part of the plant analysed, soil type and depth which sample was taken, source of soil contamination, country in which the experiment was conducted, cultivar of species used in experiment, biomass washing method prior to analysis, analytical instrument used, plant harvesting/sampling time and whether the soil used in the experiment was amended, spiked or 'natural'. If the soils used in experiments were amended, then the type of amendment and concentrations of PTE in amended soils were recorded as final soil concentrations. Where multiple values were given within the same data set for biomass concentrations the greatest value for each relevant variable, such as ‘cultivar’, or soil type or amendment rate, was selected.

The selective literature review of Miscanthus and SRC Willow was completed in the same manner as the RCG review using the same key words. Data was recorded in the same way in a replica spreadsheet. As these two species have been studied in greater details and for longer than RCG, a comprehensive review would have been outside of the scope of a study of this nature. Therefore, the review of these two species focused on soils similar to the ones concerned with this study i.e. those impacted by mining or industrial activity with a similar level of impact on the concentration of PTE in soils, such as smelter sites. Similar reviews have been conducted regarding PTE concentrations in plants used for remediation but no published work exists that comprehensively explores reported PTE concentrations found in RCG and compares those to concentrations in plants with similar uses (Gerhardt *et al.*, 2017). The statistics programme SPSS (IBM) was used to analyse all of the data from the literature review.

3.4 Results

3.4.1 PTE concentration found in RCG within literature

Table 11 provides a summary of the data in the eleven published articles that are relevant to this study in regards to the concentrations of PTE found in RCG biomass when grown in soils impacted by industry.

Table 11 Reported concentrations of phytotoxic elements found in RCG biomass. Data reported in 11 journal articles several of which have reported multiple results (Smith and Slater, 2011; Strašil, 2012; Polechońska and Klink, 2014; Badmos *et al.*, 2015; Lord, 2015a; Antonkiewicz *et al.*, 2016; Rosikon *et al.*, 2016; Mayerová *et al.*, 2017; Fijalkowski *et al.*, 2018; Usťak *et al.*, 2019; Korzeniowska and Stanislawska-Glubiak, 2019)**.**

PTE	**Min concentration mg/kg**	**Maximum concentration mg/kg**	**Mean mg/kg**	**STDev ± mg/kg**	**Data # (n=)**
Cd	0.01	1.1	0.28	0.3	9
Cr	0.45	14.6	3.59	4.3	14
Cu	3.57	26.6	8.26	6.8	11
Ni	0.1	31.6	3.68	8.9	15
Pb	0.05	64.3	5.73	14.0	21
Zn	1.2	123	40.6	34.4	22

Concentrations of Cd found in the literature search were in the range of 0.01 – 1.1 mg/kg with a mean average of 0.28 ± 0.3 mg/kg. Cadmium was the least reported element in the studies found, 9 values were recorded. The concentrations of Cd all fall within the range 0.10–1.43 mg/kg reported by the UKSHS, as reported in chapter one, suggesting that RCG does not uptake Cd in quantities greater than the 183 plants tested in the EA survey (Environment Agency, 2007). Chromium concentrations ranged from 0.45 – 14.6 mg/kg and had a mean average of 3.59 ± 4.3 mg/kg. The found concentrations of Cr were in slightly excess of the concentrations reported in the UKSHS, which had a mean of 1.6 ± 1.58 mg/kg. Concentrations of Cu found in RCG biomass had a range of 3.57 – 26.6 mg/g with a mean average of 8.26 ± 6.8 mg/kg. As with Cr the Cu concentrations from the literature search were slightly in excess of those reported by the UKSHS which had a mean value of 7.22 ± 2.28 mg/kg. Concentrations of Ni in RCG biomass found in the literature search ranged from 0.1 – 31.6 mg/kg and had a mean average of 3.68 ± 8.9 mg/kg. The mean concentration for Ni found in the UKSHS was 1.72 ± 1.12 and the greatest plant biomass concentration of Ni found by the UKSHS was 8.97.

Concentrations of Pb ranged from 0.5 – 63.4 mg/kg and had a mean average of 5.73 ± 14.0 mg/kg. The range of result and mean average for Pb found in the UKSHS was lower at 1.0–19.1 mg/kg and 1.87 ± 2.11 mg/kg. Concentrations of Zn were found to have the broadest range from 1.2 – 123 mg/kg and had a mean average of 40.5 ± 34.4 mg/kg. Zinc was the element for which the concentrations reported were the greatest out of all the PTE in the studies found, with 22 values of Zn concentrations in RCG biomass were reported. The range and mean average of Zn concentrations found in the UKSHS were very similar to those found in the literature search. The range of Zn concentrations reported in the UKSHS was 14.7–102 mg/kg with a mean average of 33.6 ± 11.8 mg/kg.

The comparison between the literature search and UKSHS reveal that RCG interacts broadly in a similar way to other plants growing ‘wild’ in the UK. However, only a loose equivalence between the two data sets can be drawn without determining the soil concentrations. For example, the greatest concentration of Pb found in the RCG was higher by a factor of approximately three when compared to that of the UKSHS and may have been the result of far higher Pb soil concentrations than were tested in the UKSHS. The mean average results found in the literature search for RCG grown in contaminated soils for the micro nutrients Cu and Zn were quite similar to those for the UKSHS (Environment Agency, 2007).

3.4.2 Soil and RCG interaction in data from literature search

In order to gain a further understanding of how RCG interacts with impacted soils in terms of biomass accumulation or exclusion of PTE the reported soil concentrations found in the literature search were plotted against the biomass concentrations. Different PTE were separated or grouped based on similar concentrations found in soil and or biomass and presented in scatter diagrams with soil and biomass concentrations as the two variables. Figure 10 shows the concentrations of Cd in RCG biomass found in the published literature.

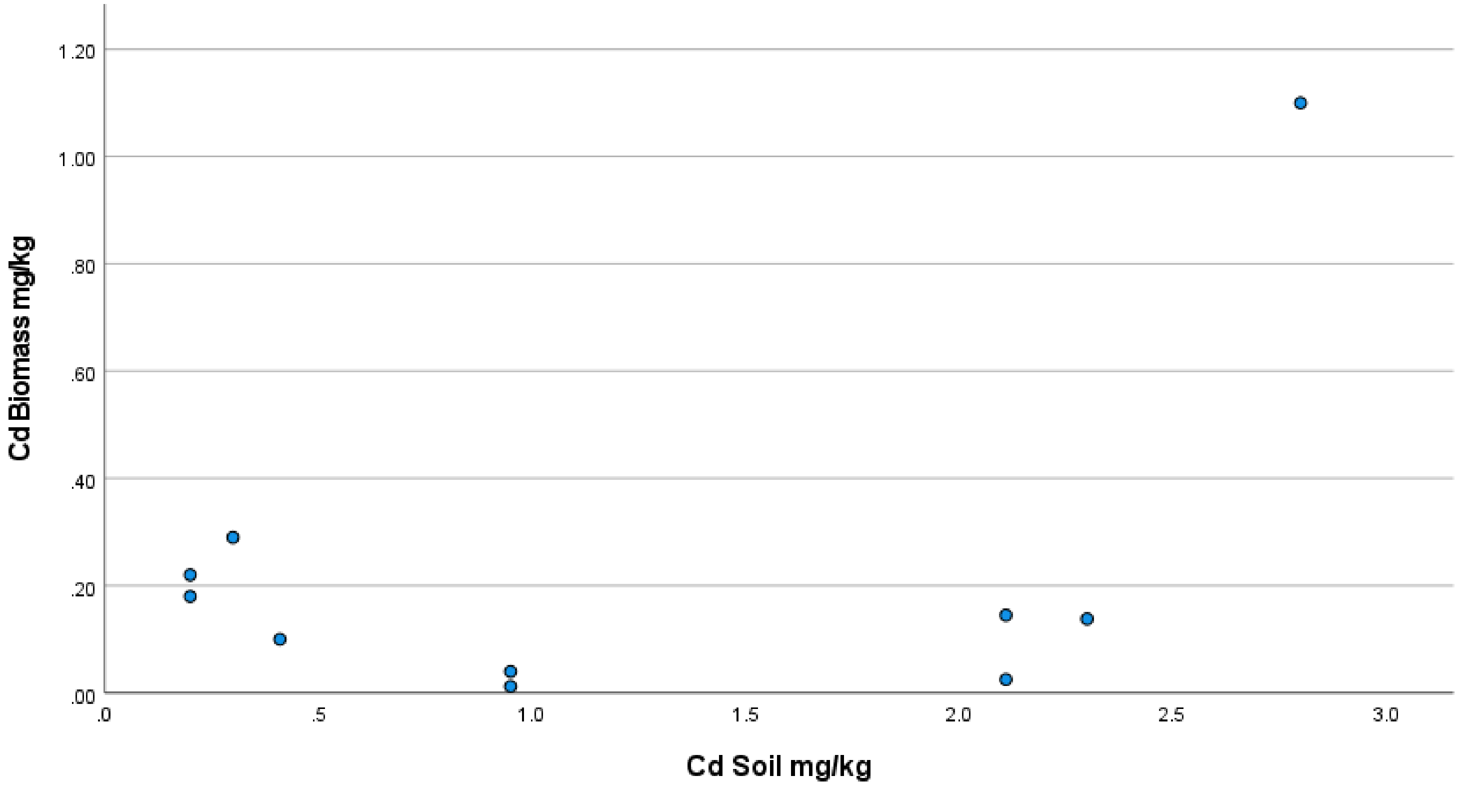


Figure 10 Concentrations of Cd in RCG biomass found in published literature and the corresponding reported soil concentrations. Data reported in 11 journal articles several of which have reported multiple results. (Badmos et al., 2015; Polechońska and Klink, 2014; Antonkiewicz et al., 2016; Lord, 2015; Korzeniowska and Stanislawska-Glubiak, 2019; Rosikon et al., 2016; Smith and Slater, 2011; Usťak et al., 2019; Strašil, 2012; Mayerová et al., 2017; Fijalkowski et al., 2018)

By looking at Figure 10 it is clear that within the limited range of the data set, no correlation was found between the Cd concentrations of biomass and soil found in the literature on RCG. Cadmium's availability to plants has been found to vary by up to a factor of 10 in different soils and is considerably influenced by pH (Smolders and Mertens, 2013b). It is therefore, unlikely to find a linear relationship in a small data set that contains analysis RCG plant and soil material from a broad range of sites. It is interesting to note that three of the data points represent RCG that was grown in some of the least impacted soils but have some of the greatest Cd biomass concentrations. As geogenic soil concentrations of Cd are typically 0.1-1 mg/kg worldwide, this indicates that anthropogenic Cd maybe less bioavailable, at least to RCG (Smolders and Mertens, 2013b). The data point with the greatest Cd concentrations (biomass 1.1 mg/kg soil 2.8 mg/kg) was from RCG biomass taken from a three year phytoextraction field trial on a Cd-Zn impacted smelter site in which RCG was found to translocate far less Cd than the majority of the 14 plant species tested (Mayerová *et al.*, 2017).

Figure 11 shows the concentrations of Cu, Ni and Pb in RCG biomass found in published literature and the corresponding reported soil concentrations. Copper shows an apparent positive correlation between soil and biomass at lower concentrations. Although one data point has a far greater amount of Soil Cu than the others (293 mg/kg) the concentrations of Cu within the biomass does not exceed the greater value of 11 mg/kg found at soil concentrations of 53 mg/kg. Copper is typically absorbed into plant life at a concentration of 6 mg/kg (Marschner, 1986; Kirkby, 2012).This suggests that there may be a threshold for Cu accumulation for RCG. Copper concentrations generally range between 4-15 mg/kg in plant shoots and Cu has been found to have little effect on plant physiology below 200 mg/kg (Oorts, 2013). It could be the case that RCG is able to exclude Cu at low soil concentrations limiting its bioaccumulation in the biomass.

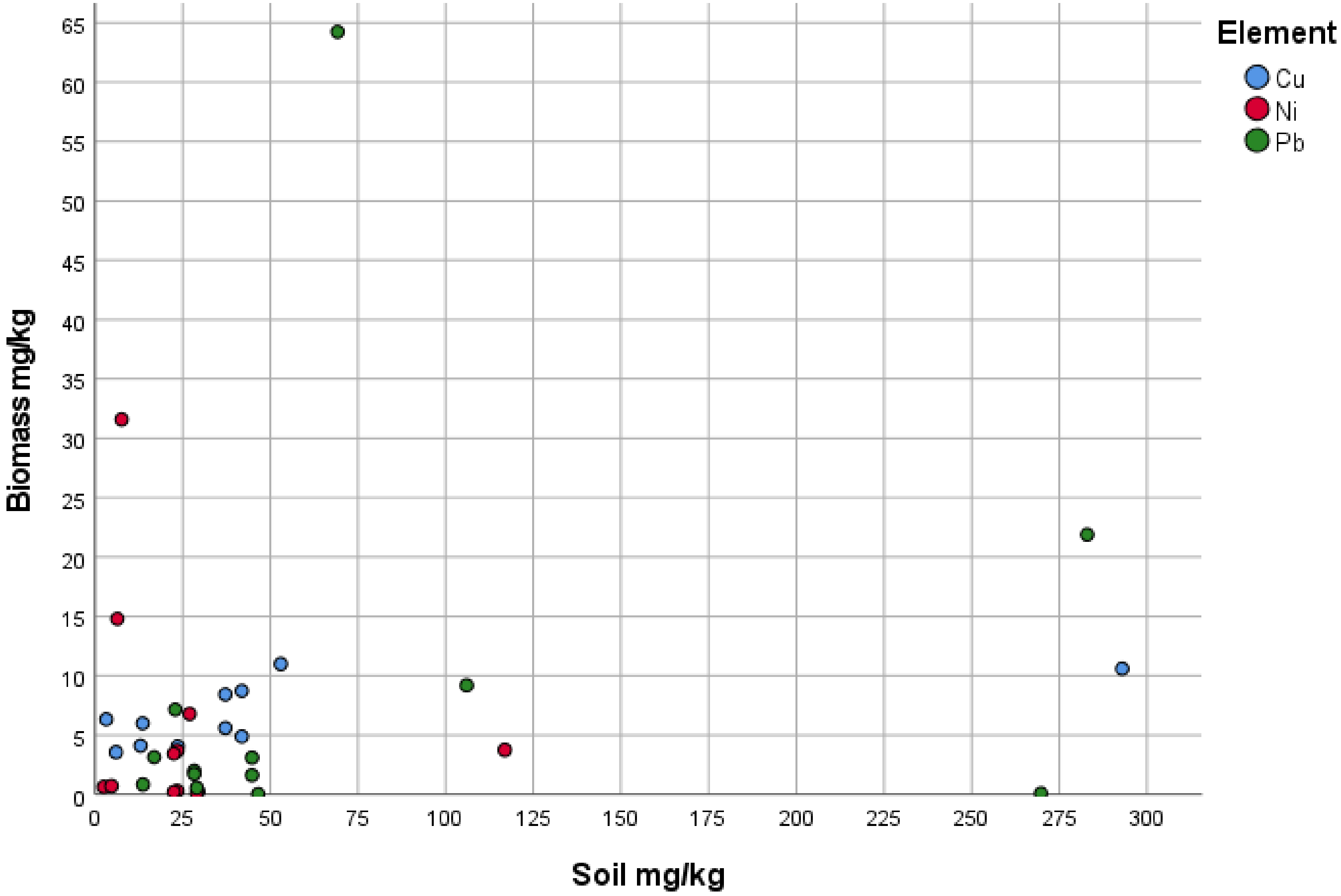


Figure 11 Concentrations of Cu, Ni and Pb in RCG biomass found in published literature and the corresponding reported soil concentrations. Data reported in 11 journal articles several of which have reported multiple results. (Badmos et al., 2015; Polechońska and Klink, 2014; Antonkiewicz et al., 2016; Lord, 2015; Korzeniowska and Stanislawska-Glubiak, 2019; Rosikon et al., 2016; Smith and Slater, 2011; Usťak et al., 2019; Strašil, 2012; Mayerová et al., 2017; Fijalkowski et al., 2018)

The relationship between soil and biomass concentrations of Pb is less strong than that for Cu but is moderately linear with a couple of outliers. The majority of the soils tested in the studies reviewed had relatively low levels of Pb, the mean content of non-industrially impacted soils has been found to be 17 mg/kg globally and more than half of the data set has soil concentrations of < 50 mg/kg Pb (Nriagu, 1978). This is a clear indication of a research gap concerning RCG and Pb impacted soils. No relationship between soil and biomass concentrations of Ni was found. The RCG biomass and soil concentrations of Zn were greater than any of the other PTE found in the literature and so the data is presented separately in Figure 12.

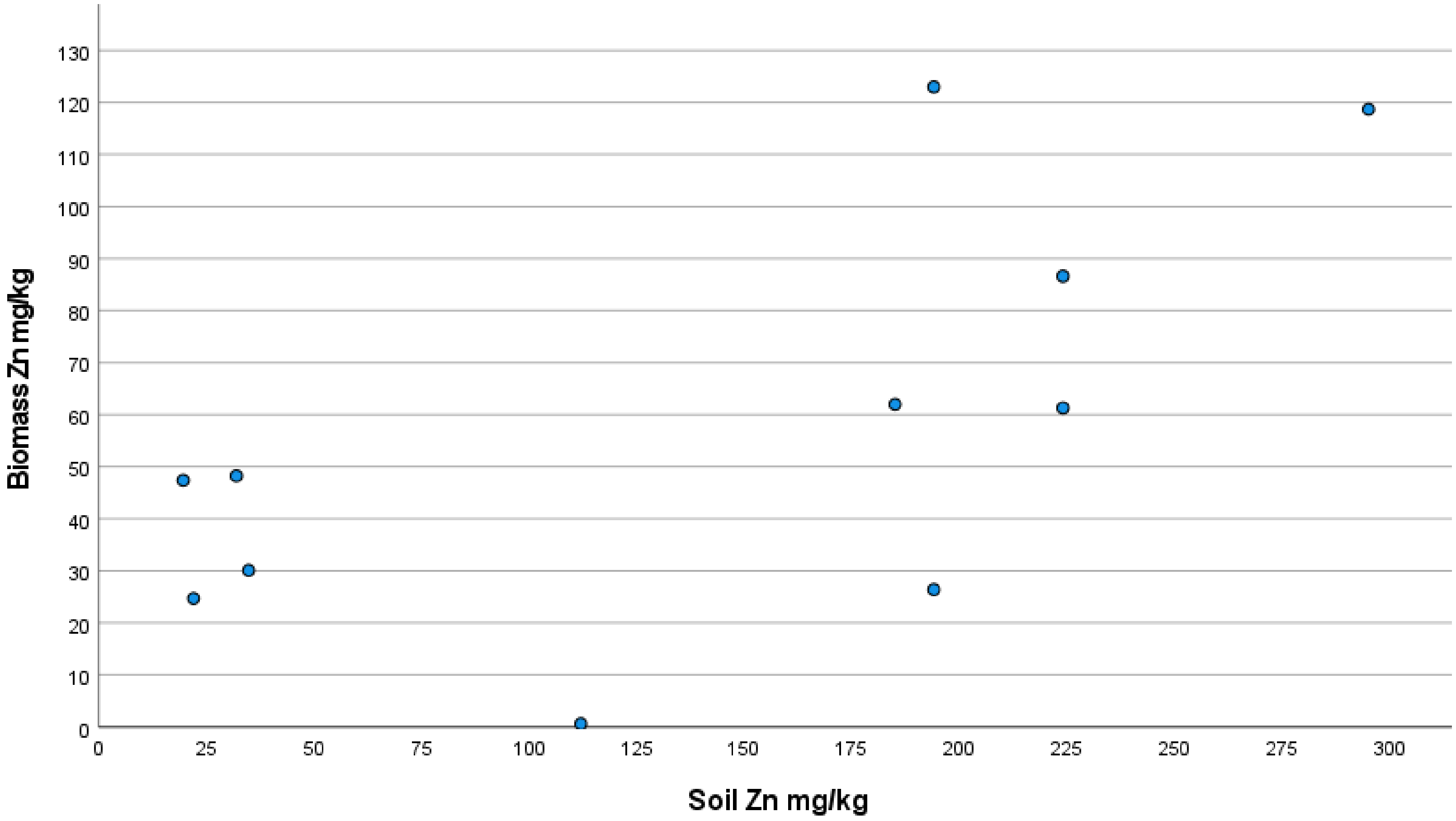


Figure 12 Concentrations of Zn in RCG biomass found in published literature and the corresponding reported soil concentrations. Data reported in 11 journal articles several of which have reported multiple results. (Badmos et al., 2015; Polechońska and Klink, 2014; Antonkiewicz et al., 2016; Lord, 2015; Korzeniowska and Stanislawska-Glubiak, 2019; Rosikon et al., 2016; Smith and Slater, 2011; Usťak et al., 2019; Strašil, 2012; Mayerová et al., 2017; Fijalkowski et al., 2018)

Figure 12 shows an apparent positive linear relationship between the soil and biomass concentrations of Zn. Zinc is a micro nutrient but is typically only absorbed in biomass to concentrations of approximately 20 mg/kg (Marschner, 1986; Broadley *et al.*, 2007; Kirkby, 2012). As with the Pb results Fig 7 highlights that RCG has not been grown in soils of particularly high Zn concentrations within the published works. The UKSHS found that soils in the UK have a mean concentration of 94.8 mg/kg Zn with the greatest value being 442 mg/kg (Environment Agency, 2007). This indicates a clear research incentive to analyse RCG biomass grown in soils with a greater concentration of Zn than found in the literature. This would also help discover if the observed linear relationship continues or if RCG has a threshold for Zn absorption.

3.5 Comparisons with PTE concentrations found in RCG and other lignocellulosic biomass

The results of the selective literature review of published studies relating to other ligno cellulosic biomass species when compared to the discussed RCG results are shown in the following figures. It should be noted that one of the studies provided a considerable amount of the data on SRC Willow concentrations of Cu, Ni and Zn as it represented the most comprehensive study of its type (Pulford *et al.*, 2002). As the same sewage sludge amended soils were used throughout that study some of the figures show results with the same soil concentrations of Cu, Ni and Zn and different biomass concentrations for each type of willow cultivar that was analysed. Figure 13 shows the concentrations of Cu in RCG, Miscanthus and SRC Willow biomass found in published literature. The Cu results shown in indicate that all three of the species analysed do not tend absorb Cu in high concentrations even in soils above the typical background concentrations for rural UK soils (19.8 mg/kg) (Environment Agency, 2007). For example, none of the data showed plants that had greatly exceeded the typical range of 4-15 mg/kg Cu for plants grown in 'un contaminated' soils (Oorts, 2013). All three species show a moderate, positive linear relationship to plant absorption rates and soil concentrations but no strong relationship was revealed. Short rotation coppice Willow was found to have the greatest Cu biomass concentrations. The ability for SRC Willow to absorb Cu at low soil concentrations has been noted by other studies (Greger and Landberg, 1999). Although RCG was found to have absorbed the least Cu it is clear that this species has not been grown on soils impacted by Cu of such high concentrations as for SRC Willow and to a lesser extent Miscanthus.

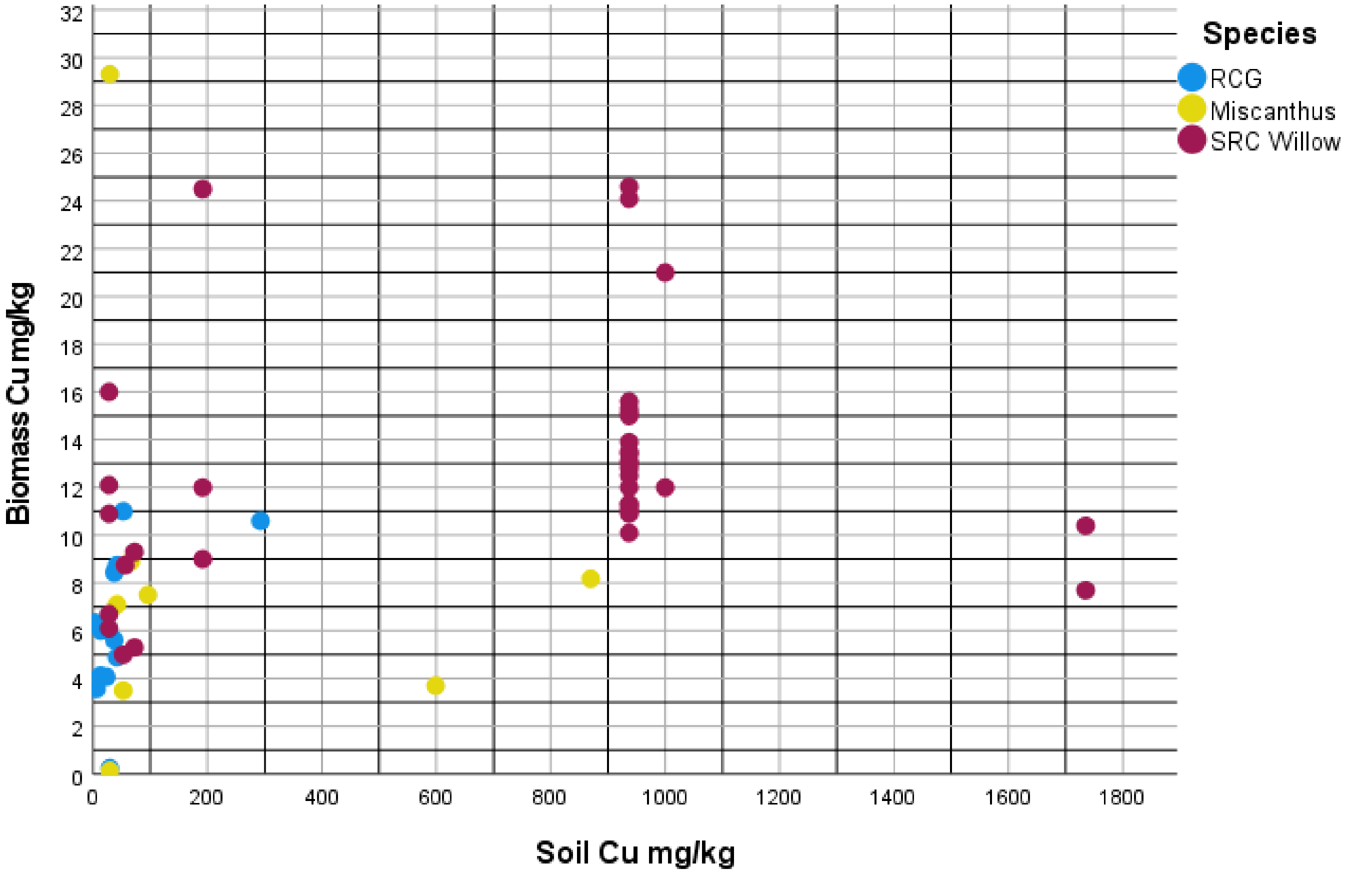


Figure 13 Concentrations of Cu in RCG, Miscanthus and SRC Willow biomass found in published literature and the corresponding reported soil concentrations (data collected from literature n=56)

The results for Ni are shown in Figure 14. Nickel is one of the least reported elements of those selected and thus the results on SRC willow from one paper make up over half of the data set (Pulford *et al.*, 2002). No linear relationship was found between the soil and plant concentrations.   As with Cu none of the species were found to absorb Ni much more above the typical range of 0.05 to 10 mg/kg for plants grown in 'un contaminated' soils (Gonnelli and Renella, 2013). Not many of the studies reviewed were associated with Ni soil concentrations much greater than the UKSHS background rate for rural soils (19.5 mg/kg) suggesting scope for testing plant interactions in more Ni impacted soils (Environment Agency, 2007). Soils are not thought to be phytotoxic at concentrations below 100 mg/kg Ni (Adriano, 2001).

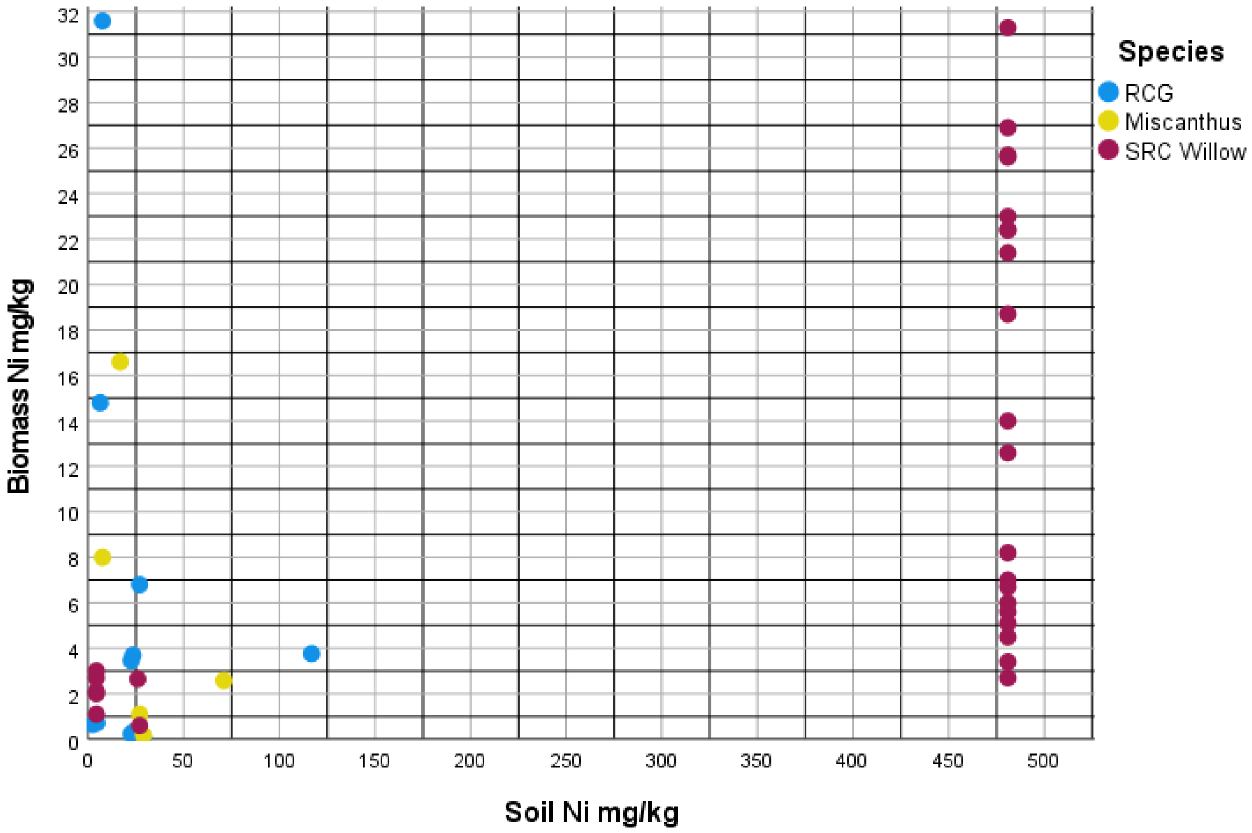


Figure 14 Concentrations of Ni in RCG, Miscanthus and SRC Willow biomass found in published literature and the corresponding reported soil concentrations (data collected from literature n=46)

The results for Pb are shown in Figure 15. Lead was found to be the least reported element of those selected for this study. The data for Miscanthus shows a moderate positve linear relationship for increasing soil and biomass Pb concentrations, SRC Willow shows no relationship. The reported highest concentrations of Pb found within Miscanthus biomass are from field trials at historic mine sites where the cultivar *Miscanthus floridulus* was planted and the above ground portion of the plant analysed (Leung *et al.*, 2007; Wang *et al.*, 2012b). *Miscanthus floridulus* differs from the more common and commercailly developed *Miscanthus x giganteus* in that it is limited by suitable climatic conditions to the South of East Asia at latitudes below 30°N (Lewandowski *et al.*, 2018). All other data regarding Miscanthus and Pb reported in Fig 10 concerns *Miscanthus x giganteus.* This may be an indication that the different plants interact significantly differently with soil Pb.

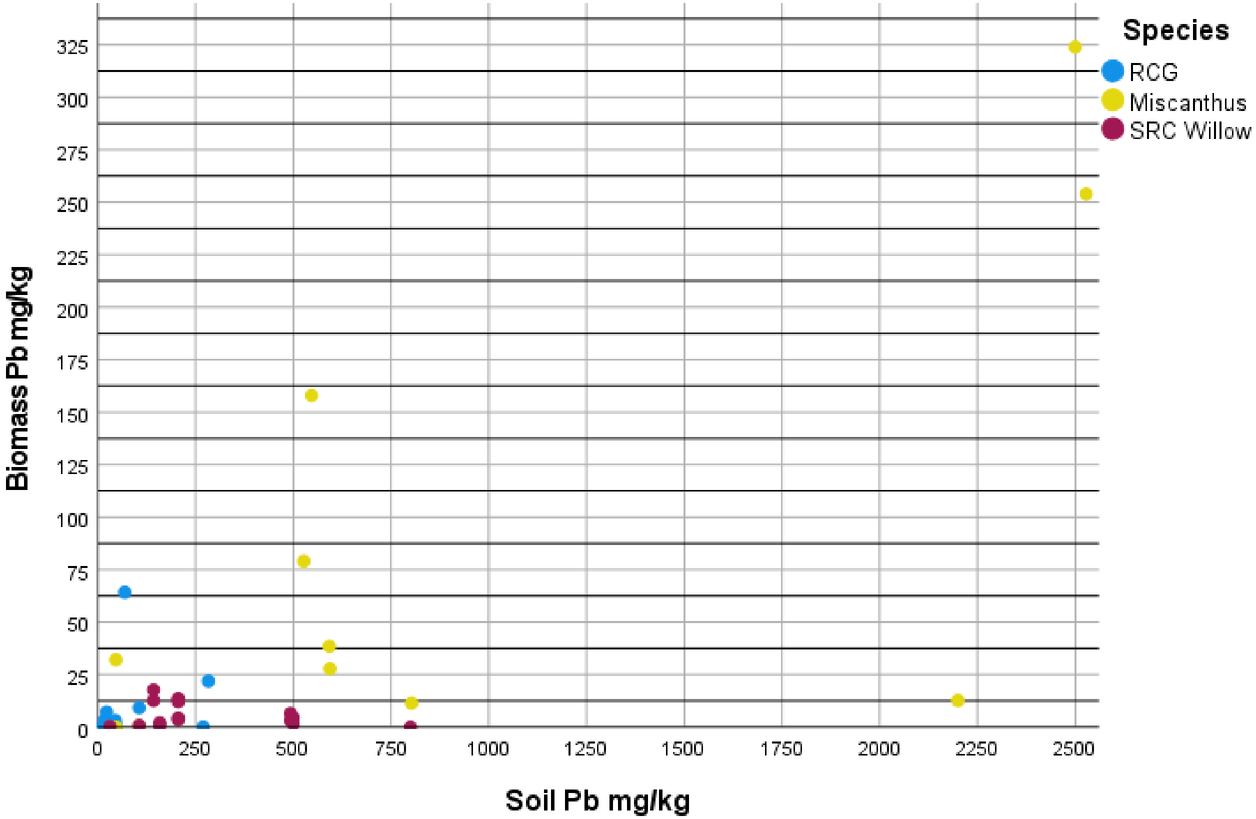


Figure 15 Concentrations of Pb in RCG, Miscanthus and SRC Willow biomass found in published literature and the corresponding reported soil concentrations (data collected from literature n=42)

Figure 16 shows the same results as Figure 15 but with the *Miscanthus floridulus* data removed for clairty. The data for both RCG and SRC willow biomass and soil concentrations show little to no linearity. Both species have been described as good phytostabilation plant options for Pb (Sylvain *et al.*, 2016). As discussed previously Pb soil concentations in which the RCG has been grown in within the published literature are generally not that much greater than non Pb impacted soils, highlighting a clear research gap. Fig 11 shows clearly the greater concentrations of Pb found in M*iscanthus giganteus* when compared to the two other species.

The results for Zn soil and biomass concentrations are shown in Figure 17. There is an apparent positive relationship for both RCG and Miscanthus up to biomass concentrations of 500 mg/kg. The relationship is strong for RCG and moderate for Miscanthus. The results for SRC willow are scattered and no discernible relationship can be determined. It is clear however, that willow is potentially able to absorb greater concentrations of Zn than the two other species.

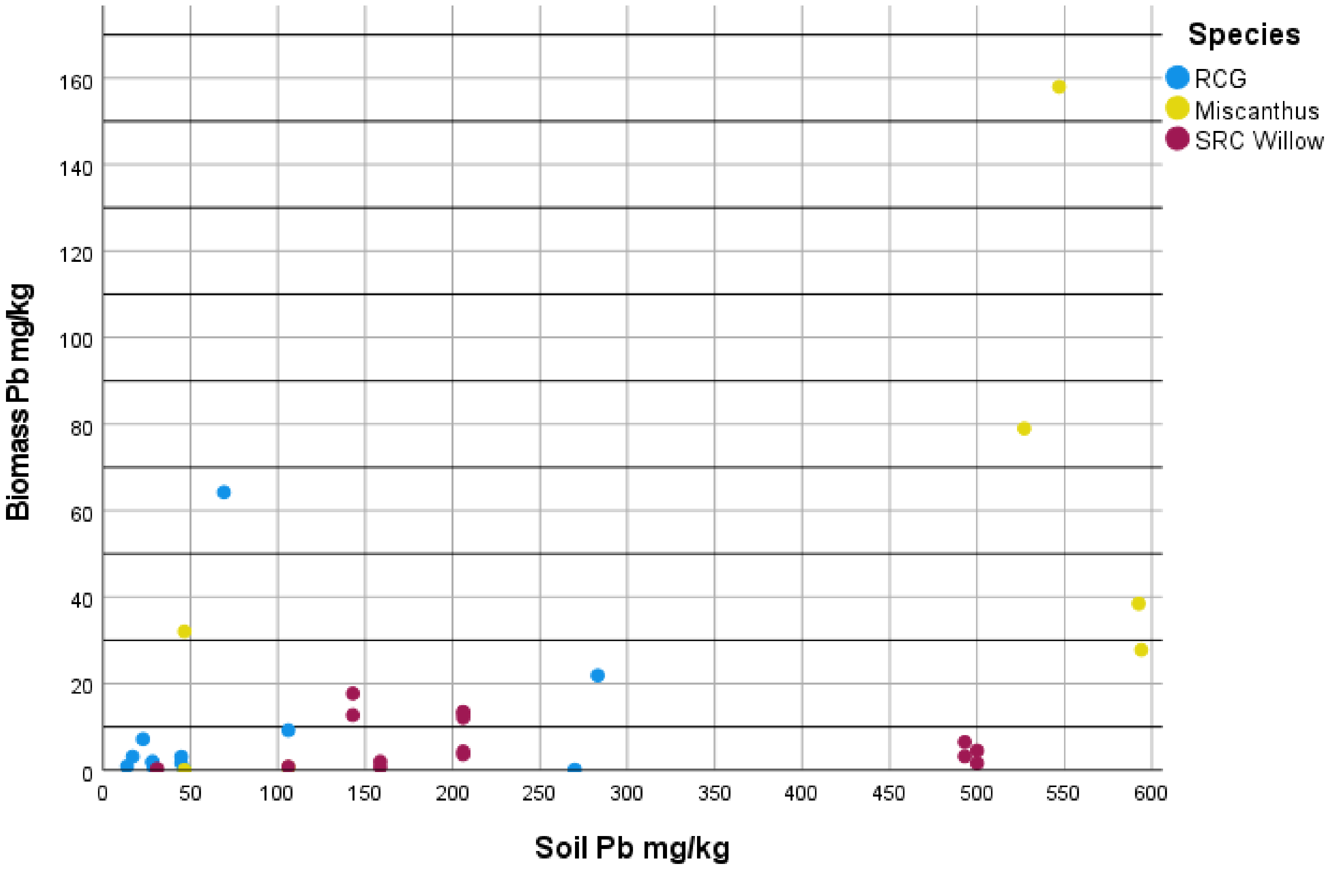


Figure 16 Concentrations of Pb in RCG, Miscanthus and SRC Willow biomass found in published literature and the corresponding reported soil concentrations with the Miscanthus floridulus data removed for clairty (data collected from literature, outliers have been removed n=40)

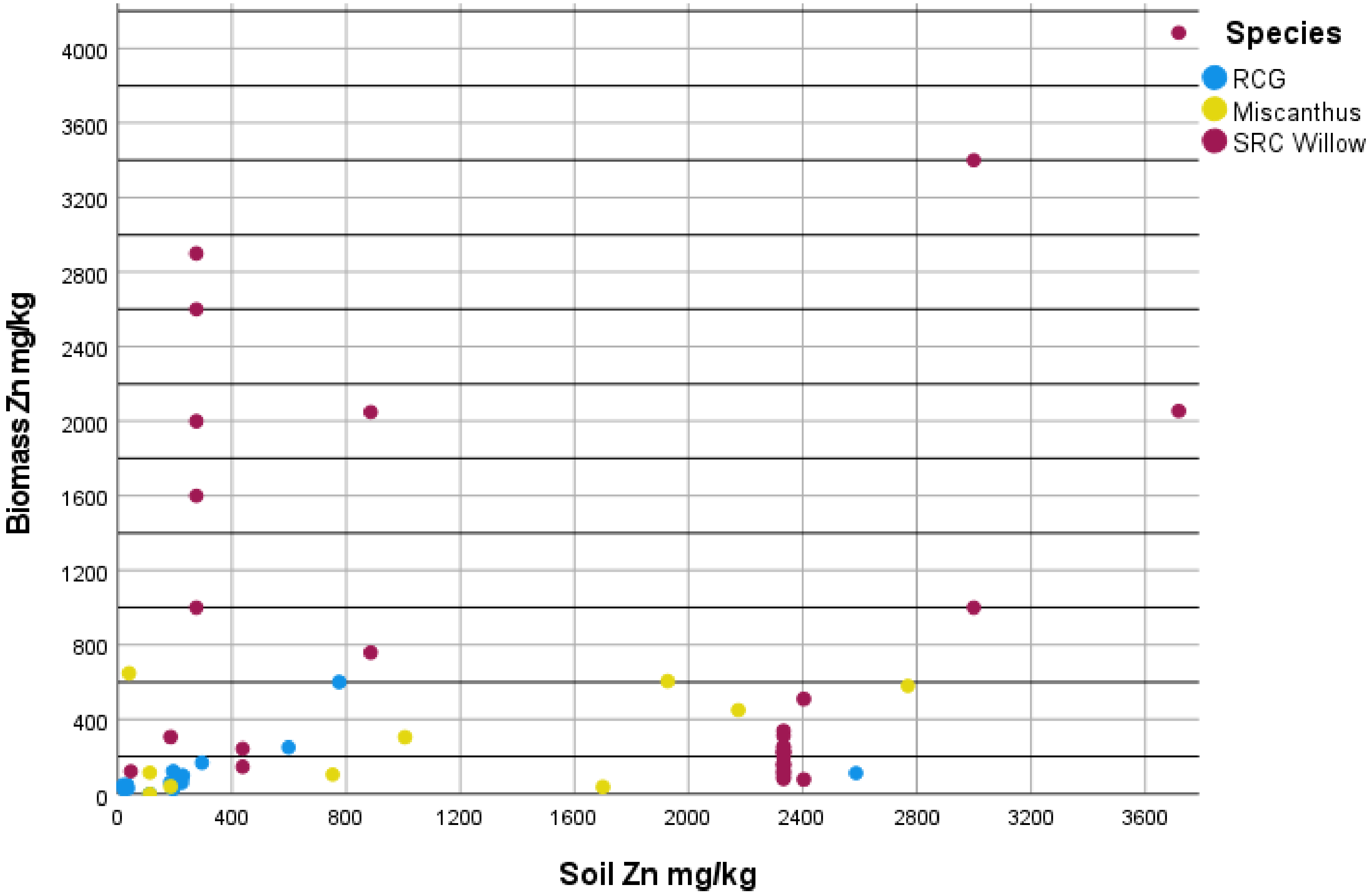


Figure 17 Concentrations of Zn in RCG, Miscanthus and SRC Willow biomass found in published literature and the corresponding reported soil concentrations (data collected from literature n=65)

3.5.1 Issues with comparison of data

Several issues arose during the data collection and comparison for this chapter that are valuable to consider when approaching a study of this nature. A key difficulty with comparisons within and between species and the way they interact with soil PTE is that often different cultivars of that species are found to absorb PTE at different rates. This is best exemplified by the broad range of results of Ni concentrations found in SRC willow which were all grown in soils with the same soil concentration (Pulford *et al.*, 2002). This is also shown in the Pb results for Miscanthus. As phytoremediation remains relatively in its infancy, many of those publishing works on the topic do not have a grasp of how important cultivar choice can be for biomass concentrations of PTE. In many papers the cultivar and its origins are not mentioned at all. The RCG data (n=11 journal articles) contained only one reference to cultivar selection and in all other articles the cultivar was either unknown, from 'uncertified seed' or the subject was not mentioned at all.

Other variables relevant to biomass absorption of PTE such as soil characteristics are more routinely mentioned and herein lies another issue with the comparison of a variable data set. As the bio availability of PTE are determined by many variables such as soil pH, sediment size fraction and cation exchange capacity. This effect means that comparisons of results of even the same species and cultivar are still difficult to confirm when the reported results come from a wide range of sites in which none are identical or perhaps even similar. For example, the RCG data set (which represents a comprehensive literature review of reported PTE concentrations in RCG) contained information on agricultural soils, mine tailings and hydrocarbon impacted sites.

Other meta data collected along with the biomass and soil PTE concentration data reveal how non standardised the analysis of biomass is. For example, the RCG data consisted of articles which contained a description of four different parts of the plant which were analysed, excluding roots. A key variable for which there is no standard practice for is the washing of the biomass before analysis. Each article had its own washing procedure description of which the following are some examples “washed carefully,” “washed in distilled water,” “brushed and washed with distilled water” and “thoroughly washed.” The majority of articles did not mention a biomass washing procedure. Biomass was also analysed with a range of different analytical methods, digestates and analytical instruments. The time of year that the plants were harvested was also regularly not mentioned despite this being a clear parameter in the absorption of PTE.

3.6 Chapter conclusions

Several conclusions can be drawn from the literature review of concentrations in RCG and the two lignocellulosic biofuel species. Firstly, that there are not any published works where RCG has been grown in soils that are highly contaminated with PTE. None of the soil data reviewed concerning RCG growth contained concentrations of PTE that would be above the UK risk assessment guideline values for their type of land use (CL:AIRE, 2014; LQM/CIEH S4ULs, 2015) and the majority were not greatly above the average for UK soils (Environment Agency, 2007). Although this limits the conclusions that can be drawn from the literature review it highlights a considerable research gap.

There is even less information available on the impact of PTE on RCG over time and vice versa, as the longest field trial found lasted just three years.

A linear relationship and threshold for RCG biomass adsorption and soil concentrations was potentially found for Cu and Zn which could be confirmed with further trials at greater concentrations. No relationship between RCG biomass concentrations and soil concentrations was found for other elements that were looked at, such as Ni. This maybe because of the various different factors involved in each of the published works reviewed and the general limitations of such a small data pool.

Differences were found in the two other lignocellulosic biofuel species when compared to RCG. However, whilst the data suggests that SRC Willow is capable of adsorbing more PTE than RCG or *Miscanthus giganteus*, it has also clearly been grown in soils with greater PTE concentrations, probably as it is a more well established species. The importance of indicating the cultivar of a species which has been used in a field or pot trial has been well highlighted by this work. Future work in this field should consider the differences in cultivar interaction with soil PTE as a key factor when designing experiments and describing results. The results of this chapter provide a clear justification for the work described in this study and will also be discussed at greater length within the conclusion.

4 General experimental procedures

4.1 Introduction

This chapter outlines the general experimental procedures and data handling employed in this work. Glassware, microwave digestion tubes and sample containers were soaked in 10 % HNO_3 (general purpose grade supplied by Sigma Aldrich, UK) overnight and thoroughly rinsed with distilled water, then in double distilled deionised water, before use. All the reagents used were of analytical grade. Sample solutions were stored in polyethylene bottles and refrigerated at 4 °C prior to analysis. All samples were weighed with a 5 figure analytical weighing balance (ME 204T/00 Mettler Toledo, Leicester, UK). Data analyses were performed with SPSS 24 (IBM). Specific aspects of experimental procedure for each experiment discussed in this study can be found within the experimental section of the relevant chapter.

4.2 pH measurement

4.2.1 Apparatus

The pH of soil samples were found using a seven Multi Mettler Taledo MPC 227 pH meter (Schwerzenbach, Switzerland). The pH probe had a three-point calibration using standards of pH 4, 7 and 10 and has an accuracy of ±0.001.

4.2.2 Analytical method

Following a method adapted from a British standard (BSEN13652:2001) approximately 5 g of air dried soil (particle size < 2 mm) was added to a 50 ml centrifuge tube (Fisher brand -Fisher Scientific, Loughborough, UK) which were then filed with 25 ml of de-ionised water to give a solid to liquid ratio of 1:5. Centrifuge tubes were then shaken at 700 oscillations per minute for 1 hour on a Stuart Orbital Shaker SSL1 (Stone, UK). Samples were then left to settle for 1 hour before the pH was recorded with the calibrated pH meter.

4.3 Determination of moisture and organic matter content

4.3.1 Apparatus

Samples were dried in a fan assisted oven (Gallenkamp hot box oven size 1, London, UK) and organic matter was incinerated in a muffle furnace (Nabertherm LT 15/12/P330, Lilienthal, Germany).

4.3.2 Analytical method

The moisture content of samples was determined in order to express the PTE concentration on a dry weight basis. Approximately 1 g of sample (sieved to < 2 mm) was weighed into a dried and pre-weighed crucible and was dried in the oven at 110 °C until at a constant weight. The crucible was then transferred to a desiccator, allowed to cool for two hours and then weighed. The percentage moisture content of the sample was estimated using Equation 2.

$$\% \, moisture \, content = \frac{(inital \, weight - oven \, dried \, weight) * 100}{intial \, weight}$$

Equation 2 moisture content

The percentage organic matter (% OM) of samples was estimated through the measurement of their loss on ignition (LOI). Crucibles containing residue from the moisture content determination were placed in the muffle furnace. The muffle furnace was programmed to ramp at 10 °C per minute, hold at 550 °C for eight hours, and then to cool to room temperature. Then the crucible with its contents was transferred to a desiccator, allowed to cool for two hours and then weighed. The percentage of organic matter was estimated using Equation 3.

$$\%OM = \frac{(oven \, dried \, wright - weight \, following \, combustion) * 100}{oven \, dried \, weight}$$

Equation 3 Organic matter content

4.4 Extraction procedures

For all the extraction and digestion procedures the glassware and storage bottles used were soaked overnight in a 5 % (v/v) Nitric acid (HNO_3) bath. Trace analysis grade HNO_3 (> 68%) (Fisher Scientific, Loughborough, UK) was used. These containers were then washed thoroughly with deionised water and dried in a closed drying cupboard. Prior to analysis and during the procedure all solutions were stored in polyethylene bottles or glassware at 4 ^{o}C. Reference materials were used as part of each analysis in order to assess the analytical performance of the procedures for quality control. The results of the analysis of the reference materials used during each analysis are presented in their relevant chapters.

4.4.1 Pseudototal digestion of soil and sediment samples

4.4.1.1 Apparatus

Pseudototal digestion of the samples was carried out in microwave digestion tubes (CEM Microwave Technology Ltd., Buckingham, UK) using a microwave digestion system (MARS Xpress, obtained from CEM Microwave Technology Ltd., Buckingham, UK).

4.4.1.2 Reagents

Aqua regia prepared by mixing PrimarPlus- Trace analysis grade hydrochloric acid (HCl) (~ 37 %) and nitric (HNO_3) (> 68 %) acid (Fisher Scientific, Loughborough, UK).

4.4.1.3 Digestion procedure

Samples were weighed to the desired mass, placed into MARS Xpress digestion tubes and the desired volume of aqua regia was added (typically 20 ml). Procedural blanks were always prepared and digested at the same time. The microwave tubes were then loosely covered with the cap and allowed to stand overnight in a fume cupboard. This was done to allow any vigorous reaction and build-up of pressure to subside. The

digestion tubes were then transferred into the microwave and the digestion was carried out in accordance with the condition stated in

Table 12.

Following the completion of the digestion programme the tubes were allowed to cool for 40 minutes. The contents of the tubes were then washed into clean 50 ml volumetric flasks which were then made up to volume with nano pure deionised water (n-DW). This solution was then filtered into 50 ml centrifuge tubes using syringe filters and stored at 4 ^{o}C awaiting analysis with ICP-OES (iCAP 6000 Series, Thermo Scientific, East Grinstead, UK). If the sample required further dilution solution, would be removed from centrifuge tubes by means of a mechanical pipette and diluted to the desired ratio, typically with n-DW but occasionally with a matrix matched solution.

Table 12 Mars Xpress "soil" method

Step	Power (W)	Power (%)	Ramp (min)	Temperature (°C)	Hold (min)
1	800	100	20	160	
2	800	100		160	20
3	800	100	20	180	
4	800	100		180	20

4.4.2 Digestion of plant material

4.4.2.1 Apparatus

Digestion of the plant samples obtained following the 10-week pot trials was conducted with a similar method to that used for the soil samples carried out in microwave digestion tubes (CEM Microwave Technology Ltd., Buckingham, UK) using a microwave digestion system (MARS Xpress, obtained from CEM Microwave Technology Ltd., Buckingham, UK).

4.4.2.2 Reagents

Nitric Acid PrimarPlus-Trace analysis grade (HNO_3) (> 68 %) acids (Fisher Scientific, Loughborough, UK).

1.1.1.1 Digestion procedure

Samples were weighed to 0.1 g and placed into MARS Xpress digestion tubes, 10 ml of HNO_3 was added and the material was left to predigest for 24 hours. The rest of the procedure was identical to that described in section 4.4.1 other than the operating parameters for the microwave oven which are outlined in Table 13. The samples were analysed using ICP-MS (Model 7700x, Agilent Technology, Chesire, UK) following digestion.

Table 13 Mars Xpress "plant" method

Step	Power (W)	Power (%)	Ramp (min)	Temperature (°C)	Hold (min)
1	900	100	15	200	
2	900	100		200	15

4.4.3 BCR sequential extraction procedure

4.4.3.1 Apparatus

A Gerhardt Rotoshake RS12 mechanical end over end shaker (C. Gerhardt GmbH and Co. KG, Königswinter, Germany) was used to perform extractions at a speed of 20 rpm with the appropriate extractant solution at a temperature of 22 ± 5 °C. The delay between extract addition and shaking was minimal for BCR sequential extraction procedure steps one to three as extractions were set to run for 16 hours over night and end at the start of the working day. The extracts were separated from the solid sample residue using a THERMO electron ALC centrifuge PK130 (Thermo Fisher Scientific Waltham, East Grinsted, UK) at 3000 g (4543 rpm) for 20 minutes.

A Digi prep Jr graphite digestion block (SCP Science, Montreal, Canada) was used to heat solutions in step three of the procedure. Following tests with a thermometer the temperature control of the block was set at 106 °C to achieve a reagent temperature of 85 °C.

4.4.3.2 Reagents

Solution A (acetic acid, 0.11 mol l^{-1}): 25 ml of glacial analytical grade acetic acid (Arcos Organics, Geel, Belgium) was added to 0.5 l of n-DW in a 1 l graduated polypropylene bottle and made up to volume with n-DW. 250 ml of this solution (acetic acid, 0.43 mol

l^{-1}) and was diluted with n-DW to 1 l to obtain a final acetic acid solution of 0.11 mol l^{-1}.

Solution B hydroxylamine hydrochloride, 0.5 mol l^{-1}): 34.75 g of analytical grade hydroxylammonium chloride (Alfa Aesar, Massachusetts, United States) was dissolved into 400 ml of n-DW. This solution was transferred into a 1 l calibrated flask and 25 ml of 2 mol l^{-1} HNO_3 (prepared by weighing from a suitable concentrated solution) was added by means of a calibrated pipette. The flask was then made up to volume with n-DW. This solution was always prepared on the same day that the extraction was performed.

Solution C (hydrogen peroxide, 300 mg g^{-1}, 8.8 mol l^{-1}): Analytical grade hydrogen peroxide (Fisher Scientific, Loughborough, UK), was used as supplied by the manufacturer (30% 8.8 mol L^{-1}) i.e. that is acid-stabilised to pH 2–3.

Solution D (ammonium acetate, 1.0 mol l^{-1}): 77.08 g of analytical reagent grade ammonium acetate (Fisher Scientific, Loughborough, UK) was dissolved in 900 ml of n-DW. The pH of this solution was adjusted to 2.0 ± 0.1 with concentrated HNO_3 and made up to 1 L with n-DW.

Aqua Regia: As described previously in section 4.4.1

Blanks: Procedural blank solutions were prepared for each step by adding 20.0 ± 0.1 ml of solution A for step one, 20.0 ± 0.1 ml of solution B for step two and 25.0 ± 0.1 ml of solution D for step three to centrifuging tubes which were then carried through the complete procedure and analysed at the end of each extraction step. The procedural blanks also represent regent blanks.

4.4.3.3 Sequential extraction procedure

Step 1 (Exchangeable fraction)

An approximately 0.5 g test portion was accurately weighed into a 50 ml centrifuge tube that contained 20 ml of solution A and immediately placed on the end over end shaker for 16 hours. The sample was then centrifuged to separate the supernatant liquid from the solid residue. The liquid was decanted into a polyethylene bottle and refrigerated at 4 °C prior to analysis. The residue was washed by adding 20 ml of n-DW and shaken for 15 minutes. After centrifuging at 3000 g for 20 minutes the supernatant was carefully decanted and then discarded.

Step 2 (Reducible fraction)

A 20 ml volume of freshly prepared solution B was added to the washed residue from step 1 in the same centrifuge tube. The sample was then shaken, centrifuged and stored as in step 1.

Step 3 (Reducible fraction)

A 5 ml volume of room temperature solution C was added, slowly in small aliquots to avoid vigorous reactions, to the washed residue from step 2. The centrifuge tube was covered loosely with its lid and left to digest for 1 h with occasional manual shaking. The digestion was then continued for a further 1 hour in the graphite block digester which bought the temperature of the solution up to 85 ± 2 °C. The caps of the centrifuge tubes were removed to allow for the reduction in volume to 1.5 ml. A further 5ml of solution C was then carefully added and the covered samples were headed for a further 1 hour at 85 ± 2 °C. After removal of the lids the volume was reduced to less than 1 ml, with care not to take to complete dryness. The resulting solution was then allowed to cool before 25 ml of solution D was added to the moist residue. The centrifuge tubes were then shaken, centrifuged, the solution decanted and stored for analysis, and the residue washed as in step 1.

Step 4 (Residual fraction)

1 ml of n-DW was added to mobilise the residue in centrifuge tubes. The sample was then transferred to Mars Xpress vessels and 10 ml aqua regia was added. This solution was left to pre-digest overnight before being placed in the microwave oven and digested using the procedure described in section 4.4.1. Digestates were made up to volume in 50 ml volumetric flasks following digestion and filtered as previously outlined.

4.5 Analyses of digests and extracts

Digests of plant material were analysed for PTE content using ICP-MS (Model 7700x, Agilent Technology, UK). Digests and extracts of soils were analysed for PTE content using ICP-OES (iCAP 6000 Series, Thermo Scientific, Waltham, MA USA). The operating conditions for each instrument are shown in Table 14.

Table 14 Operating conditions for ICP-OES and ICP-MS

PARAMETERS	ICP - OES	ICP – MS
RF power, w	1150	1550
Plasma gas flow rate, l/min	15	15
Auxiliary gas flow rate, l/min	0.5	0.9
Nebulizer argon gas flow rate, l/min	0.5	0.85
Purge gas	Nitrogen	Nitrogen
Plasma gas	Argon	Argon
Internal standard	- 1000 mg/L Y in HNO_3	^{209}Bi, ^{115}In, ^{72}Ge and ^{45}Sc

4.5.1 Calibration of the Instruments

Multi-element (Cd, Cr, Cu, Ni, Pb, and Zn: 10 mg/l in 2 % HNO_3) standards (Fisher Chemical, New Hampshire, United States) were used to prepare the working standard solutions by accurately measuring the appropriate amounts using micropipettes (Thermo Scientific, Vantaa, Finland) to provide the desired elemental concentrations of 10 and 0.1 ppm. Standards were prepared in 2 % aqua regia and in the extractants used in the BCR sequential extraction procedure. Calibration standards were matrix matched with the matrix of the samples from the BCR sequential extraction procedure during analysis e.g. a acetic acid, 0.11 mol l^{-1} solution for samples from Step 1. The concentrations of the multi-element standard solutions used as calibrants are listed in Table 15.

Table 15 Concentration of multi-element (Cd, Cr, Cu, Ni, Pb, and Zn) used to prepare calibrants.

Sample ID	**Matrix**	**Desired concentration ,ppm**	**Stock Concentration, ppm**	**Dilution Factor**	**Dilution Factor for analysis**
Standards in Solution A High	acetic acid, 0.11 mol l^{-1}	10	1000	100	2
Standards in Solution A Low	acetic acid, 0.11 mol l^{-1}	0.1	10	100	2
Standards in Solution B High	hydroxylamine hydrochloride 0.5 mol l^{-1}	10	1000	100	2
Standards in Solution B Low	hydroxylamine hydrochloride 0.5 mol l^{-1}	0.1	10	100	2
Standards in Solution C High	ammonium acetate, 1.0 mol l^{-1} at pH 2	10	1000	100	2
Standards in Solution C Low	ammonium acetate, 1.0 mol l^{-1} at pH 2	0.1	10	100	2
Standards in AQ High	(Aqua Regia) 3 parts HCL (15ml) to 1 part HNO3 (5ml)	10	1000	100	2
Standards in AQ Low	(Aqua Regia) 3 parts HCL (15ml) to 1 part HNO3 (5ml)	0.1	10	100	2

4.6 X-ray computed tomography

4.6.1 Apparatus

Grass samples were scanned with a Nikon Metrology X-ray micro CT (XCT) system (Tring, UK) with 180 kV X-ray source, then these 2D projections were reconstructed into 3D volumes using Nikon CT Pro software.

4.6.2 Operating conditions

The scan parameters used for the XCT are summarised in Table 16. Three scans were completed during this study. The first with eight grass samples which were unwashed and dried to a constant weight at 100 ^{0}C and the second and third scans with grass samples that were washed with deionised water and dried to a constant weight at 100 ^{0}C. Calibrations standards were made using dissolved lead acetate at concentrations of 1000, 100 and 10 mg/kg.

Table 16 Scan parameters for X-ray computed tomography system

		Resolution	**Energy**	**Current**	**Exposure**	**Projections**	**Angular Step**	**Reconstructed Length (suitable for analysis)**
Sample	Scan #	[microns]	[kV]	[micro Amps]	[milliseconds]	[#]	[degrees]	[mm]
Unwashed	1	60	85	176	2000	3141	0.115	96
Washed	1	70	92	174	1415	1595	0.226	119
	2	22	80	188	2000	3141	0.115	32.56

4.7 Data handling

4.7.1 Analyte concentration

The two analytical instruments used throughout this study ICP - MS and ICP - OES measured the concentration of the analyte in µg/l of the solution. These values were converted to mg/kg of the sample dry weight using Equation 4.

$$\text{Concentration (mg kg)} = \frac{\text{analyte concentration (µg l)} * \text{volume (ml)} * \text{dilution factor}}{\text{mass of sample (dry weight)} \ (g)}$$

Equation 4 Conversion from µg/l to mg/kg

4.8 Statistics

Statistical Package for the Social Sciences (SPSS) (IBM, Armonk, New York,) was used for all statistical tests. The null hypothesis for each test was rejected if the p-value < 0.05.

4.8.1 T-test

The t-test abbreviated from "hypothesis test statistic" was first derived by Helmert and Lürothis and later named by William Sealy Gosset who published it in English in 1908 in the scientific journal *Biometrika* using his pseudonym "Student" (hence ‘student’ t-test) (Helmert, 1876; Lüroth, 1876; Student, 1908). The t-test is a statistical test that is used to determine whether the means of two groups are the same (within random error) or significantly different. A null hypothesis is set, that is there is no difference between the two sample means, other than one due to random error. The t test is often used in hypothesis testing to determine whether a treatment has had an effect on the population of interest, or whether two groups are different from one another. The T-test can only be used when comparing the means of two groups and is a parametric test of difference which assumes that the data are independent, normally distributed and have a similar amount of variance.

As this study applied the t-test to data from two different populations, two-tailed t-test (also called independent t-tests) were used. The two-tailed t-tests were applied to the data as the null hypothesis used in this study aimed to tests whether a treatment had a significant effect on a group rather than whether the treatment caused a group to either just increase or just decrease when compared to a differently treated group (one-tailed t-test). Equation 5 shows the formula for the two sample t-test. In this formula, t is the t-value, x_1 and x_2 are the means of the two groups being compared, s^2 is the pooled standard error of the two groups, and n_1 and n_2 are the number of observations in each of the groups.

$$t = \frac{x_1 - x_2}{\sqrt{(s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right))}}$$

Equation 5 two tailed t-test formula

4.8.2 Mann-Whitney U test

The Mann-Whitney U test is a nonparametric test that allows the median of two independent groups to be compared by their rank (Mann and Whitney, 1947; Corder and Foreman, 2011). The Mann-Whitney U Test is a nonparametric version of the independent samples t-test and so can be applied to data that is not normally distributed.

In this study non-parametric tests were applied under two circumstances;

1. If the data samples showed skewed distributions as skewness makes parametric tests less powerful because the mean is no longer the best measure of central tendency as it is strongly affected by the extreme values. Non-parametric tests work well with skewed distributions and distributions that are better represented by the median.
2. If the size of the data set was small, as the application of nonparametric tests is a more suitable option with smaller data sets where parametric tests may not

be able to validate the distribution of the data. There is no upper or lower limit of how many data points that is necessary in order to conduct the Mann-Whitney U test (Mann and Whitney, 1947; Corder and Foreman, 2011).

In the Mann-Whitney U test all the values of sample A are listed and the number of values in sample B that are greater than each value of sample B are counted. The sum of the counts is the test statistic. The null hypothesis is that the two samples come from the same population (i.e. have the same median). The null hypothesis is rejected if test statistics are less than or equal to the critical value at the given significant level. The p-value approach can also be used. The Mann-Whitney U test (at 0.05 significance level) was used in this study to determine if there were differences in the dry weight of biomass for plants grown under different growing conditions. Equation 6 shows the equation for the Mann-Whitney U test where U = the Mann-Whitney statistic; m= number of samples drawn from population X; n = number of samples drawn from population Y, and Rx and Ry is the sum of ranks attributed to population X and Y. The U statistic is then calculated as the minimum U statistic of both X and Y. The p value can then be calculated based on the comparison between the critical value (the cut-off values that define regions where the test statistic is unlikely to lie) and the U value. If the U value is less or equal to the critical value, then the null hypothesis can be rejected and vice versa.

$$Ux = mn + \frac{m(m+1)}{2} - Rx$$

$$Uy = mn + \frac{n(n+1)}{2} - Ry$$

$$U = \min(Ux, Uy)$$

Equation 6 Mann-Whitney U test

4.8.3 Analysis of variance

When determining whether there are any statistically significant differences between the means of three or more independent groups, an analysis of variance (ANOVA) test can be used. The ANOVA test can be thought of as an extension of the t-test for two independent samples to more than two groups. The reason for running an ANOVA rather than many two-group t tests is that, as the number of groups increases, the number of two-group comparisons increases. An increase in the number of significance tests conducted on the same data increases the risk of falsely concluding significance when there is no real effect present, this is known as a controlled or fixed effect factor. The risk of inherent random errors in the measurements is also increased. The original concept for the ANOVA test was developed by the English statistician Sir Ronald A. Fisher who applied the test mostly to agricultural experiments (Fisher, 1925).

The one-way ANOVA does not look at the differences between pairs of group means; instead, it looks at how the entire collection of group means are spread out and compares that to how much those means might spread out if all the groups were sampled from the same population, thus separating variation (Montgomery and Runger, 2010). The ANOVA test looks for variance in the data in two ways; the variation within samples and variation between samples. The test provides a statistic called the F ratio, which is the ratio of how much variability there is between the groups relative to how much there is within the groups. The null hypothesis in ANOVA is always that there is no difference in means. The test is an omnibus test statistic and so is unable to show which specific groups were statistically significantly different from each other. The test can only show that at least two groups are significantly different. To determine which specific groups differed from each other a post hoc test is applied. Post hoc tests are applied in an attempt to control the experiment wise error in the same manner that the one-way ANOVA is used instead of multiple two group t tests. Post hoc tests reduce the family wise error rate and thus the chance of obtaining false positive results also known as a type one error.

This study applied the Bonferroni post hoc correction to data analysed with the ANOVA test. The Bonferroni correction is a multiple test correction, it counter acts the problem of experiment wise errors by testing each individual hypothesis as shown in Equation 7, where a = the desired overall alpha level (in the case of this study equivalent to the p value - 0.05) and *m* = the number of tests (Bonferroni, 1935; Miller, 1966).

$$\frac{\alpha}{\mathrm{m}}$$

Equation 7 Bonferroni post hoc

Whilst reducing the chances of a type one error, the Bonferroni correction can increase the chance of type two errors. Type two errors can occur when a test fails to reject a false null hypothesis resulting in false negative results. This post hoc test can therefore reduce the statistical power of the ANNOVA making it harder to find the true effect. The results from the Bonferroni test should be viewed therefore as relatively conservative .

5 Trial site survey and characterisation of soils and amendments

5.1 Aims and objectives

The following chapter includes the results of a desk-based search on the geology, ecology and history of Whiteheaps mine site, the analysis of the impact the mine site may be having on stream sediments, and the characterisation of the mine soils and organic waste amendments which will be used throughout this study.

Whiteheaps mine and washing complex was selected for this study as an example of a former mine site that had the potential for a phytostabilisation remediation plan involving the use of reed canary grass. The site is representative of typical historic lead mines in the UK and so is an ideal location for research focusing on historic mine remediation. The aim of this chapter is to provide the reader of this study with an understanding of the materials used throughout this study i.e. the mine soils at Whiteheaps and the organic waste amendments i.e. green waste compost (GWC) and drinking water treatment residue (DWTR). This chapter also aims to assess the impact that Whiteheaps may be having on the sediments of the local water course and wider catchment area. Specific objectives were:

- An evaluation of the key literature associated with Whiteheaps mine practically its geology and history.

- The identification of two areas within the mine complex and assessment for their suitability for providing material for pot trials and locations for field trials.

- The characterisation of the relevant spoil materials at Whiteheaps historic mine and the organic waste soil amendments (GWC and DWTR). This objective was met through analysis of the spoil material and amendments for their elemental content, nutrient content and their physical properties.

The following is an account of the Whiteheaps mining complex that serves as an introduction to the chapter. The account of the mining complex has been formed from previous studies, books on mining, websites created by mining historians, reports written on the area and personal communication with mining enthusiasts (Pickin, 1992; Burrows, 1981; Dunham, 1948; Fairbairn and Northern Mine Research Society, 2000; Chaffee, 1994; Morrison, 1998; Geology North, 2021; Lord, 2015; Milburn, 1987).

5.1.1 Geology of the area surrounding Whiteheaps mine complex

The Whiteheaps mine and washing complex is situated in the Northern Pennines on the boundary between the counties of Northumberland and Durham, and lies on the banks of Bolts Burn (NGR: NY946465) within the Derwent valley (Pickin, 1992). The Northern Pennines are well-known as an area of Pb-Zn-Ag-Ba-F mineralisation and historic mining activity (Dunham, 1948). The mineral veins and deposits in this area are known collectively as the Northern Pennine Orefield (NPO) and represent the second largest area (1500 square miles) of carbonate-hosted lead-zinc mineralisation in the British Isles after the Irish Midland Orefield, as shown in Figure 18 (Dunham, 1948).

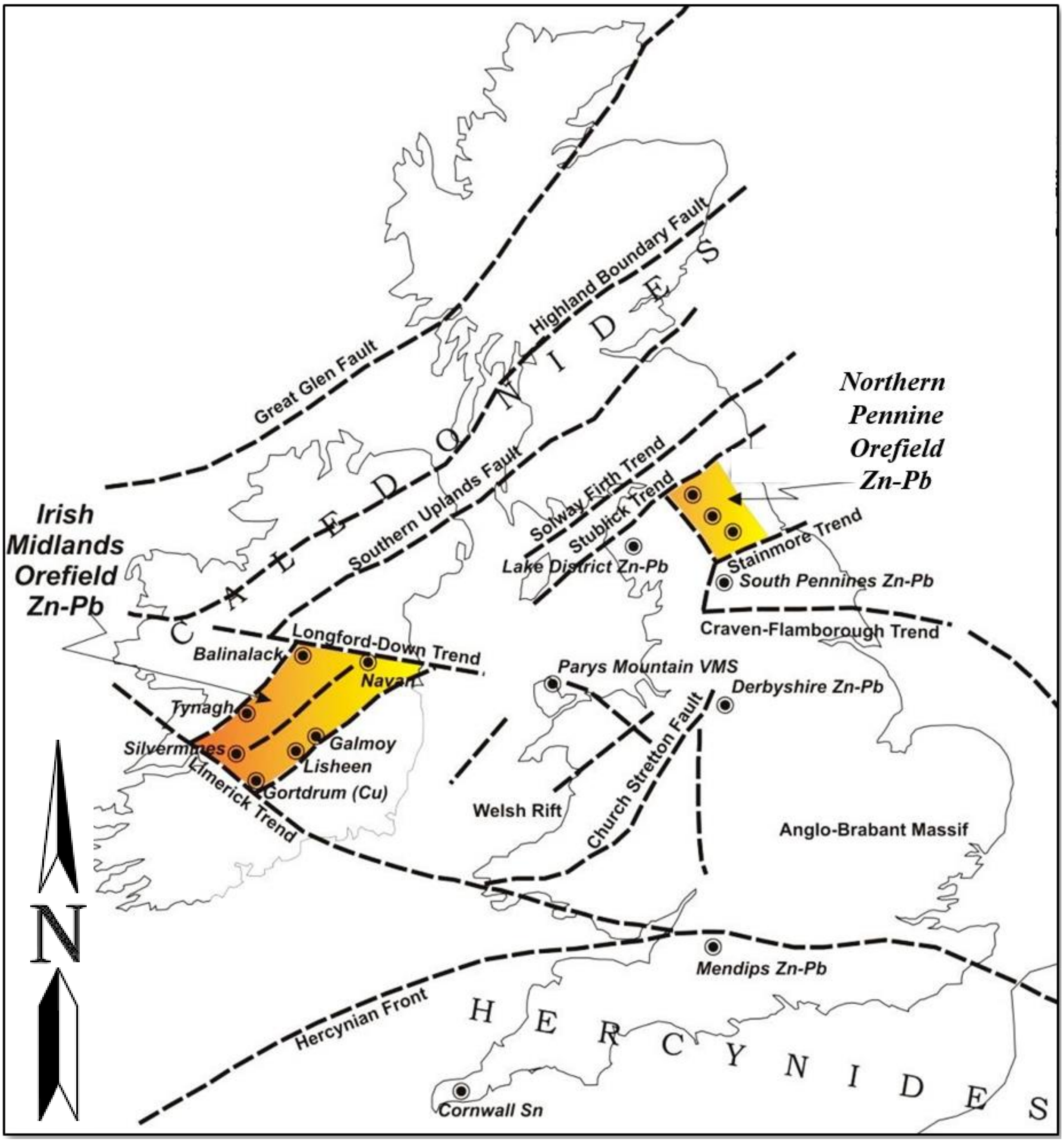


Figure 18 Location of the North Pennine Orefield in relation to the Irish Midland Orefield, where mineralisation appears to have taken place in a similar way **(Geology North, 2021)**

The NPO is subdivided into nine areas; Escarpment, Alston Moor, West Allendale, East Allendale, Weardale, Derwent Valley, Teesdale, Haydon Bridge area and Durham Coalfield (Dunham, 1948). Whiteheaps mine lies in the Derwent valley which is situated in the Derwent catchment and is shown in Figure 19. Formed approximately 290 million years ago, the lead ore and other minerals are found within steeply dipping mineral veins which typically tread NE to SW with a few exceptions.

The surface rock formations in the Derwent valley belong to the Carboniferous Limestone Series, consisting mainly of sandstone beds. The sandstone beds occur between the Crag and Upper Felltop Limestone (Burrows, 1981). The formations were laid down in delta channels during the Rogerley and Coalcleugh marine transgressions. All strata in the catchment are overlain by the Millstone Grit series and underlain by the Great Limestone. Most mineralisation has occurred in and around the Great Limestone; however, mineral deposition was not confined to strata at this level. Mineralising fluids often force their way upward through the hard beds resulting in deposition at higher stratigraphic levels (Dunham, 1948). The Hunstanworth and Shildon veins, the most exploited in the Derwent valley, were formed in this way(Burrows, 1981). The formation of ore shoots at high stratigraphic levels has been attributed to the thick sandstone beds in the Derwent catchment (Burrows, 1981). Most of the veins in the vicinity of Whiteheaps are dominated by fluorspar. Samples taken in 1941 from the Red Vein which lies west of Whiteheaps Shaft had 91.2 % fluorspar, 7.8 % silica, 1 % calcium carbonate and 2 – 3 % sulphides (Fairbairn and Northern Mine Research Society, 2000). These findings correspond with Derwent Mining Company records which show that galena content was rarely above 6 % (Fairbairn and Northern Mine Research Society, 2000). Other metalliferous ores such as chalcopyrite (Cu), sphalerite (Zn), and iron minerals (pyrite, siderite and limonite) have also been shown to be present in the Derwent valley, although not in commercial quantities. (Dunham, 1948; Chaffee, 1994; Fairbairn and Northern Mine Research Society, 2000).

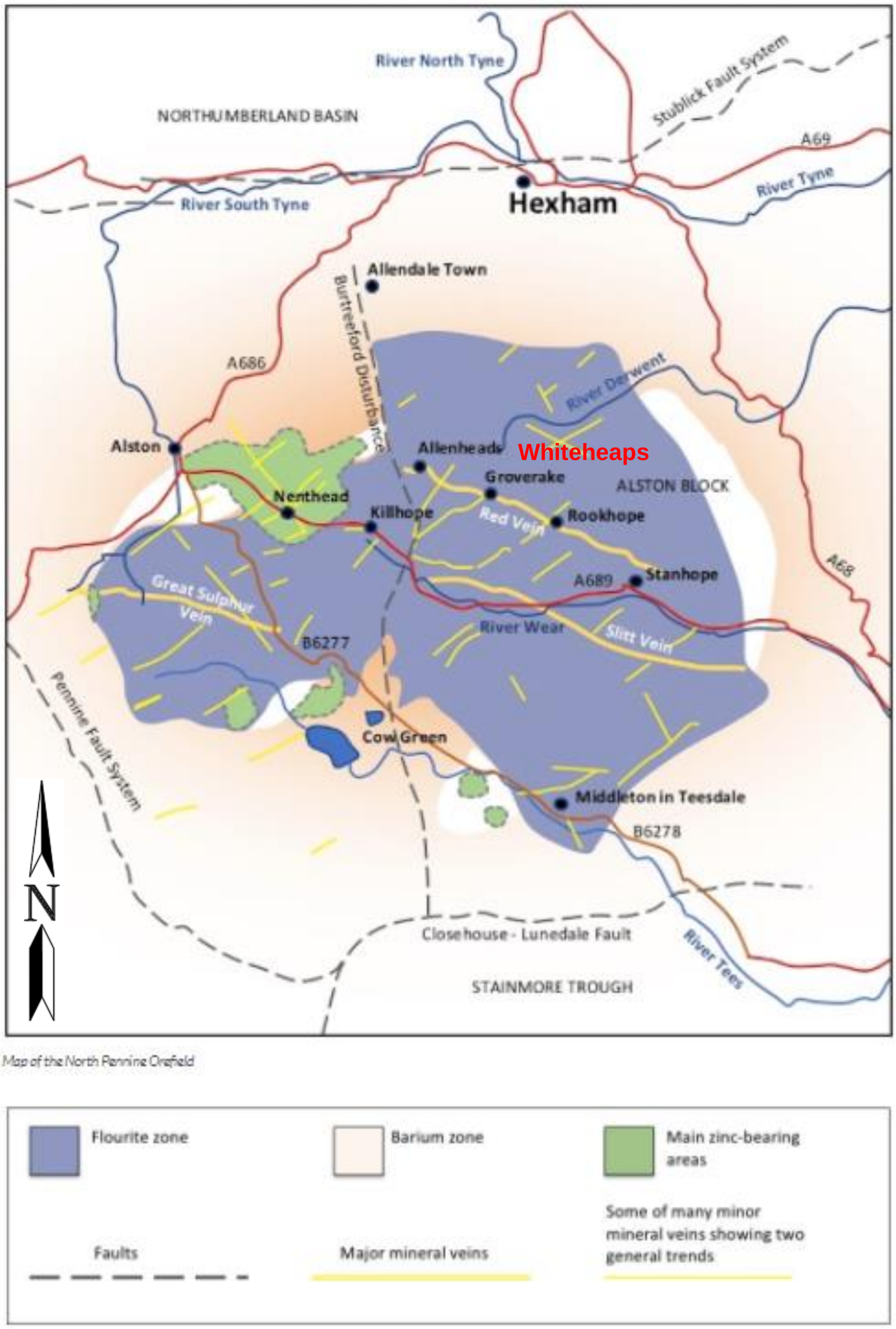


Figure 19 Geological map of the North Pennine Orefield, with location of Whiteheaps mine included in red text

(Geology North, 2021)

5.1.2 Mining history at Whiteheaps

For much of the 18th and 19th centuries, Great Britain was the principle producer of Pb and Zn in the world. At that time, the North Pennine Orefield was the key area for production (Dunham, 1948). Historical records for mining in the area date back to the 12th Century when deposits in the NPO were worked for Silver (Ag) and Pb (Burrows, 1981). Lead was the major ore mined in the Derwent catchment until late 19th century, when a use was found for fluorspar. Other commercially mined minerals include sphalerite (zinc ore), iron ores and barium minerals such as baryte and witherite.

Mining for Pb took place at Whiteheaps from at least 1690 when the first recorded lease of the mine can be found (Pickin, 1992). Production of galena (PbS) and at a later date fluorspar (CaF_2) continued from this time until operations ceased in 1920 (Pirt and Dodds, 2002). In 1938 the mines were reopened to allow for the production of fluorspar for use as a flux for smelting iron in the steel industry (Burrows, 1981). During this time the treatment plant at Whiteheaps was used to process fluorspar from other British Steel mines in the area (Burrows, 1981). The imported ore typically came from the Groverake Mine in the Rookhope Valley for which the gross mineralogy is very similar to Whiteheaps (Brian Young, Friends of Killhope, personal comm.) Mining at Whiteheaps finally ceased in 1989, the ore was regraded across the site and the buildings were removed (Pickin, 1992).

Mineral processing operations at Whiteheaps were typical of the time and involved dammed water (Sikehead dam) used to drive a water wheel that operated a ‘crusher’ that would break up ore (typically consisting of five percent lead ore) into uniform sizes so that it could be separated in a process named ‘washing’ or ‘dressing’ (Pickin, 1992, Milburn, 1987). The principle of the dressing process simply relied on the higher specific gravity of lead ore (galena) to waste material when crushed (Pickin, 1992). The galena would then be smelted on site at a high temperature (Morrison, 1998). Dressing floors were typically situated near a water source to power crushing wheels and remove waste. Waste material at Whiteheaps was probably originally piled up around the dressing floors; this was typical of lead mines at the time (Pickin, 1992). Following mechanisation of this process in the 19th century, the crushing of the ore produced a fine material known as 'slimes' which was 'washed' to discard the lighter waste material (Pickin, 1992; Morrison, 1998). This fine material was often dumped in 'Slime pits' and re processed to salvage the remaining ore (Morrison, 1998). Unwanted material from this process at Whiteheaps was later reprocessed for fluorspar over the course of the 20th Century (Chambers, 1992). Although the processes for crushing and separating the ore became mechanised, the locations at which these processes were carried out remained relatively similar throughout the operation of the mine and are shown in Figure 20 and Figure 21 (Chambers, 1992). Figure 20 shows that the majority of the mine workings and tailings piles were located around the bottom on the valley next to Bolts burn. A washing floor can also be seen at the northern end of the site.

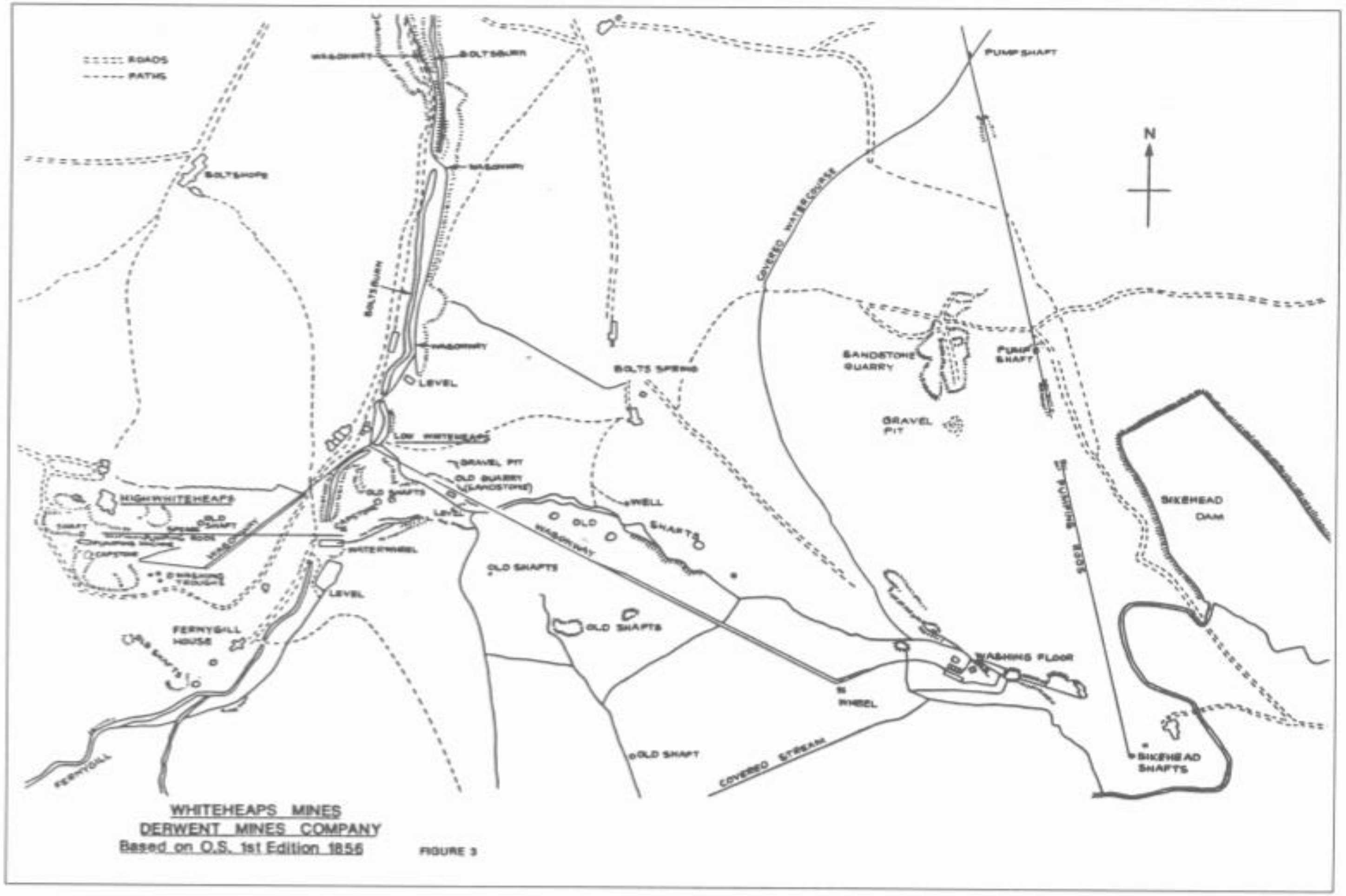


Figure 20 Photocopied Plan of Whiteheaps Mine site (Derwent mines company) Based on O.S 1st Edition circa 1856 (Chambers, 1992)

The map in Figure 21 shows a surface plan of Whiteheaps in the 1980s drawn by a chemist (George Pickin) who worked on the site for British steel over the 1970s and 80s. This image is reproduced here with permission from his family (Pickin, 1992). It is clear that the settling ponds, where the finest crushed ore would have collected, were situated just after the culverted entrance of Bolts Burn. It is also clear from fig 4 that during the 1970s and 80s the mine waste was distributed across the site with a waste dump situated to the north west of the site and the 'crushing' and 'settling tanks' in the centre. Pickin also records that there were further settling ponds and tips to the south east of the site, downstream of Bolts Burn (Pickin, 1992). Following the closure of the mine site in 1989 the buildings were broken down and removed and the mine waste was used in the regrading (the process of raising and/or lowering the levels of land) of the site, giving an appearance described as like 'a desert' (Fairbairn and Northern Mine Research Society, 2000). The regrading process resulted in the distribution of crushed waste across the site. During a personal communication between members of a local mine enthusiast group (Friends of Killhope) and the author of this study, it was claimed that top soil was brought in to cap the site following its closure by a company named Sherburn Stone Group, but this is currently unconfirmed (Brian Young, Friends of Killhope, personal comm., 2020)

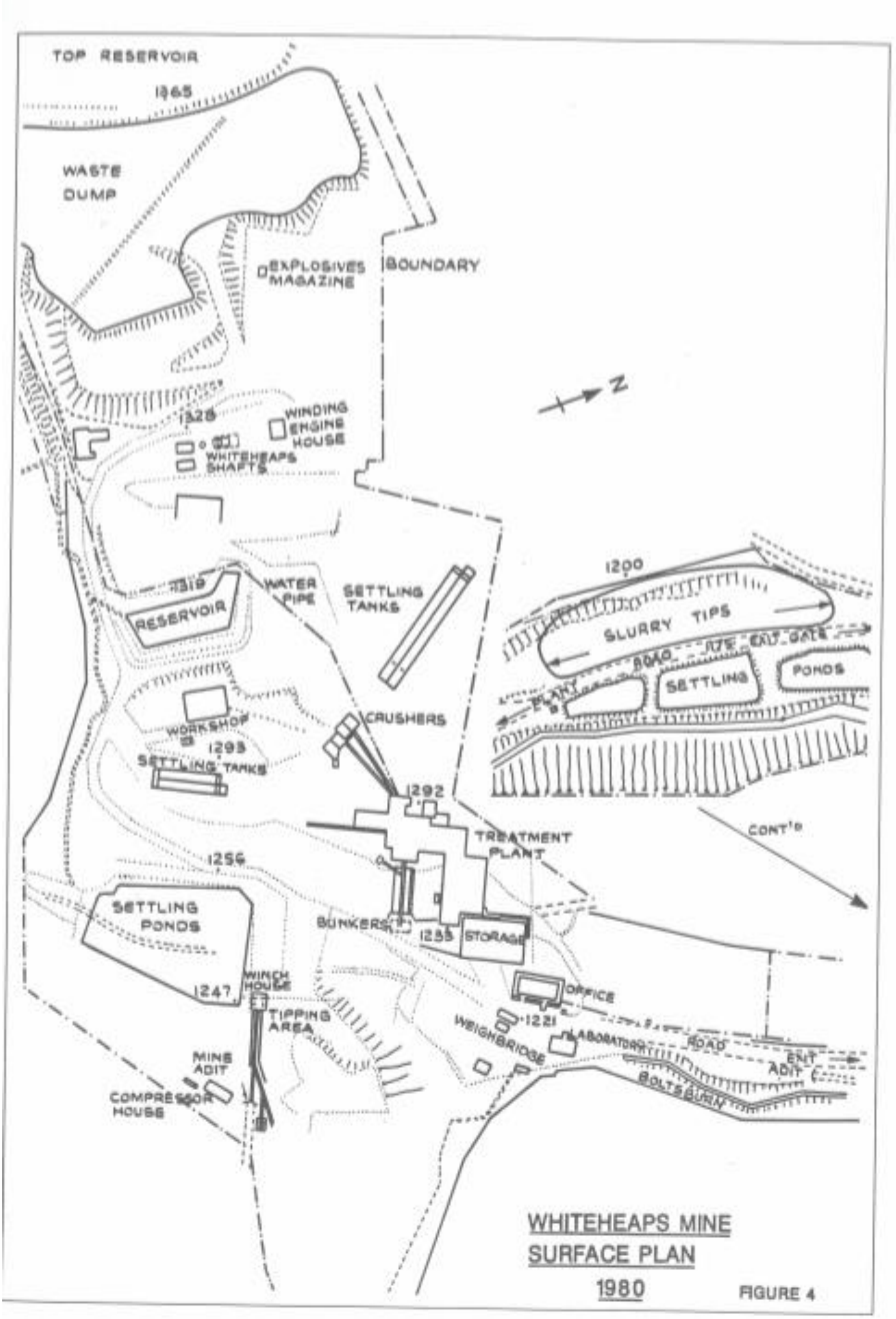


Figure 21 Photocopied surface plan of Whiteheaps mine complex 1980 **(Pickin, 1992)**

5.1.3 Derwent catchment and reservoir

Whiteheaps mine site is located within the catchment of the Derwent reservoir and lies to its East. The Derwent reservoir is 5.6 km long, has a surface area of 4 km^2, a maximum capacity of 50,000,000 m^3 and slopes to a maximum depth of 30 m near the dam. The reservoir and its tributaries are shown in Figure 22. The average rainfall of the catchment is 953 mm. Bolts Burn is one of the head waters, and the largest tributary, of the River Derwent which feeds the reservoir. Land use in the Derwent Catchment is primarily arable, with large areas of grassland and moorland used for sheep grazing. The main use of the river and reservoir today is for recreation including boating and angling. The primary abstractor of water is Northumbrian Water Limited, associated with public water supply. Small, local abstractions are made for domestic and agricultural uses. The river and reservoir water are brown in colour as a result of the high quantities of peatland in the draining area.

The catchment of the reservoir has experienced a degree of industrialisation due to the exploitation of its mineralisation. The catchment has been found to contain at least 40 mine shafts or adits, 4 smelt mills and 2 older bale sites from the mining, processing and smelting of Pb, or the mining and processing of fluorspar between 1475 and 1987 (Lord, 2015b). Fairbairn lists 26 levels and 24 shafts in the Derwent Valley as a whole, and of these, 20 levels and 20 shafts have been confirmed as lying in the catchment area (Fairbairn and Northern Mine Research Society, 2000).

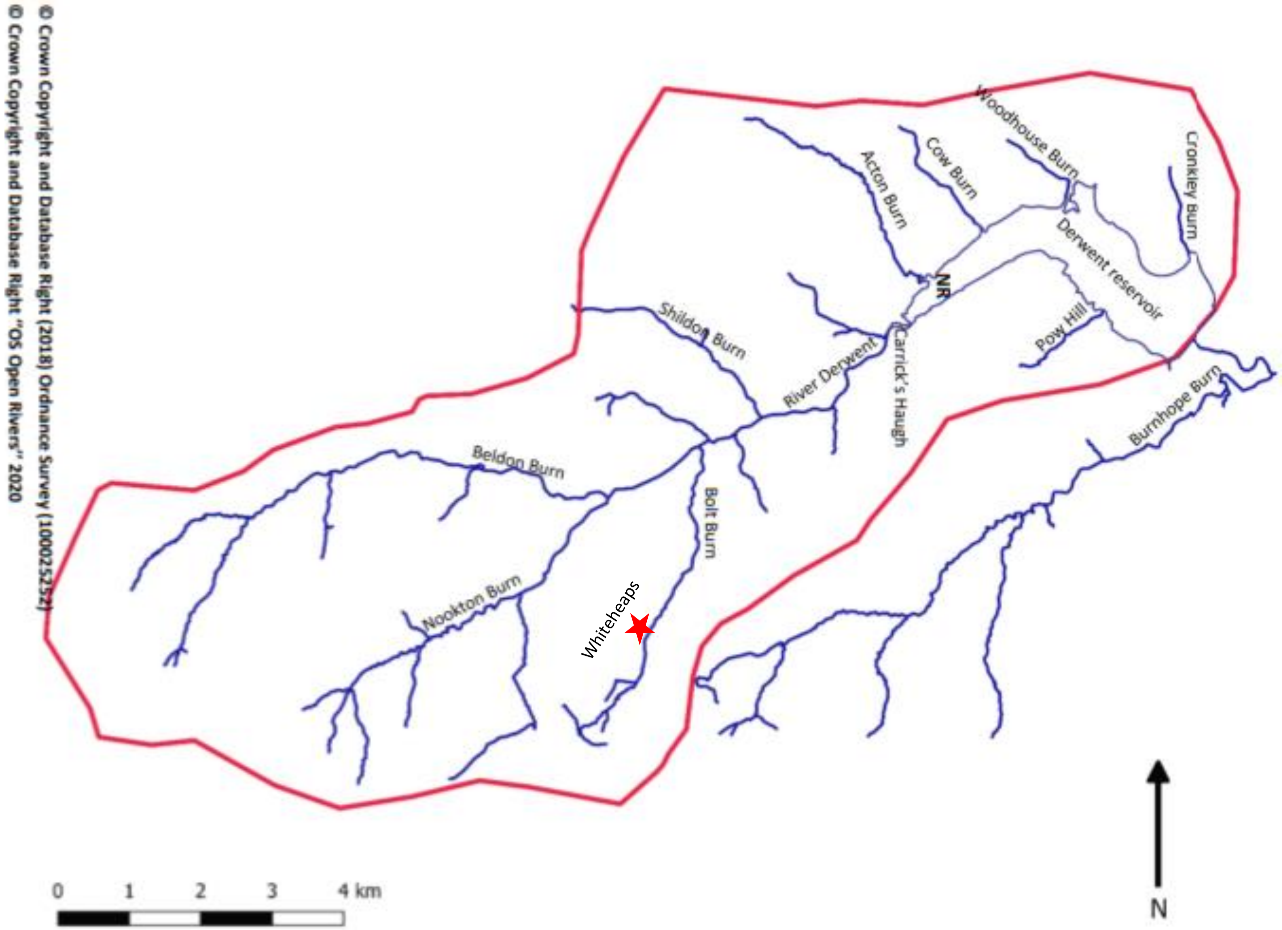


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Figure 22 Map of the Derwent Reservoir and catchment with Whiteheaps mining complex indicated with a star. The red line shows the draining area of the catchment. NR: nature reserve.

These include the Shildon Mine first recorded in 1475, through to the Whiteheaps Mine complex which as discussed closed recently, in 1989. In addition Fairbairn listed 2 bale sites (historical open smelting pyres) and 4 smelt mills located in the catchment, including two situated NE of the Derwent Reservoir (Fairbairn and Northern Mine Research Society, 2000). A comprehensive study of contamination levels in sediment, water and plants above, below and within the Derwent Reservoir showed that 7 0% of Zn, 97 % of Cd and 89 % of Pb total load entering the reservoir was retained in bottom sediments (Harding and Whitton, 1978). Total annual deposition to the reservoir sediments were presented, from which total loads entering the reservoir were calculated as 7.9 t Zn, 114 kg Cd and 2.0 t Pb per annum. Harding and Whitton also refer to unpublished work which showed that metal pollution originated mainly from Bolts Burn (Harding and Whitton, 1978). This study suggested that the origin of Zn and Cd was their dissolution in mine water pumped from an adit to drain the mine workings, whereas particulate Pb was associated with the discharge from the fluorspar processing plant located at Whiteheaps, which was still operational at the time.

An unpublished report (Aldred, 2013) that includes a description of flow and load calculations and source apportionment for Zn, Cd and Pb in the Upper Derwent, also found that Bolts Burn was a significant contributor to water metal loadings (Aldred, 2013; Lord, 2015). This report was obtained from the Environment Agency through a formal Environmental Information Request described by Lord (2015). Averaged total Zn and Pb concentrations in water samples from the upper River Derwent taken from this report are shown in Figure 23.

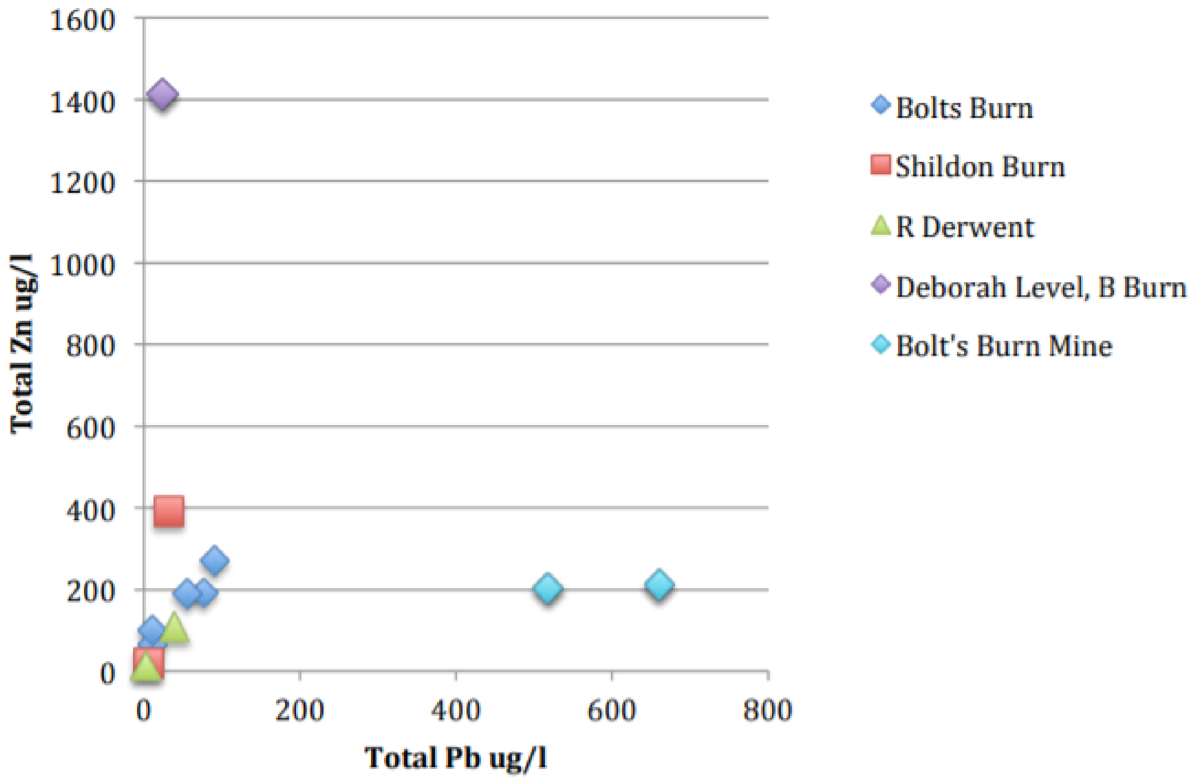

***Figure 23 Averaged total Zn and Pb concentrations in water samples from the upper River Derwent. Data from Aldred* (Aldred, 2013)**

5.2 Experimental

5.2.1 Pseudo-total concentrations of PTE in initial samples from Whiteheaps mine site

In June 2018 a site walkover on Whiteheaps mine complex was conducted to assess site suitability for phytostabilisation field trials; this involved taking six soil samples at locations at which a trial was considered to be suitable. Approximately 1 L soil sample was taken using a cleaned trowel at each of the sites and stored in a plastic container. Bulk samples were then stored in a refrigerator at 4 ºC. Each of the six soil samples were then homogenized using the coning method, and an approximately 20 g sub sample was taken. Each sub sample was then air dried for three weeks. Approximately 0.1 g of each soil sub-sample was then digested in a ‘reverse’ Aqua Regia mix of 2 ml HCL and 8 ml HNO_3. The method used during digestion followed procedures set out in British Standard BS EN 13650 (BSI Standards Limited, 2001). A CEM Mars 5 Microwave was used during the digestion process. After digestion, analysis of the ‘pseudo total’ concentrations of selected elements were carried out by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (iCAP 6000 SERIES, Thermo Scientific, UK) as described in the general experimental procedures chapter.

5.2.2 Collection and analysis of mine soil bulk sample

A 50 L bulk sample was collected in August 2018 in preparation for the pot trials discussed in the following chapter. At each location 10 x 5 L buckets were filled with equal amounts of material taken from 10 cm depth of soil from 10 places across both plots, using a clean trowel. Samples from buckets were then added to two 25 L containers by trowel in turn so as to provide an initial homogenisation of the bulk sample. A 1 L sample from each bulk sample was removed for analysis and sent to a commercial laboratory (NRM Labs) for the determination of soil characteristics, nutrient levels and the concentration of PTE.

5.2.3 In situ bulk density (dry)

Two determinations of bulk density were made at two locations at the whiteheaps mine site. In situ bulk density determinations were made using the excavation method

(BS EN ISO 11272:2017) but substituting water for sand as replacement material. A volume of 0.5-1 L was excavated in a levelled area, lined with plastic sheet and then filled with a measured amount of water. The mass of water required to completely fill the void was determined gravimetrically to the nearest gram using a portable balance. A density correction was applied for water at 10 °C. The excavated soil was transferred to a weighed plastic bag, field-weighed to determine wet mass, and sealed for transport to the laboratory. In order to determine final (or dry) bulk density, the entire contents of the bagged samples were transferred to aluminium trays for drying at 105 °C until no further mass loss occurred, and the weights were recorded.

5.2.4 Bolts Burn sediment analysis

Two sediment samples were collected from Bolts Burn in 2020. Bolts Burn is a large stream that flows into the Derwent reservoir. The stream flows through the southern end of the Whiteheaps mine site and is culverted. A gold panning sediment suction pump was used to collect two sediment samples (one upstream of the mine site and one below), following a method described in published literature involving similar sediment analysis (Lord and Morgan, 2003; Shepherd *et al.*, 2009). The gold panning suction pump was capable of lifting uncemented sediment particles with a diameter smaller than coarse gravel (< 20 mm) as re-suspended slurry from between boulders or by excavation of the streambed, and was effective in water depths approaching 1 m. The sediments were field sieved to < 150 μm (clay and fine silt fractions) using a stainless steel British Standard sieve and kept under 15 cm of their own river’s water in a high density polyethylene bucket. On return to the laboratory, the samples were allowed to settle, then excess water was decanted and the sediment was stored in the cold room at 4 °C. The sediment samples were then dried to a constant weight, homogenised and crushed in a ball mill (Retsch PM100, Hope Valley, UK). The samples were prepared for analysis of their pseudo-total concentrations of PTE following the method outlined in section 4.4.1. Subsamples of 0.25 g were analysed in triplicate for both samples alongside CRM 701. An ICP-OES (iCAP 6000 SERIES, Thermo Scientific, UK) was used to determine the samples pseudo total concentrations of PTE as described in the general experimental procedures chapter.

1.1.1 Organic waste amendment collection and preparation

Soil amendments were collected on site during August 2018 in 2 x 25l containers. The DWTR was collected from Mosswood Water Treatment Works (WTW) (Northumbrian Water Ltd.) At Mosswood water treatment works (WTW) the coagulant ferric sulfate is added to screened water to encourage flocculation of suspended colloids. Sodium hydroxide is then added and the resulting waste flocs are removed as a ferric hydroxide sludge which is temporally stored on site for dewatering and then taken from the site as a waste by-product (Johnson *et al.*, 2018).

The GWC (BSI PAS100: 2005) was processed at Codlaw Hill (DJ and SJ Recycling) using the windrow composting system, which involves the piling of waste in long narrow rows or piles followed by maturation and screening of the material (Maheshwari *et al.*, 2014).

Both amendments were collected from large piles of processed material. Small amounts of soil amendment were added to 25 L containers from different parts of the piles to provide a representative sample. A 1 litre sample of each amendment was collected from both 25 litre containers of each amendment and sent for commercial lab analysis (NRM Labs) for the determination of soil amendment characteristics, nutrient levels and the concentration of PTE. Bulk amendments were then refrigerated at 4°C in sealed containers.

5.3 Results

5.3.1 Pseudo concentrations of PTE in initial samples from Whiteheaps mine site

The location of the six sample locations is shown in Figure 24. Previous sampling had been carried out by Northumbrian Water ltd, and the sample locations for this work are also shown. The certified reference material (AEA-413 Algae) was also digested and analysed in the same way to provide confidence in the results. This certified reference material was selected due to the absence of a material with a better matrix match to the samples. The results of the pseudototal concentrations of PTE in the certified reference material and their indicative values can be found in Table 17. The reference material was analysed only once i.e. not replicated (n=1). The recovery difference (%) between the obtained values of the reference materials and the certified values were low, indicating confidence in the method for determination of pseudototal PTE.

Table 17 Results from reference material analysis (AEA-413) by ICP – OES (n=1)

	As	Cd	Ni	Pb
Obtained value, mg/kg	106	192	101	222
Certified value (AEA-413 Algae reference material), mg/kg	127	204	113	242
Recovery difference, %	17	6	11	8

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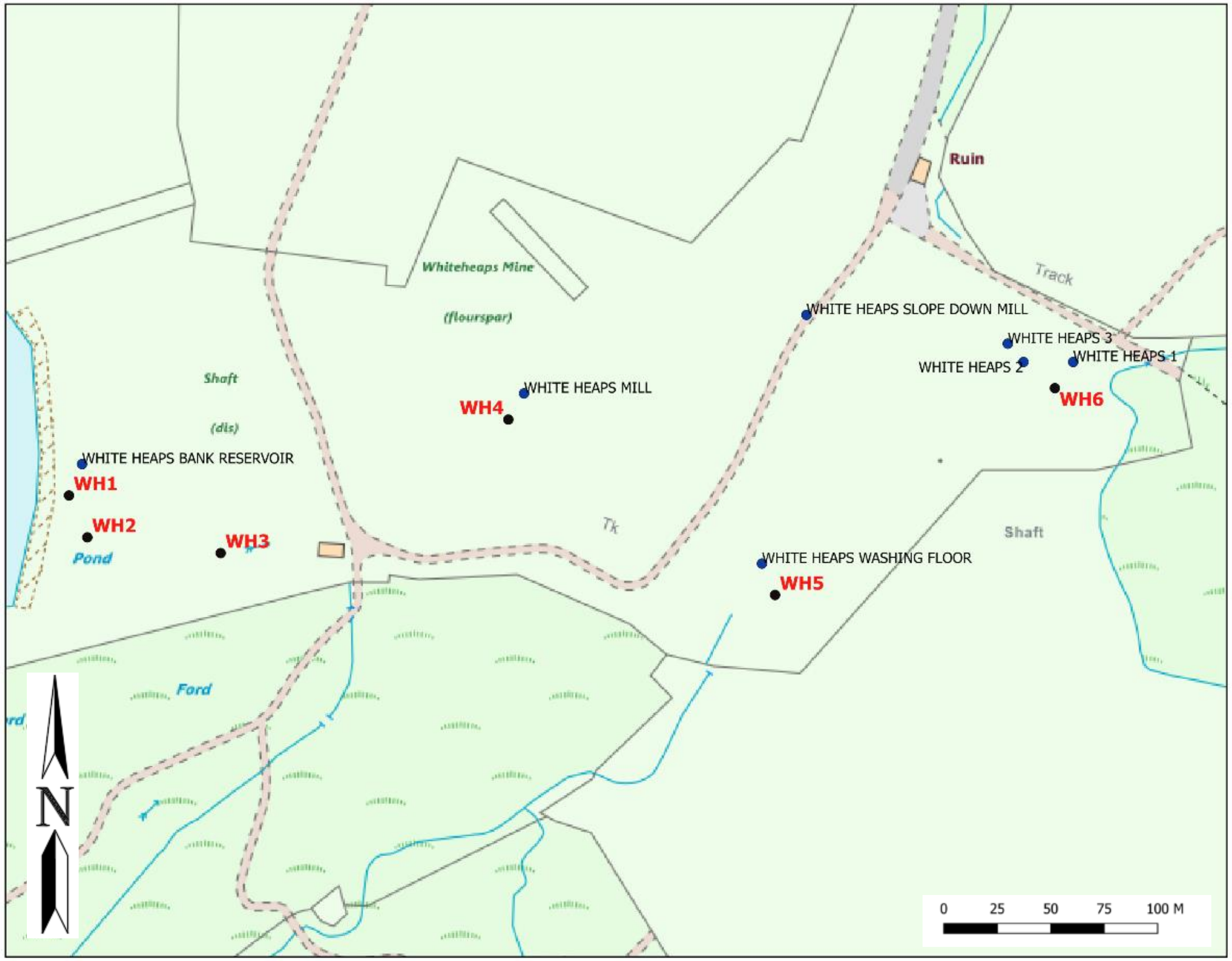


Figure 24 Sample locations at Whiteheaps historic mine site used during this study (WH1-6) with sample locations of a previous study by Northumbrian Water

All six samples were successfully analysed for pseudo-total concentrations of As, Cd, Cr, Cu, Pb and Zn, the results of which are shown in Table 18. The analysis of the samples was carried out without replicates due to time and budget constraints. The intention for the results was to provide an indication of the distribution of PTE across the site.

Table 18 Pseudototal results from ICP – OES analysis of six mine soil samples taken from Whiteheaps. All results shown are in mg/kg dry weight. Samples not analysed with replicates as results were intended to supply an initial indication of concentrations throughout site.

Site ID	As	Cd	Co	Cr	Cu	Ni	Pb	Zn	Grid Ref
WH1	37	2	5	22	793	10	13700	577	NY945465
WH2	24	5	8	23	421	14	5640	1290	NY945465
WH3	39	6	7	19	605	9	14300	2440	NY946465
WH4	24	5	6	27	260	12	7600	1370	NY947465
WH5	74	22	50	45	1770	84	6730	14300	NY948465
WH6	41	1	2	21	213	3	14300	174	NY949466

The concentrations of PTE were found to be both in the typical ranges for historic metalliferous mining tailings in England (Maskall and Thornton, 1998; Hudson-Edwards *et al.*, 2008) and above concentrations found in typical soils present in upland areas located near to the site in Scotland (Towers *et al.*, 2006). High concentrations of PTE associated with historic metal mining were found in all of the six samples analysed. Concentrations of Zn showed the greatest variance across the mining site. Of particular note were sites WH5 (14390 mg/kg Zn) and WH6 (174 mg/kg Zn) with WH5 having approximately 88 times the Zn concentrations of WH6.

The trend for the WH5 site to have greater PTE concentrations is also found in the results for Cu and Ni. This may be explained by several factors. Site WH5 sits at the lowest altitude of all the sites and would therefore receive the greatest amount of leachate run off from the tailings. The site was also used as a 'Washing/Dressing Floor' during earlier mining operations as shown in Fig 3. This is the area where the mined ore would be crushed and waste spoil discarded and would therefore contain a greater amount of fine crushed substrate with high PTE concentrations (Morrison, 1998). A historical map of the mine site (fig 3) dated 1860 shows buildings potentially used in this process; furthermore, as the 'Washing/Dressing Floor' was typically built at the nearest lowest point from the mines and close to running water, the former use of site WH5 can be assumed (Morrison, 1998). The site at WH5 was later used as a 'settling pond' (shown in Fig 4) which is a concrete structure where the fine crushed material would be left to settle out of the water as a pollution control measure. This use would have also resulted in greater concentrations of PTE impacting the area, studies that have analysed mine wastes at different particle sizes have shown the finer the size fraction of the waste material the greater the concentration of PTE (Davies and White, 1981b).

Site WH3 was found to have the higher Pb concentrations (14300 mg/kg) but relatively lower Zn concentrations (2440 mg/kg) when compared to site WH5. Overall the data obtained correlates well with results from a previous study of PTE concentrations at Whiteheaps conducted by Northumbrian Water Ltd (NWL), which can be found in Appendix 10.1. For example, Pb concentrations found in both studies do not exceed 19000 mg/kg, and are also not found to be less than 5000 mg/kg. However, there are some discrepancies when comparing the two data sets. For example, this study found far greater concentrations of Cd and lower concentrations of Ni when compared to that conducted by NWL. Discrepancies in the results are likely to be due to the heterogeneity in PTE concentrations typical on historic mining sites (Rodríguez *et al.*, 2009). The lack of replicates and appropriate quality control during the initial sample analysis means that further sample analysis is required to provide reliability.

5.3.2 Field trial site suitability

Site suitability for phytostabilisation field trials was assessed through a combination of the data obtained through PTE analysis described previously and a walk-over field survey. Some of the potential sites were found to have factors that would make field trials less appropriate. For instance, site WH6 was found to have a steep gradient, greater exposure to weather and rabbit habitation as shown in Figure 25, making it unsuitable for a preliminary field trial.

Sites WH3 and WH5 were determined as the most suitable for field trial sites due to their fulfilment of a number of conditions including accessibly, topography and concentrations of PTE. Site WH3 and WH5 also show variance in proportions of PTE and potential variance in topography, moisture content and substrate composition among other factors.

Figure 25 Sample site WH6 NY949466 with steeper gradient and greater rabbit habitation than other potential sites.

5.3.3 Characterisation of the two selected mine soils to be used in further trials

The results of the commercial analysis and the in situ bulk density analysis of the two mine soil bulk samples used throughout this study are shown in Table 19.

Table 19 Chemical characteristics of the mine soils used in this study WH3 and WH5. All results are on a 'dry matter' basis

Determined	Unit	WH3	WH5
pH	Value	6.7	7.1
Textural Classification	%	Sandy Silt Loam	Silty Clay Loam
Sand (2.00-0.063mm)	%	45	14
Silt (0.063-0.002mm)	%	41	61
Clay (<0.002mm)	%	14	25
In situ bulk density (dry)	g/cm3	1.84 ±0.003 (n=2)	1.40 ±0.001 (n=2)
Copper	mg/kg	545	890
Zinc	mg/kg	1850	5150
Lead	mg/kg	13900	9110
Arsenic	mg/kg	40.7	66.2
Cadmium	mg/kg	5.07	6.3
Chromium	mg/kg	18.6	23.1
Nickel	mg/kg	<10	27.6
Nitrate Nitrogen	mg/kg	<1	<1
Ammonium Nitrogen	mg/kg	<1	<1
Total Nitrogen	% w/w	0.09	0.14
Total Phosphorus	mg/kg	135	223
Available Phosphorus	mg/l	<2.5	<2.5
Total Potassium	mg/kg	4980	9750
Available Potassium	mg/l	30	34
Available Magnesium	mg/l	69	58
Oven Dry Matter	%	77.7	83.9
Organic Matter	%	8.7	2.2

The pH of the mine soils was found to be circum-neutral for both WH3 (pH 6.7) and WH5 (pH 7.1). Although this is lower than the optimal pH for the growth of reed canary grass (RCG), which has been found to be in the range of 7.7 - 8.2, it is clear that the site is not affected by an acidic pH potentially increasing the mobility of PTE (Young, 2013;

Usťak *et al.*, 2019). The difference in the physical form of the mine soils gives an indication of their origins and historical uses, as shown in Figure 26 Photocopied surface plan Whiteheaps mine complex 1980 with field trial locations (Chambers, 1992)Figure 26.

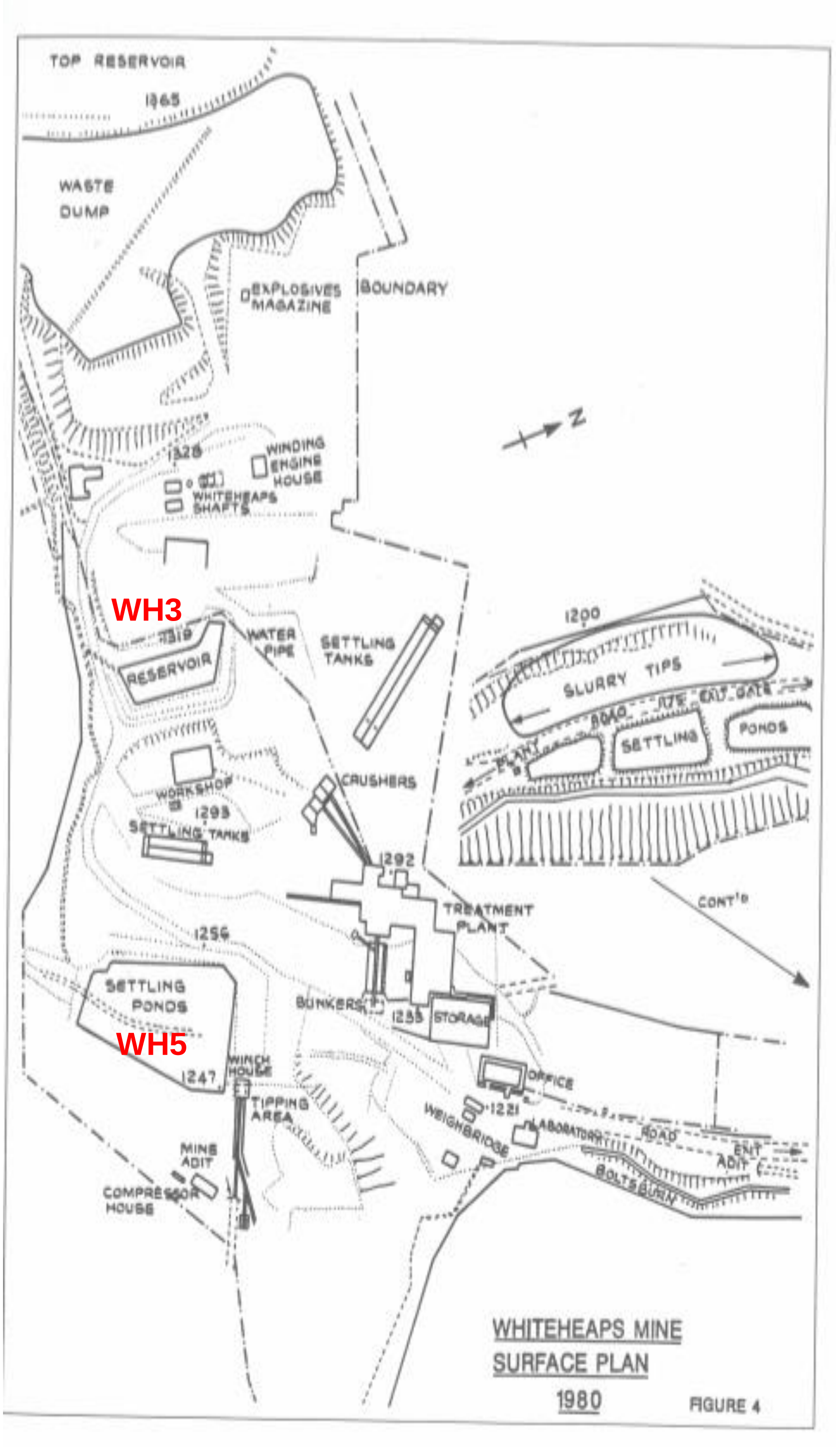


Figure 26 Photocopied surface plan Whiteheaps mine complex 1980 with field trial locations (Chambers, 1992)

Site WH3 was found to be mostly composed of material with a size fraction associated with sands. The material of this site is most likely made up of regraded crushed ore. At WH5, material was processed at the dressing floor and then reprocessed as fluorspar 'slimes' in the 'settling ponds' (shown in fig 9); this location was found to be majority silt and some clay. Although fine tailings can have much better water retention capacity, they are very liable to surface compaction and even cementation processes which can combine to form an impenetrable surface barrier limiting plant establishment (Tordoff *et al.*, 2000).

The bulk density measurements for WH3 and WH5 were 1.84 ±0.003 and 1.40 ±0.001 g/cm^3 $^($n=2) respectively. McGreger *et al*. found a similar density range in a study researching the properties of sulphide-bearing tailings (McGregor and Blowes, 2002). The textual classification for WH3 is a sandy, silt loam which typically has a bulk density of 1.55 – 1.75 g/cm^3 according to the on the work of Morris and Lowery (Morris and Lowery, 1988). The counterintuitive result for site WH3 to have both the greater bulk density and particle size suggests that the site is more compacted than WH5. There is evidence of farm vehicles driving on site WH3, shown in Figure 27, and the regrading process at the closure of the mine complex may have led to compaction.

Figure 27 Sample site WH3 NY946465

The chemical analysis of the two selected mine soils show that the sites are impacted with several PTE by the mine workings, with values in excess of relevant soil guidelines. The values for Cu are in excess of the agricultural soil guidelines (100 mg/kg) for both WH3 (545 mg/kg) and WH5 (890 mg/kg) at a pH >5.5 (CoGAP, 2009). The found values for Zn are also well in excess of the agricultural soil guidelines (200 mg/kg) for both WH3 (1830 mg/kg) and WH5 (5150 mg/kg) at a pH >5.5(CoGAP, 2009). Concentrations of Pb were found to be in excess of the category 4 screening levels (displayed in the previous chapter) (Commercial) for both WH3 (13,900 mg/kg) and WH5 (9110 mg/kg) (CL:AIRE, 2014). The concentrations of As, Cd, Cr and Ni were all found to be within the limits of relevant guideline values discussed previously for commercial land (DEFRA, 2012; CL:AIRE, 2014; LQM/CIEH S4ULs, 2015). All investigated PTE apart from Cr are above the average for rural soils as found by the UK Soil and Herbage Pollutant Survey (Environment Agency, 2007).

As the mine soils are well in excess of some of the regulatory limits designed to safeguard human health from chronic exposure to soil contamination for some of the PTE investigated, it is clear that the site qualifies for further investigation and management for remediation (Environment Agency, 2009).

Nutrient analysis of the mine soils showed that total nitrogen was found to be well below levels ideal for plant growth, which is typical of historic mine sites (Tordoff *et al.*, 2000; Trzcińska and Pawlik-Skowrońska, 2008). Site WH3 was found to have slightly less total nitrogen, at 0.09 % w/w, than site WH5 at 0.14 % w/w. The plant-available forms (ammonium or nitrate) of nitrogen were both found to be below the limits of detection for sites WH3 and WH5. Reed canary grass has been reported as very sensitive to nitrogen (N) fertilization (Landström *et al.*, 1996; Lewandowski *et al.*, 2003; Wrobel *et al.*, 2009). The levels of P and K in the mine soils showed a similar pattern, where already low total nutrient concentrations were found to be mostly in 'unavailable' forms. Site WH5 was found to have a slightly greater concentration of total potassium when compared to site WH3 and does have more vegetation growing in the area, shown in Figure 28. The concentrations of available P and K are so low that according to the DEFRA index for soil nutrient analysis found in the RD209 fertilizer manual both soils would obtain an index level of 0 (DEFRA, 2010). This means that the addition of P and K would be 'highly likely' to result in a yield increase response for grass crops (DEFRA, 2010). The concentrations of available Mg conversely are just within the RD209 fertilizer manual index level of 2 meaning that further addition of Mg is unlikely to result in a yield response (DEFRA, 2010).

The presence of more vegetation at site WH5 may also be the result of a water feed from the previously discussed adit which flows across the WH5 site. Through the bulk density measurements, site WH5 was found to have a moisture content of 25 % whereas site WH3 was found to have a moisture content of 10 %.

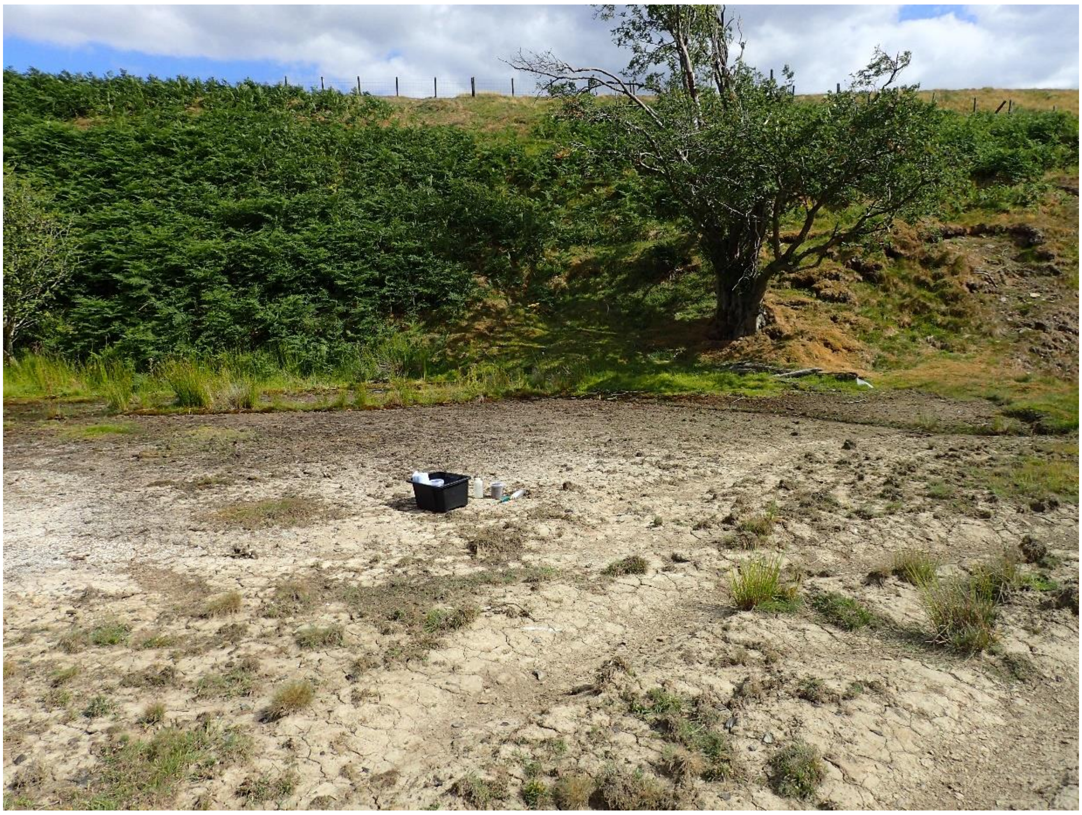


Figure 28 Sample site WH5 NY948465

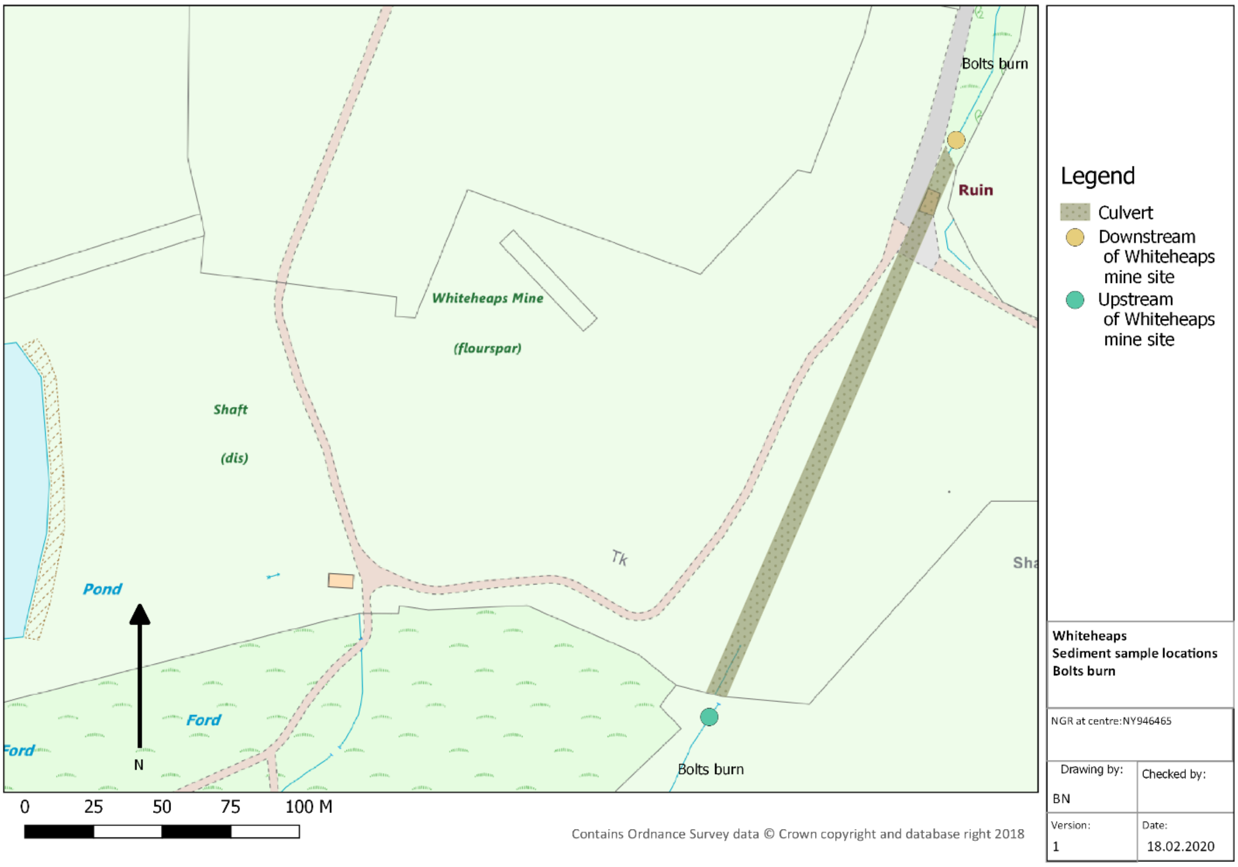
5.3.4 Botanical survey results of selected sites

The following is a summary of a botanical report conducted on the Whiteheaps mine site by Dr Janet Simkin MCIEEM, in May 2019 at the request of Northumbrian Water ltd (Simkin, 2019). The full report can be found in Appendix 10.5. The report was conducted in order to determine if field trials would disturb metallophyte species that are indicators of calaminarian grasslands. The survey found Whiteheaps mine complex to have little calaminarian grassland or other vegetation characteristic of metal-contaminated ground due to disturbance caused by the reprocessing of the tailings for fluorite and the landscaping of the site following the mines closure.

The report concluded that the area around site WH5 exhibited some wetland plants such as toad rush (*Juncus bufonius*) due to it being fed by water from the mine workings (adit). The area around site WH3 was found to be made of a fine mineral substrate, with small stones and slag fragments sorted to the surface. The site was described as very dry and well compacted by vehicles and rabbits making it an unsuitable site for the growth of reed canary grass. The site was found to have sparse vegetation which was connected to soil compaction. No species of conservation importance were found.

5.3.5 Bolts Burn sediment analysis

In order to determine the impact that the unvegetated mine spoil may be having on the water course (Bolts Burn) connected to the Whiteheaps mine site, sediment samples were taken and analysed for their concentrations of PTE. The location of the two sampling points for the sediment samples taken from Bolts Burn are shown in Figure 29. The culvert was found to be intact and constructed in a way that the stream could not run over across the mine site.



Contains Ordnance Survey data © Crown copyright and database right 2018

Figure 29 Map of Whiteheaps mine site with location of sediment sample sites and culverted section of Bolts Burn

5.3.6 Quality control for pseudototal analysis of sediment samples

The CRM (701 lake sediment) was used to assess the analytical performance of the pseudo total determination of PTE in the two sediment samples. The amounts of pseudo total PTE in CRM 701 were certified values. All analyses were carried out in triplicate. A blank solution was also analysed at regular intervals during the ICP-MS analysis as a quality check. The pseudo total PTE concentrations, standard deviations and % recoveries are listed in Table 20. The % recoveries for the pseudo total concentration were all below ± <25%, indicating good result precision.

Table 20 Comparison of the obtained and certified pseudo total values of PTE (mg/kg) in the CRM 701 lake sediment (n=3)

Element	**Mean Obtained values mg/kg**	**Standard Deviation mg/kg**	**Certified values mg/kg**	**Uncertainty of indicative value mg/kg**	**Recovery %**
Cd	14.6	0.3	11.7	1	125
Cr	296	9.7	272	20	109
Cu	266	3.8	275	13	97
Ni	105	3.0	103	4	102
Pb	148	1.9	143	6	103
Zn	518	6.2	454	19	114
-	-		-	🟩	± <25%
-	-		-	🟧	25% - 50%
-	-		-	🟥	± 50+%

5.3.7 PTE in the Bolts Burn sediment

Table 21 provides the pseudo total values for PTE found in the Bolts Burn sediment samples. The analysis was carried out successfully with very little variation in the distribution of the analytes found between the replicates of the samples. In the absence of UK fresh water sediment guidelines and in preference to the previously discussed soil quality guideline values, (DEFRA, 2012; CL:AIRE, 2014) the sediment data set was evaluated using the Ontario aquatic sediment quality guidelines (Persaud *et al.*, 1993).

The Ontario aquatic sediment quality guidelines establish three levels of effect - No Effect Level (below the lowest effect level), Lowest Effect Level and Severe Effect Level. The guide states that if an element concentration is found below the' No Effect Level' it is at a level which is so low that no contaminants are passed through the food chain, whereas if an element concentration is found to be above the Lowest Effect level, the PTE may be having long-term effects on sediment-dwelling organisms (Persaud *et al.*, 1993). The guide explains that whilst contamination in sediment that exceeds the Lowest Effect Level may require further testing, at the Severe Effect Level a management plan may be required that includes controlling the source of the contamination and removing the sediment (Persaud *et al.*, 1993).

Table 21 Comparison of the pseudo total values of PTE (mg/kg, n = 3) found in Bolts Burn sediments taken below and upstream of Whiteheaps historic mine site, standard deviation (SD; ±)

Bolts Burn sediment analysis					Ontario threshold values	
Element	Upstream of Mine	SD ±	Below mine	SD ±	*Lowest effect level (LEL)*	**Severe effect level (SEL)**
Cd	0.40	±0.01	0.40	±0.00	*0.6*	**10**
Cr	**121**	±1.17	**133**	±0.76	*26*	**110**
Cu	10.7	±0.06	*30.6*	±1.96	*16*	**110**
Ni	*35.6*	±0.47	*32.2*	±1.03	*16*	**75**
Pb	*161*	±2.57	**1240**	±9.52	*31*	**250**
Zn	*538*	±12.1	*690*	±3.47	*120*	**820**

The 'below mine' values for Cd and Ni shown in table 4 were found to be very similar to the upstream values. The mean concentration of Ni was found to be slightly lower (32.2 mg/kg) in the sediments taken from below the mine when compared to those upstream (35.6 mg/kg) of the mine site. The value for Cd in both the sediment samples was found to be lower than the Ontario threshold value (OTV), suggesting that the presence of these elements are having little to no effect on sediment dwelling organisms.

A study conducted by Obolo analysed sediments in rivers feeding into the Derwent reservoir and found pseudo total Ni concentrations ranging from 11.6 – 39.9 mg/kg (Obolo, 2019). Although the found values for Ni in both sediment samples are above the OTV LEL, the results suggest that the Ni concentrations found are background concentrations reflecting the local geography. Soils in England have been found to have Ni concentrations ranging from 2.1 – 89.0 mg/kg (Environment Agency, 2007). The OTV guidelines suggest that In areas where local background levels are above the LEL, the local background level will form the practical lower limit for management decisions (Persaud *et al.*, 1993). The concentrations of Cr, Cu and Zn were found to be slightly elevated in the below mine site sediment samples when compared to the upstream samples, with Cu showing the most considerable increase of the 3 analytes (Upstream – 10.7 mg/kg, Below mine site – 30.6 mg/kg). All three of the elements exceeded the OTV LEL, with Cr exceeding the SEL value.

The analyte which showed the greatest increase in concentration in the below mine site sample was Pb. The concentrations of Pb were enriched in the below mine site sample by a factor of almost 10 (1240 mg/kg) when compared to that of the upstream sediments (161 mg/kg). The found values for Pb exceed the SEL by nearly 1000 mg/kg, resulting in Pb becoming the element associated with this study that is of most concern for sedimentary lifeforms. The below mine site Pb concentrations found by this study are also consistent with previous similar studies which have found that streams and rivers in former lead mining areas have fine-grained sediments that typically contain more than 600 mg/kg Pb (Shepherd *et al.*, 2009).

The pseudo total concentrations of PTE in river sediments impacted by historic mining operations found within the literature are listed in Table 22. Comparing the results of the sediment analysis obtained by this study with those published in the literature shows that whilst the sediments impacted by Whiteheaps do not have values as great as those found elsewhere, they are within the broad range of typical found values.

Table 22 Concentration (mg kg-1) of potentially toxic elements in river sediments that are impacted by historical mining

Rivers	Potentially toxic elements				Ores mined in the catchment	Reference
	Cd	Cu	Pb	Zn		
River Twymyn, Wales, UK	1.5 - 44	44 - 2560	593 - 6410	159 - 6960	Pb and Zn	(Byrne *et al.*, 2010)
River Wear, UK		10 - 340	20 - 15000	40 - 1500	Barite, fluorite, Pb and Zn	(Lord and Morgan, 2003)
Mala Panew River, Poland	0.18 - 559	3.97 - 483	36 - 3310	126 - 11200	Pb and Zn	(Aleksander-Kwaterczak and Helios-Rybicka, 2008)
River Tinto, Spain	1 - 23	1800 - 26500	3200 - 16500	600 - 67300	Au, Ag, Cu, Pb and Zn	(Galán *et al.*, 2003)
Lahn River, Germany		18 - 152	19 - 232	63 - 741	Cu, Fe and Pb	(Martin, 2019)
Przemsza River, Poland	13 - 154		670 - 9450	765 - 28200	Au, Pb and Zn	(Strzebońska *et al.*, 2017)
River Derwent, UK	0.6 - 13.8		96 - 3120	82 - 2760	Pb and fluorite	(Burrows and Whitton, 1983)

The increase of Pb concentration in the samples taken below the mine site suggest that the Whiteheaps mine site is enriching Bolts Burn and thus the Derwent reservoir with Pb. Although the culverted part of the stream may avoid the majority of the tailings, photographic evidence of run off from the mine site into the burn can be seen in Figure 30.

Figure 30 Evidence of erosion and run off from Whiteheaps mine site into Bolts Burn, resulting from the movement of water due to rainfall. Blue arrows show noted direction of travel of eroded sediment (February 2020)

Greater concentrations of both Pb and Zn have been found in Bolts Burn sediments further downstream from Whiteheaps in previous studies. Lord (2015) sampled Bolts Burn at the confluence before it joins the river Derwent and found Pb concentrations of 8600 mg/kg and Zn concentrations of 1280 mg/kg. This study analysed river sediments across the Derwent reservoir catchment and found that Bolts Burn had the greatest Pb concentrations (Lord, 2015b). High concentrations of fluorspar were also found in Bolts Burn sediments, and the study concluded that Bolts Burn is a major source of particulate Pb contamination, probably the result of the active disposal of finely ground wastes from the fluorspar processing plant at Whiteheaps. (operational 1930-1980).

It is important to note that the described sediment collection method focuses on capturing deposited particulate matter, and no effort has been made to analyse dissolved concentrations of elements within the water of Bolts Burn itself, or to recover suspended particulate matter for analysis. Harding and Whitton hypothesised that whilst Pb mostly occurs as particulate material and is deposited rapidly (particularly as sediment in the Derwent reservoir), Zn is dissolved and remains in suspension throughout the watercourse (Harding and Whitton, 1978). The method described in this study also does not take into account sediment accumulation rates, so the time span of contamination (years/decades/centuries) represented by the two samples is unknown (Shepherd *et al.*, 2009). However, the increase in Pb concentrations in the below mine site sediment sample still provide a clear justification for the implementation of a management plan that aims to stabilise the eroding mine waste to prevent further enrichment of the waterway.

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5.4 Organic waste soil amendments

In order to provide vital nutrients and potentially reduce the mobility of PTE, two organic waste/waste-derived amendments were selected that were produced locally to Whiteheaps historic mine site. As discussed in section 2.19 of this study, organic waste amendments are increasingly being seen as a sustainable option for contaminated land remediation (Bolan *et al.*, 2011) .

5.4.1 Green waste compost

The PAS100 GWC used throughout this study was processed at Codlaw Hill (DJ and SJ Recycling) using the windrow composting system (shown in Figure 31), which involves the piling of waste in long narrow rows or piles, followed by maturation and screening of the material (Maheshwari *et al.*, 2014). The results from commercial analysis of a sub sample taken from the bulk samples of the GWC used throughout this study are presented in Table 23.

Figure 31 Codlaw Hill (DJ and SJ Recycling) processing green waste compost using the windrow composting system

Table 23 Chemical characteristics of the green waste compost (GWC) used throughout this study. All concentration results are on a 'dry matter' basis

Determined	Unit	GWC
pH	Value	8.2
Copper	mg/kg	72.1
Zinc	mg/kg	293
Lead	mg/kg	159
Arsenic	mg/kg	9.03
Cadmium	mg/kg	0.55
Chromium	mg/kg	25.8
Nickel	mg/kg	23.8
Nitrate Nitrogen	mg/kg	<10
Ammonium Nitrogen	mg/kg	23.8
Total Nitrogen	% w/w	1.23
Total Phosphorus	mg/kg	2170
Leachable Phosphorus	mg/kg	65.2
Total Potassium	mg/kg	6360
Leachable Potassium	mg/kg	2630
Oven Dry Matter	%	62.7
Organic Matter	%	26.4
Organic Carbon	%	15.3
Conductivity 1:6	uS/cm	1260

The pH of the GWC was found to be slightly alkaline at pH 8.2. Gurusamy *et al.*, found a pH range of 7.9 - 8.7 when testing four different finished green waste composts, suggesting that the compost used in this study has a typical pH for GWC (Gurusamy *et al.*, 2021).

By comparing this analysis with the PAS 100 parameters for PTE found in the previous chapter, it is clear that the GWC used in the study is PAS 100 compliant in this regard. Despite this, the GWC used in this study is clearly impacted by Pb (159 mg/kg) and Zn (293 mg/kg). Zinc is the potentially toxic element that according to this analysis is closest to the threshold of the PAS 100 upper acceptable limit. The conductivity of the GWC was found to be slightly lower than the typical values found by a recent review, which suggests that 2000 uS/cm is average for municipal organic waste composts (Gondek *et al.*, 2020). As the GWC used in this study has a conductivity of 1260 uS/cm it would be classed as having a 'light' degree of salinization and so good for plant growth (Gondek *et al.*, 2020). A 'heavy' degree of salinization for composts (8000 uS/cm) could be the result of high concentrations of Na+ or other ions, which can be detrimental to plants due to their ability to accumulate in plant tissue and interfere with root uptake of water (Gondek *et al.*, 2020)

Table 23 shows the typically low availability of nitrogen (ammonium and nitrate nitrogen) in the GWC, although there is substantially more nitrogen in organic forms (Total-N minus Available-N) that could become available through mineralization. The Carbon to Nitrogen ratio for the GWC is 12.4 (C15.3: N1.23) indicating that the material is well composted (Sullivan *et al.*, 2018).

The analysis in shows that whilst the GWC has reasonable concentrations of total P and K, the majority of both macro nutrients is not in a plant available form. The Nutrient management guide (RB209) produced by the Agriculture and Horticulture Development Board (AHDB) suggests that 3000 mg/kg of Phosphate (PO_4^{3-}) and 5410 mg/kg of Potash (K_2O) is typical for UK-produced GWC. The analysis of the GWC used in this study shows that the concentration of leachable Phosphate was 65.2 mg/kg and the concentrations of leachable Potassium was 2630 mg/kg. Hoitink and Poole (1980) suggested that compost should have a moisture content of less than 40 %, and the analysis shows that the GWC tested are just within this limit (moisture content 38.3 %) (Hoitink and Poole, 1980).

5.4.2 Drinking water treatment residue (DWTR)

The DWTR used throughout this study was collected from Mosswood Water Treatment Works (WTW) (Northumbrian Water Ltd.) (shown in Figure 32) . At Mosswood WTW the coagulant ferric sulfate is added to screened water to encourage flocculation of suspended colloids. Sodium hydroxide is then added and the resulting waste flocs are removed as a ferric hydroxide sludge which is temporally stored on site for dewatering and then taken from the site as a waste by-product (Johnson *et al.*, 2018). The results from the commercial analysis of a sub-sample from the bulk samples of the DWTR used throughout this study are presented in Table 24.

Figure 32 Collection of drinking water treatment residue bulk sample from Mosswood water treatment works (Northumbrian Water Ltd.).

Table 24 Chemical characteristics of the drinking water treatment residue (DWTR) used throughout this study. All concentration results are on a 'dry matter' basis

Determined	Unit	DWTR
pH	Value	5.2
Copper	mg/kg	27.8
Zinc	mg/kg	595
Lead	mg/kg	50.5
Arsenic	mg/kg	6.32
Cadmium	mg/kg	1.5
Chromium	mg/kg	25.7
Nickel	mg/kg	164
Nitrate Nitrogen	mg/kg	<10
Ammonium Nitrogen	mg/kg	<10
Total Nitrogen	% w/w	1.07
Total Phosphorus	mg/kg	887
Leachable Phosphorus	mg/kg	<0.01
Total Potassium	mg/kg	369
Leachable Potassium	mg/kg	194
Oven Dry Matter	%	24
Organic Matter	%	30.5
Conductivity 1:6 [Fresh]	uS/cm	192

The pH of the DWTR was found to be acidic, at pH 5.2. Finlay (2015) tested 9 different DWTR samples taken from WTW in the NE of England and found that all were acidic with pHs ranging from 4.1-7.2 (Finlay, 2015). This was attributed to the coagulant chemicals, and can be explained by the acid-generating nature of the formation of iron oxyhydroxides.

The PTE concentrations found in the DWTR used in this study are mostly below the PAS100 thresholds, and also within the ranges reported within relevant literature discussed in the previous chapter (BSI, 2018; Finlay, 2015; Kyncl, 2008; Ippolito *et al*., 2009; Sarkar *et al*., 2007). The concentrations for all of the PTE analysed in the DWTR used throughout this study are within the Sewage Sludge Directive 86/278/EEC limits for soils receiving sludge amendment, meaning that an environmental permit could be sort for its application to land. The concentrations of Ni and Zn were found to be out with the PAS100 limits, and greater than those found in the majority of other studies. Elliot *et al*., did report similar high levels of Ni and Zn in Fe Oxide-based DWTR, and suggested that metals found in the residue came predominantly from the coagulant chemicals used in the treatment process (Elliott *et al.*, 1990). As previously discussed, Mosswood WTW treats the water coming out of the Derwent reservoir, the catchment of which is dominated by historic mines, smelters and tailings piles. Lord estimated that 3.0 t per annum of dissolved Zn currently enters the Derwent Reservoir each year (Lord, 2015b). It is likely that the majority of the Zn found within the DWTR originates from the dissolved Zn found in the reservoir.

Table 24 also shows that the total N content for the DWTR is 1.07 %, which is similar to values reported in the literature (Elliott *et al.*, 1990; Sarkar *et al.*, 2007; Finlay, 2015) and greater than the typical soil concentrations of 0.02 -0.5% found in NE England at a depth of 0-15cm (Emmett *et al.*, 2007). The high total N is like caused by the natural organic matter (NOM) that is removed from the raw water and concentrated in the residue as part of the treatment process (Elliott *et al.*, 1990). However, almost none of the total nitrogen found in the DWTR is in plant available forms; indeed, both ammonium and nitrate nitrogen values were found to be below the limits of instrument detection.

Previous analysis of the Mosswood DWTR has found total K values of 833 ± 565 mg/kg (Finlay, 2015) and the analysis carried out for this study falls within that range (369 mg/kg). Whilst the leachable K value was found to be over half of the total at 194 mg/kg, the K values are well below a desired concentration for a soil amendment

(AHDB, 2021). The total P value for the DWTR (887 mg/kg) was found to be greater than 9 other DWTRs that were analysed in North East England (Finlay, 2015) but much smaller than those reported within the international literature. For example Sarakar *et al* found 3540 mg/kg P in DWTR and Makris found concentrations of up to 11,000 mg/kg P in a study looking at long-term (months) P retention (Makris *et al.*, 2005; Sarkar *et al.*, 2007). The leachable P value found in the DWTR used in this study was below the limit of detection for the instrument (<0.01 mg/kg). The very low levels of available nutrient concentrations are also reflected in the low conductivity of the DWTR (192 uS/cm) and suggests a low concentration of cations which may be beneficial to plant growth, such as calcium (Ca^{2+})), magnesium (Mg^{2+}), aluminium (Al^{3+}) and manganese (Mn^{2+}) (Gondek *et al.*, 2020).

5.5 Chapter conclusions

This aim of this chapter was to provide a characterisation of the site (Whiteheaps), the mine soils used throughout this study and the impact they may be having on the wider catchment. The chapter also aimed to show the initial analysis of the organic waste amendments. The results found here will guide the conclusions of the following three chapters.

It is clear from the review of literature concerning Whiteheaps, together with the sediment and mine soil analysis, that the site is capable of impacting local waterways and the Derwent reservoir. The findings of the walk over survey found that the primary mobilisation mechanism for PTE entering Bolts Burn is through the erosion of waste mining material by the movement of water. This suggests that stabilising the mine soils should be a primary focus for remediation, and justifies experiments that focus on novel stabilisation techniques such as phytostabilisation.

Through the characterisation of the Whiteheaps mine soils this study has found low levels of nutrients and high levels of PTE, particularly Pb and Zn. Whiteheaps has also been found to be sparsely vegetated. These findings give a clear indication that a successful phytostabilisation experiment would require the addition of vital nutrients and a plant species that is able to tolerate high concentrations of PTE in the soil. The physical characterisation and botanical survey suggest that site WH3 will not be ideal for revegetation and that site WH5 is likely to provide a better substrate for plant growth. The two selected amendments for use throughout this study were found to have differing qualities. The amendments have different pH's, concentrations of PTE, conductivity and total/available nutrients. The GWC was found to be PAS100 compliant for PTE, and the concentrations within the DWTR were found to be within the Sewage Sludge Directive 86/278/EEC limits. The lack of available nutrients within the DWTR is likely to make it a less effective soil amendment when compared to the GWC. The findings of this chapter provide a clear justification for phytostabilisation trials using the Whiteheaps mine soils and the discussed organic waste amendments, and provide a foundation for the three following chapters, which concern the pot and field trial results.

6 The effect of organic waste amendment on the growth and PTE concentrations of reed canary grass when grown in mine soils over a 10-week pot trial

6.1 Aims and objectives

The aim of this chapter was to evaluate the effect that two organic waste soil amendments (green waste compost and drinking water treatment residue) have on the growth and PTE concentrations of reed canary grass.

Specific objectives were the:

- Determination of the potential for RCG to establish on amended mine spoil samples from Whiteheaps mine site. This objective was met through an assessment of the results of laboratory pot trials.

- Evaluation of the impact the addition of organic amendments had on the mobility of PTE within mine soils. This objective was met through the analysis of the biomass produced in the pot trials.

6.2 Introduction

This chapter focuses on the results of a laboratory pot trial in which reed canary grass was grown in the mine soils and organic waste amendments that were discussed in the previous chapter. A pot trial, in this instance, is a method used to assess the quality of an unknown soil by determining the emergence and early growth response of plants compared to that of plants grown in a reference or standard control soil.

The use of laboratory pot trials to determine the impact of a contaminant or soil amendment on plant growth is common (Ogunkunle and Beckett, 1988; Vamerali *et al.*, 2009; Karami *et al.*, 2011; Badmos *et al.*, 2015; Reverchon *et al.*, 2015). Pot trials have become popular in the last 50 years due to their ability to remove problematic features of field trials, such as inter-site climatic variations, differences in the handling of experiments at various sites due to differences in custom or training and problems of logistics or expense (Ogunkunle and Beckett, 1988). Whilst pot trials can reduce variability in results due to non-specific and human factors they are susceptible to several issues that can cause the results to not be applicable to the outside world. For example, soil-site interactions such as climate and the rooting behaviour differences between potted and 'wild' plants may not be taken into account. Ogunkunle and Beckett, found that laboratory pot trials often used disturbed homogenised soils that have been processed in a way (dried and sieved) that may result in unpredictable effects such as the mineralisation of organic matter (Ogunkunle and Beckett, 1988). In a large scale review concerning the remediation of PTE through immobilisation Bolan et at., highlight the need for more field trials rather than pot trials for these reasons (Bolan *et al.*, 2014). Ogunkunle and Beckett advised that using undisturbed soil cores rather than processed soil from a site is the best option when trying to obtain results that are comparable to field trials (Ogunkunle and Beckett, 1988). Despite these criticisms pot trials remain a valuable way of obtaining results within a limited time frame and budget and are the basis for many plant, amendment and soil contamination research.

Pot trials are often used as an initial study to help determine the best approach when planning a field trial. Mendez and Maier point out that little is known on the ranges of metal tolerance in species suitable for growth on mine tailings and the minimum amounts of organic amendments required for successful plant establishment (Mendez and Maier, 2008). It is therefore, still necessary to carry out a pilot study or pot trials for each specific historic mine site to increases the chances of successful field trials.

A glasshouse pot trial conducted by Badmoss *et al*., studied the addition of 90 and 180 t ha−1 (wet weight) GWC and DWTR to contaminated soil obtained from former industrial site with PTE and hydrocarbon contamination (Badmos *et al*., 2015). The addition of both DWTR and GWC was found to increase soil nutrient status (organic matter, total N, total P and soil nitrate) of the contaminated soil and the dry biomass of plants grown after a 30 day period by 120-337% when compared to an unamended control (Badmos *et al*., 2015). The DWTR amendment was found to have a less positive effect on both soil quality and plant growth when compared to the DWTR. One of the recommendations of the study was that pot trial experiments should consider the nutrient status and availability of amendments, an area Badmos *et al*, claim is often overlooked in published work which tends to focus on vegetation growth and bioavailability of the toxic compounds.

A greenhouse pot trial lasting four months was conducted by Karami *et al*., to assess the impact that GWC and Biochar amendments had on regulating the mobility of Cu and Pb in historic copper mine soils which were planted with perennial ryegrass

(Karami *et al.*, 2011). The amendments were applied at rates of 20 and 30 % v/v (not stated if AR or DW). Although, ryegrass shoot Cu and Pb concentrations were found to be reduced in plants produced in both types of amended soils the GWC was found to a more suitable amendment for encouraging plant growth as well as reducing PTE mobility (Karami *et al.*, 2011).

The approach taken in this study was a modified version of the pot trial procedure described in BS ISO 11269-2:2005. This British standard is designed for evaluating the quality of soils of different origin carrying unknown contaminants through an assessment of their impact on the growth of the above ground portion of the plant. The standard advises the use of a 'standard soil' alongside the contaminated soils as a way of revelling whether factors not connected with soil contamination such as the pot trial growing conditions are effecting plant growth. The use of unamended soils in experiments concerning organic waste amendments is commonly used as a control in published pot trials (Badmos *et al.*, 2015; Reverchon *et al.*, 2015).

6.3 Experimental

6.3.1 Bulk sample collection

Following initial site survey and sample characterisation discussed in the previous chapter WH3 and WH5 were chosen for supplying bulk material for pot trials and field trial locations. A 50 L bulk sample was collected in August 2018 in preparation for pot trials. At each location 10 x 5 L buckets were filled with equal amounts of material taken from the first 10 cm depth of soil from 10 places across the both plots using a clean trowel. Samples from buckets were then added to two 25 L containers by trowel in turn so as to provide an initial homogenisation of bulk sample. A 1 L sample from each bulk sample was removed for analysis and sent to a commercial laboratory (NRM Labs) for the determination of soil characteristics, nutrient levels and the concentration of PTE. The results of this analysis have been discussed in section 5.3.3. Bulk samples for pot trials were refrigerated at 4°C.

6.3.2 Bulk sample preparation

The two bulk samples (WH3 and WH5) were air dried for three weeks at 20 – 25 °C in a fume cupboard in shallow trays to remove moisture. A clean cement mixer was used to homogenise each bulk sample following drying. Bulk samples were mixed in the cement mixer for 15 minutes. This was carried out in a fume cupboard to avoid dust creation. Bulk samples were then sieved with a 2 mm sieve and refrigerated at 4°C. Particles with a diameter > 2 mm, rocks and biological debris were removed and disposed of.

6.3.3 Pot trial experimental design

To investigate the optimum amount of GWC and DWTR, target application rates of 0%, 20% and 30% (amendment as received weight equivalent/soil dry weight) were used in the current study. The experimental design for the pot trial was factorial (apart from the unamended compost control) and laid out in a randomised complete block. There were three factors; plants (RCG); waste amendments (GWC – 38% water content and DWTR – 75% water content); and target amendment application rates of 0, 20 and 30% (amendment as received weight equivalent/soil dry weight). As suggested in BS ISO 11269-2:2005 a separate growing media was used to provide an indication that the growing conditions of the pot trial were appropriate. John Innes No.1 young plant compost (produced by Arthur Bowyer) was selected as the ideal growing media and purchased from Amazon.com (Bunt, 1976).

The treatments were replicated in triplicate, thus making 33 experimental units (Table 25). The 33 experimental units were arranged in four propagators; each propagator had at least one replicate of each treatment so as to minimise variability due to treatment arrangement. The pots in each propagator were reorganised randomly every week, following watering.

Table 25 Factorial design of pot trial experiment growing reed canary grass in amended mine soils

Growing media	Pot trial experimental units				
	DWTR 20%	GWC 20%	DWTR 30%	GWC 30%	Un-amended soil
WH3	3	3	3	3	3
WH5	3	3	3	3	3
John Innes No1 young plant compost	-	-	-	-	3

Notes: 20% (amendment as received weight equivalent/soil dry weight), 30% (amendment as received weight equivalent/soil dry weight). Drinking water treatment residue (DWTR) moisture content - 76%. Green waste compost (GWC) moisture content - 38%.

6.3.4 Pot trial establishment

The following method was used to amend soils for the pot trial. A 10 cm diameter plastic pot was filled with dried unamended mine soil. Then either 20% or 30% of the mass of dried soil was removed from the pot and replaced with the equivalent mass of ‘as received’ amendment. The weights of the soils and amendments were recorded and are shown in Table 26. Contents of each individual pot were then removed from the pot, homogenised through cone and quartering method and replaced back in the same pot. This work took place in a fume cupboard to avoid cross contamination due to dust creation. Unamended pots of bulk sample and pots with an ideal growing medium (John Innes compost No.1) were used as a control. All pots were filled with 300 mL of growing media level to the brim of the pot, gently tapped to settle, but were not forcibly compacted.

As discussed in the previous chapter the two mine soils used in this study have different bulk densities. This was unfortunately not taken into account when amending the mine soils. The WH3 soils has an average bulk density of 1.84 g/cm^3 (n=2 ± 0.005) whereas the WH5 soils has an average bulk density of 1.40 g/cm^3 (n=2 ± 0.002). The difference in density of the two mine soils has an effect on the weight of the soils filing the pots and thus the amount of amendment added. For example, it took an average of 337g (n=12 ± 27) to fill a pot with WH3 soils and an average of 290g (n=12 ±29) to fill a pot with WH5 soils. This weight difference led to a difference in amendment application rate as WH3GWC30% received 170 t/ha and WH5GWC30% received 149 t/ha.

Table 26 Weights recorded during pot trial establishment with the moisture contents and calculated dry amendment application's rates of amended soils

Sample	Target as received amendment rate, %	Weight of dry mine soil filling 300mL pot , g	As received amendment added following removal of equal mass of dry soil, g	Dry weight of amendment added to pot, g	Dry amendment application rate, %	Dry weight amendment application rate t/ha	As received amendment application rate t/ha
WH3DWTR	20	316	63.2	15.2	4.8	24	99.4
WH3DWTR	30	360	108	25.9	7.2	41	170
WH3GWC	20	310	62	38.4	12.4	60	98
WH3GWC	30	360	108	67.0	18.6	105	170
WH5DWTR	20	264	52.8	12.7	4.8	20	83.0
WH5DWTR	30	315	94.5	22.7	7.2	36	150
WH5GWC	20	264	52.8	32.7	12.4	51	83.0
WH5GWC	30	315	94.5	58.6	18.6	92	149

1.1.2 Nutrient loadings in amended soil used for pot trials

The treatment rate of the amendments with the moisture content taken into account and an target application rate in t/ha for 20% and 30% amendment rate/soil weight are as is found in Table 27.

Table 27 Application rate of amendments used in pot trials in t/ha on a dry weight basis

Amendment rate (%)	**Dry matter content (%) as received**	**Dry weight application rate t/ha**
WH3 DWTR 20%	24	24
WH3 GWC 20%	63	60
WH3 DWTR 30%	24	41
WH3 GWC 30%	63	105
WH5 DWTR 20%	24	20
WH5 GWC 20%	63	51
WH5 DWTR 30%	24	36
WH5 GWC 30%	63	92

The data in Table 26 and Table 27 show that the different as received amendment rates resulted is very different dry application rates once moisture content of the amendment is taken into account. In order to maintain the structure of the amendments (DWTR becomes irreversibly hardened following drying) application of wet amendment by mass within the pots was required.

As the dry weight rates are so different from one another the nutrient loadings for all the types of amended growing media are therefore also different. The 20% and 30% (amendment as received weight equivalent/soil dry weight) amendment rates for GWC and DWTR for both WH3 and WH5 soils can be found in Table 28 and Table 29. The nutrient concentrations of the two amendments and the mine soils are discussed in much greater depth in the previous chapter. Table 28 and Table 29 represent the calculated nutrient loadings in the amendments at the given application rates and are not an estimate of nutrient concentrations in amended soils. As discussed in the previous chapter concentrations for available N and P are within the unamended mine soils were found to be below the limits of detection for the instrument (LOD = <2.5 mg/l for P and <1 mg/l for NO^{-}_{3} and NH^{+}_{4}). Concentrations of K for the mine soils were found to be very low (WH3 30 mg/l WH5 34 mg/l). Concentrations of total N for the mine soils were also found to be low (WH3 0.09 % w/w and WH5 0.14 % w/w).

Table 28 Calculated nutrient loadings for pot trials soils (WH3 and WH5) amended with GWC at target application rates of 20% and 30% (amendment as received weight equivalent/soil dry weight)

Determined	GWC analysis (mg/kg) dry matter basis	Dry weight amendment loadings in kg/ha for 60t/ha which is represented as 20%	Dry weight amendment loadings in kg/ha for 105t/ha which is represented as 30%	Dry weight amendment loadings in kg/ha for 51t/ha which is represented as 20%	Dry weight amendment loadings in kg/ha for 92t/ha which is represented as 30%
Mine soil		WH3	WH3	WH5	WH5
Total nitrogen	12300	738	1292	627	1132
Ammonium nitrogen	23.8	1.43	2.50	1.21	2.19
Nitrate N	<10	-	-	-	-
Total P	2170	130	228	111	200
Water soluble P	62.5	3.75	6.56	3.19	5.75
Total K	6360	382	668	325	585
Water soluble K	2630	158	276	134	242

Table 29 Calculated nutrient loadings for pot trials soils (WH3 and WH5) amended with DWTR at target application rates of 20% and 30% (amendment as received weight equivalent/soil dry weight).

Determined	DWTR analysis (mg/kg) dry matter basis	Dry weight amendment loadings in kg/ha for 24t/ha which is represented as 20%	Dry weight amendment loadings in kg/ha for 41t/ha which is represented as 30%	Dry weight amendment loadings in kg/ha for 20t/ha which is represented as 20%	Dry weight amendment loadings in kg/ha for 36t/ha which is represented as 30%
Mine soil		WH3	WH3	WH5	WH5
Total N	10700	257	439	214	385
Ammonium N	<10	-	-	-	-
Nitrate N	<10	-	-	-	-
Total P	887	21	36.4	18	32
Water soluble P	<0.01	-	-	-	-
Total K	369	8.86	15.13	7.38	13.3
Water soluble K	194	4.66	7.95	3.88	6.98

Note: Red font indicates an unknown value below the limits of detection for the analysis

Table 28 and Table 29 show that the levels of available nutrients in the amended soils were markedly lower in those amended with DWTR than with GWC. Although the two amendment have been found to contain similar total N (GWC 1.2% and DWTR 1.1%), the available N in the DWTR was found to be below the limit of detection. The GWC also contains greater concentrations of both P and K when compared to the DWTR. The lack of water soluble P in the DWTR is likely to be a result of the ability for the residue to bind P into less extractable forms (Dayton and Basta, 2001).

The lower concentrations of available nutrients in DWTR combined with the lower application rates (due to lower dry matter content) when compared to the GWC, means that loadings of essential elements in the DWTR amended growing media will be much lower than in the GWC amended growing media. The impact of the differences in the bulk densities of the two mine soils is also clear from Table 28 and

Table 29 as the WH3 soils clearly received greater concentrations of N, P and K. By looking at the data in these tables it is reasonable to assume that soils amended with GWC will produce greater biomass than soils amended with DWTR and that amended WH3 soils produce greater biomass than amended WH5 soils.

As the differences between the amendment application rates has now been made clear the nomenclature for the amendment rates will be 20% and 30% for all amended growing media e.g. WH3DWTR20% and WH5GWC20%.

6.3.5 Water holding capacity

Water holding capacity (WHC) of growing media for each factor was determined by adapting the method found in Annex C of BS EN ISO 11269-2:2013.

A 300 mL plastic pot was filled with each growing medium option to an equal mass and volume as the pots used in the trial. The pot was saturated with water over the course of three days. Water content for each pot was topped up as required. Excess water was allowed to drain. Following complete saturation, the pots were allowed to drain for two hours. Samples of 1 g were then weighed in triplicate and then dried to a constant mass at 105 °C.

The WHC (Cw), was calculated as a percentage of dry mass, using Equation 8 :

$$Cw = \frac{ms - md}{md} * 100$$

where:

ms is the water-saturated soil sample mass;

md is the dry mass of soil.

Equation 8 water-holding capacity (WHC) calculation

Once 100% WHC was determined the moisture content of each pot could be maintained at 60% water holding capacity. This represents the ideal moisture content for RCG growth which has also been used in similar experiments (Mårtensson *et al.*, 2017b). A WHC of 60% was maintained by weighing several randomly selected pots daily and adding DI water on a balance to a saucer on which the pot sat until the desired weight was achieved. The DI water then moved from the saucer up through the soil by capillarity action (the tendency of a liquid to rise or fall as a result of surface tension).

6.3.6 Pot trial planting and growing conditions

The trials took place in a thermostatic electric propagator (Stewart Ltd, Banbury, UK) under 36W grow lamps (Sylvania, Erlangen, Germany). Each pot was planted with 10 seeds of RCG in a circular pattern with one seed in the middle to make maximum use of space. The seeds planted were of the RCG genetic variety 'Pedja' (Bright Seeds Ltd, Salisbury, UK). Pedja is currently the most popular commercial RCG seed variety used in the UK and has been selected for traits that provide cover for game birds.

Growth conditions were assessed weekly using a digital LCD thermometer hygrometer and humidity meter (SINBURY-HOM-16936, Salford, UK) and a light meter for measuring luminosity (Sunche – HS1010, Shanghai, China). Average temperature ranged from 26-28°C with little variation. Light intensity was maintained at 1500 Lumen m^2 and humidity at 80% relative humidity. The propagators were located on benches in a laboratory with a typical temperature of 20 - 22°C. Grow lamps were turned on for 16 hours per day on an automatic timed switch to simulate summer growing conditions. Main lighting in the laboratory was turned off at night for typically 10 hours per day. Figure 33 shows an image of the pot trial.

Figure 33 Layout of the pot trials including propagators and grow lighting.

6.3.7 Growth analysis

Success of plant species and amendment/amendment rate has been measured by a number of tests as described in BS EN ISO 11269-2:2013. To compensate for non-germinating seeds, a higher number of seeds (10) were planted in each pot than plants that were required for the test. Following first emergence, germination rate of 10 seeds was recorded at 5 and 13 days. Seedlings were then thinned out to five evenly spaced representative specimens of the plants per pots. This was also done to reduce the chance that the density of plants in a test vessel limits normal growth. Seedlings were removed with tweezers.

Following a 12-week growth period pot trials were completed. The above-ground portion of each plant was removed by cutting the stem at 1cm above the base of plant. Care was taken to not contaminate biomass sample with soil from pot. Number of plants per pot was recorded. Plants were soaked in DI water for 20 minutes and then rinsed three times. Plants were then dried on paper towels for a further 20 minutes before measurement of stem length. The combined above ground fresh weight (FW) of all 5 plants produced in each pot was then measured on a five figure balance and recorded. Plants were then oven dried at 40^{o}C for 72 hours in an oven following desiccation to a constant dry weight (DW). The DW was then measured on a five figure balance and recorded. Dried samples were then stored in sealed sample bags and refrigerated at 4 °C awaiting analysis.

All soil from pots was air dried in a fume cupboard for three weeks at approximately 20 °C. Soils were then sieved with a 2 mm sieve to remove organic debris including roots, placed in sample bags and refrigerated at 4 °C in preparation for the BCR modified sequential extraction procedure discussed in the following chapter and section 4.4.3.

6.3.8 Biomass analysis

All plant material analysed as part of this chapter were digested and analysed according to the conditions described in section 4.4.2. Samples were weighed to 0.1g and digested with a MARS 5 microwave digestion system (MARS Xpress, obtained from CEM Microwave Technology Ltd., Buckingham, UK) in 10ml of HNO_3 (> 68 %) acid (Fisher Scientific, Loughborough, UK).

6.4 Results and discussion

6.4.1 Germination rate

Germination rates of plants grown in pot trials were recorded at day 5 and 13 following first visible seedling emergence and are shown in Figure 34. The John Innes No1 young plant compost (control) provided the greatest mean average (n=3 pots) germination followed by the unamended WH3 mine soil pots.

Consistently high germination rates (+60%) in the John Innes No1 young plant compost control pots suggest adequate growth conditions and experimental design. All soils resulted in significantly (t test, $P<0.05$) lower germination rates than that of the control. Considerable variability as indicated by high standard deviation within the treatments was found. Soils amended at the highest rates had some of the lowest germination rates and most variable results. The effect that the amendment of the mine soils is having on the germination of RCG is not clear from the results of this test. For instance, the unamended soils taken from site WH3 have a greater mean average germination rate than that of all amended soils. An increase in moisture content resulting from greater water holding capacity in the amended soils may be responsible for the reduction in germination rate. The review of literature on the botany of RCG found in chapter two describes how soil seed contact improves the germination of RCG seeds. The WH3 mine soil has the greater bulk density and thus may provide improved seed contact compared with the amended soils.

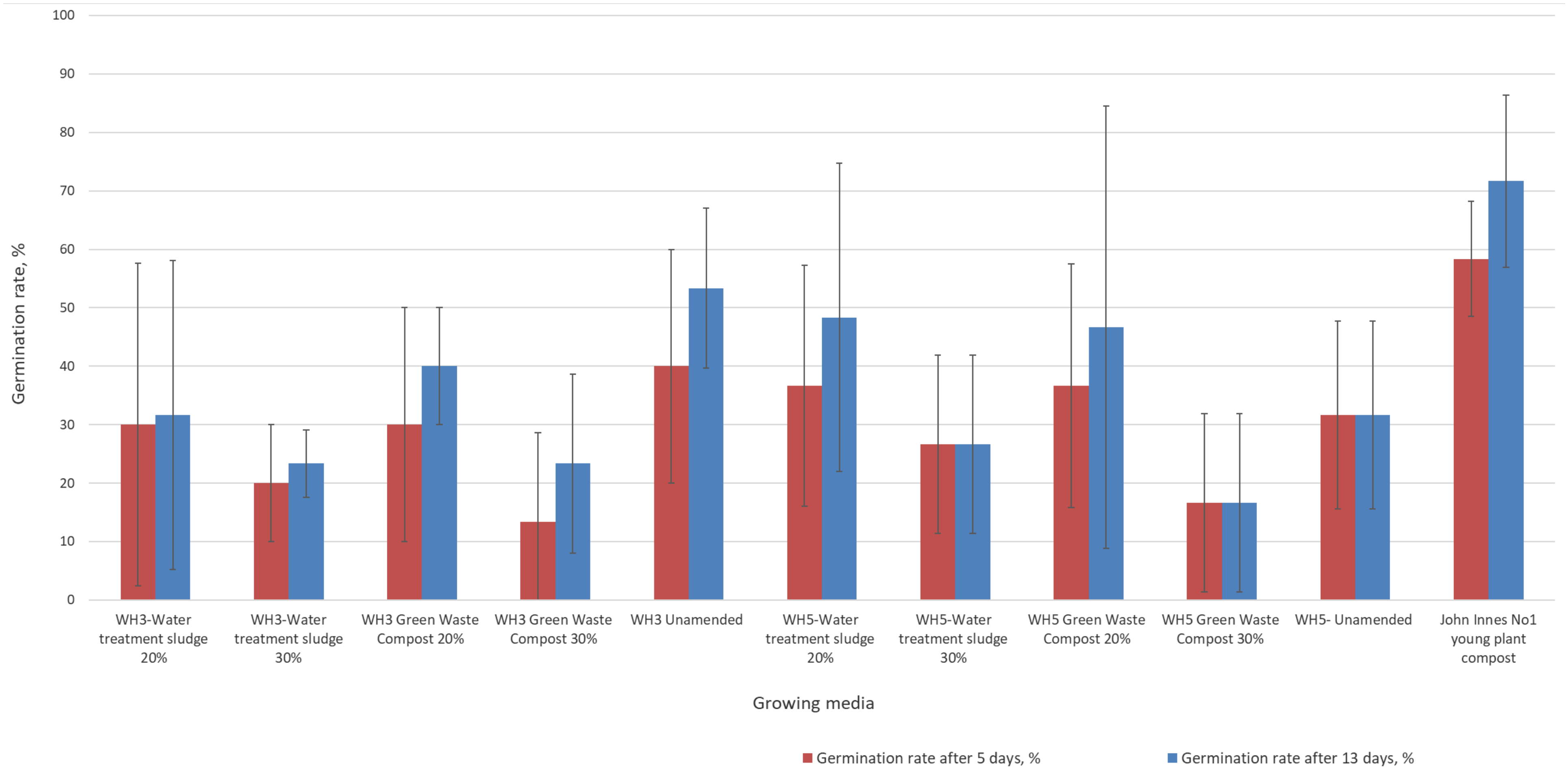


Figure 34 Germination rates of 10 Phalaris arundinacea seeds planted in different growth media (n=3 pots) recorded at 5 and 13 days following first recorded emergence. Error bars represent one standard deviation.

6.4.2 Survival rate

Plants were thinned to 5 or less following emergence of 50 % of the seedlings in the control (13 days) as described in ISO 11269-2:2012. The survival rates were determined at the conclusion of the pot trial following a 10-week growth period and are shown in Figure 35.

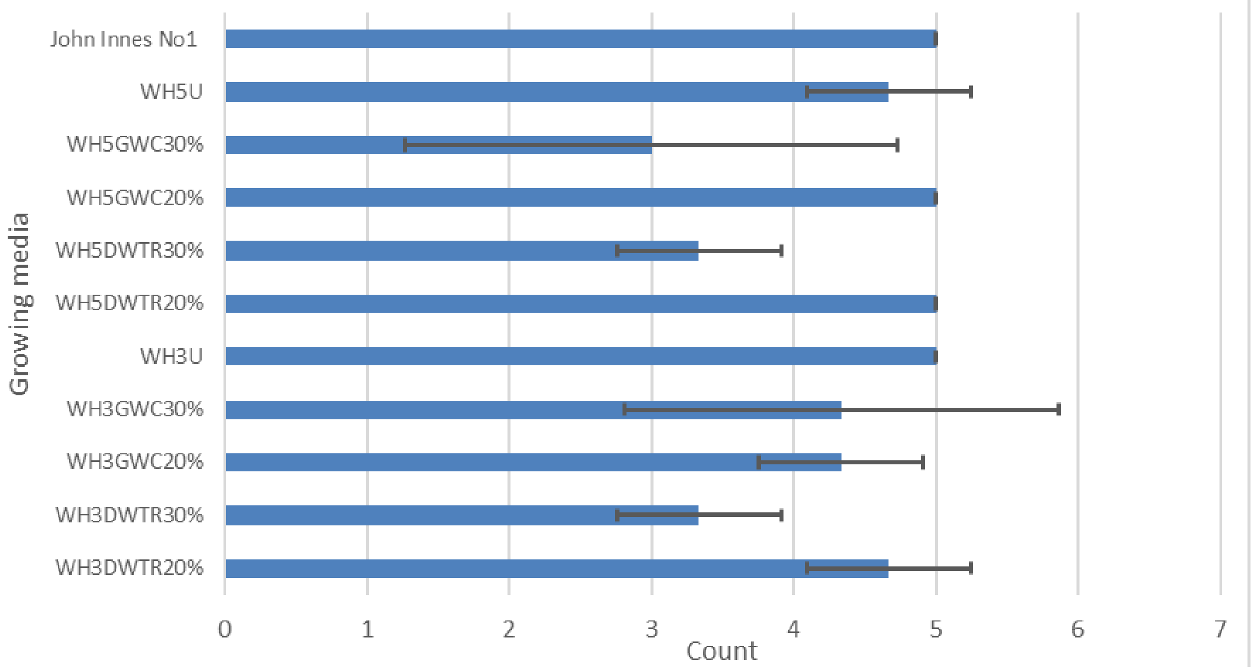


Figure 35 Survival rates of RCG plants following a 10 week pot trial

The survival rates of RCG plants at the conclusion of the pot trial confirm that RCG is able tolerate high levels of PTE in soils as described in the literature (Neuschütz and Greger, 2010b; Badmos *et al.*, 2015; Jensen *et al.*, 2018b). Plants grown in both amended and unamended soils survived equally with no clear trend between them. Three pots had seeds which germinated after the 13-day thinning out and thus provided survival rates above 5 plants. None of the plants growing in the John Innes No1 control soil died during the trial. As none of control plants died it is unlikely that competition between plants resulted in a lowering of survival rates in the amended soils.

It is important to note that the survival rate of plants in this instance has no bearing on the plants health or the biomass produced. For instance, Figure 36 shows five unhealthy withered plants per pot growing in 3 pots of unamended WH3 mine soil next to pots containing 4 ±2 healthier and larger plants growing in WH3 soils with a target amendment rate of 30%. Further pot trials could indicate if the observed effect is significant i.e. if RCG plants, albeit withered ones, are more likely to survive when grown on unamended soils.

Figure 36 Five RCG plants grown per pot in pot trials over 10 weeks in an unamended mine soil (WH3) (left) and a soil (WH3) amended with 30% green waste compost (right)

6.4.3 Above ground plant dry mass

At the conclusion of the pot trial the plants were harvested by cutting the stem of 2cm above the soil. Care was taken to not allow the soil to come into physical contact with the biomass. Shoots and leaves were washed with fresh deionized water three times. Following this washing procedure no visible residual soil was observed on any of the biomass material. Plants were then dried at 40°C in an oven resulting in desiccation to a constant dry weight (DW). The mean DW of the above ground biomass produced per pot (n=3 pots) are shown in Figure 37.

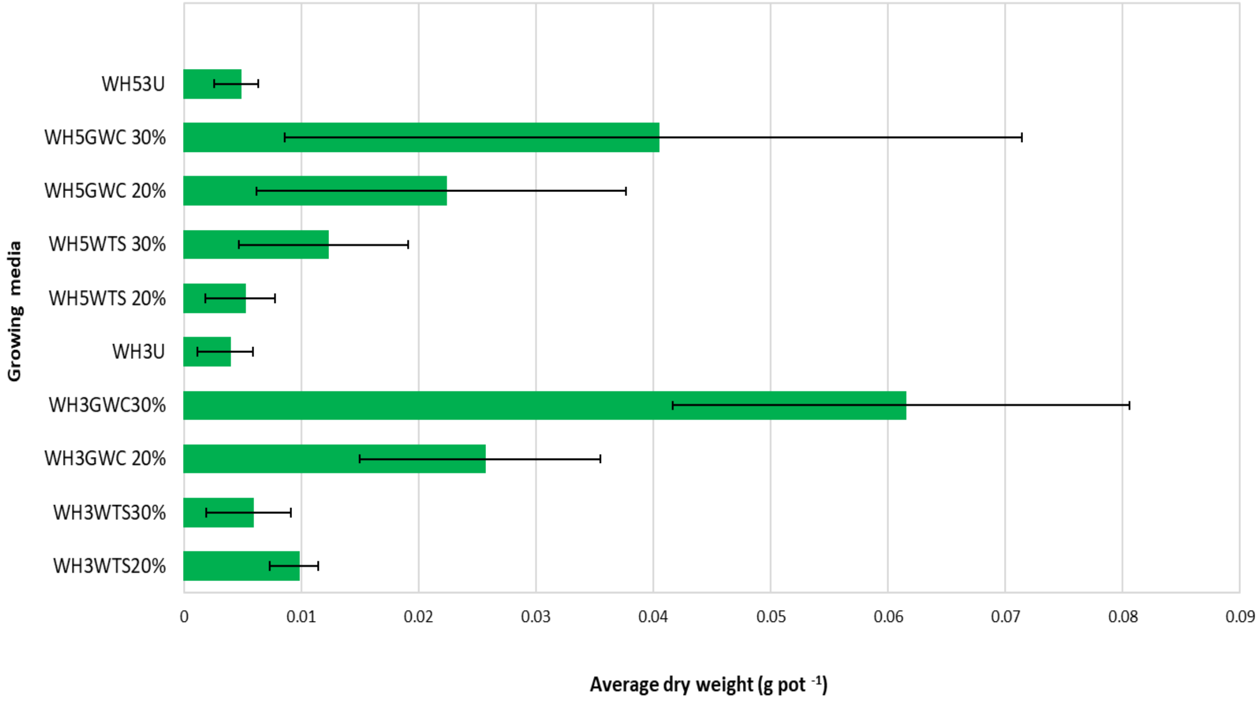


Figure 37 Dry weights of up to 5 reed canary grass plants grown in unamended mine soils and those soils amended with green waste compost (GWC) and drinking water treatment residue (DWTR) following a 10 week growth period Notes: (n = 3); mean, U = Unamended soils

The average moisture content of above ground biomass produced in the pot trial was 86.9%. ±6.1. The moisture content of biomass was not impacted by the size of the plant or amendment type in an observable way. The John Innes No.1 compost produced a significantly larger (Mann Whitney U Test p= 0.05) mean (should be median) average amount 0.27 g ±0.06 (n=3 pots) of DW biomass than all other growing media's indicating that growing conditions during the pot trial were optimal for RCG. The results for the compost control were not included in Fig 5 so that the other results were more clear. Soils amended with GWC consistently produced more biomass than soils amended with DWTR. Unamended soils produced the least biomass. Statistical analysis (SPSS) of the biomass DW results showed that only the biomass produced by the WH3 mine soil amended with 30% GWC was significantly greater than the unamended control (Mann Whitney U Test p= 0.05). When statistical tests were adjusted by the Bonferroni correction for multiple tests this pot treatment was found to also not be a significant result at a significance of p = 0.254. This is likely due to the low sample number (n=3 pots each containing 5 plants) and large standard deviation within results. However, the observed differences between the different pot treatments and the biomass they produced are very visually clear as shown in

Figure 38.

WH3U 	**WH3DWTR20%** 	**WH3DWTR30%** 	**WH3GWC20%** 	**WH3GWC30%** 
WH5U 	**WH5DWTR20%** 	**WH5DWTR30%** 	**WH5GWC20%** 	**WH5GWC30%** 

Figure 38 Biomass production of mine soils amended with 20% and 30% (amendment as received weight equivalent/soil dry weight) green waste compost and drinking water treatment residue following a 10 week pot trial

Amended mine soils from site WH3 regularly produced more biomass than amended soils from site WH5. As described in the previous chapter the Pb concentrations found in the pot trial soils from sites WH3 and WH5 were 13900 mg/kg and 9110 mg/kg respectively. The greater DW biomass production from amended WH3 soils suggests that the high concentrations of this phytotoxic element are potentially not the main factor limiting plant growth. Although the concentrations of nutrients and organic matter are lower in unamended WH3 soils when compared to WH5 (see chapter 4) the amendment rate for WH3 was slightly higher. For instance, for soils amended with 30% GWC (amendment as received weight equivalent/soil dry weight) the dry weight application rate for WH3 was 105 t/ha and 92 t/ha for WH5.

Zn concentrations in unamended WH3 soils are considerably lower than that of WH5 1850mg/kg and 5150 mg/kg respectively. High levels of Zn in soil inhibit many plant metabolic functions, resulting in retarded growth, chlorosis and early senescence (Nagajyoti *et al.*, 2010). Some of the negative impacts on biomass production in higher Zn soil concentrations arise from a plant Fe deficiency as hydrated Zn^{2+} and Fe^{2+} ions have similar radii (Marschner, 1986).

Although Zn concentrations may be the factor that resulted in observed DW biomass production differences, several studies have found that RCG has an tolerance for soil contaminated with high concentrations of Zn (Bary *et al.*, 2009; Rosikon *et al.*, 2016). Rosikon *et al.*, found that RCG biomass production was not hindered when grown in soils amended with sewage sludge that had Zn concentrations of 2590 mg/kg (Rosikon *et al.*, 2016).

6.4.4 Tolerance index

The tolerance index (TI) is calculated as the mean weight (biomass) of a plant grown in the presence of PTE divided by the mean weight of a control. The TI expressed in Equation 9 is an adaption of the TI for root elongation first applied by Wilkins (Wilkins, 1978). The TI is used in the current study as a way to summarise the effects the amendments had on biomass production and thus giving some indication on the amendments ability to reduce the impact of PTE.

$$\mathrm{TI} = \frac{Yield\ of\ biomass\ of\ treated\ soils\ (g)}{Yield\ of\ biomass\ of\ SRS\ compost\ control\ \ (g)} * \ 100$$

Equation 9 tolerance index calculation

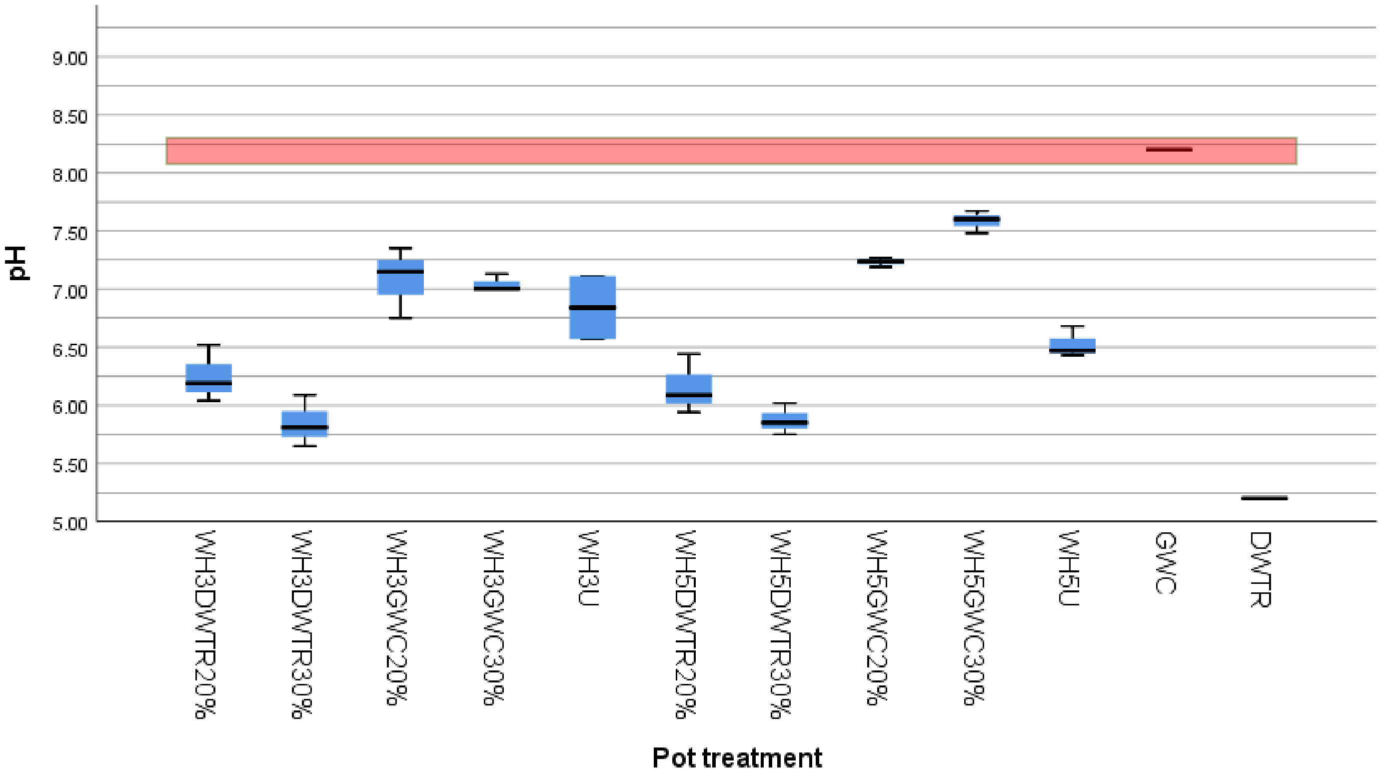
Table 30 show the results of the TI when applied to the RCG grown in amended soils compared to that grown in the John Innes No1 young plant compost (John Innes No1 young plant compost) (John Innes No.1). Green waste compost at both amendment rates on both mine soils had a far greater impact on plant tolerance to PTE than DWTR. However, some of the reduction in growth from plants grown in soils amended with DWTR may be connected to the far lower concentrations of available nutrients as shown by comparing results in Table 28 and Table 29.

Table 30 Tolerance Index for RCG produced in a 10 week pot trial grown in soils amended with green waste compost (GWC) and drinking water treatment residue (DWTR).

Sample	Dry biomass (g)	Tolerance Index, %
WH3 DWTR 20%	0.009 ± 0.00	3.5
WH3DWTRS30%	0.005 ± 0.00	2.1
WH3GWC20%	0.025± 0.01	9.5
WH3GWC30%	0.061± 0.02	23
WH5DWTR20%	0.005± 0.00	1.8
WH5DWTR30%	0.012± 0.01	4.5
WH5GWC20%	0.022± 0.02	8.3
WH5GWC30%	0.040± 0.03	15.1
John Innes No1 young plant compost	0.265± 0.06	100

6.4.5 pH of soils following pot trial

The pH of the soils following the 10-week pot trial was recorded and is the results are presented in Figure 39. Included in the results is the pH of both amendments, which was recorded as part of their initial characterisation and so these two samples have not been through the pot trial process. As pH can have a large impact on the mobility of PTE in soils it is an important parameter to note when considering bioavailability and fractionation. Young states that “Soil pH value has a greater influence on the solubility of heavy metals than any other factor” (Young, 2013. pp 67).



🟥 ***=Optimal soil pH for RCG*** (Usťak *et al.*, 2019).

Figure 39 Bar chart showing mean (n=3 pot soils) pH of mine soils amended with green waste compost (GWC) and drinking water treatment residue (DWTR) following a 10 week pot trial. The pH of the amendments is also shown.

The consistency and low variability of the pH recordings taken from three replicate pots indicates that amendments were added evenly across the entire pot trial experiment. The GWC has the highest pH at 8.2 and DWTR the lowest at 5.2. A pH of 8.2 indicates a matured compost in which the organic acids produced in the initial stages of the aerobic decomposition process have been neutralised by microbial activity. The relatively lower pH of the DWTR at 5.2 is likely the result of coagulant chemicals used in the water treatment process, as ferric sulfate is a corrosive acidic liquid which forms ferric hydroxide floc (Brandt *et al.*, 2017).

The pH of the soils following the pot trial were clearly influenced by their respective amendment of the soil e.g. the introduction of 30% DWTR lowers the pH of both WH3 and WH5 soils. Although unamended soils from site WH3 were found to have a slightly higher pH value that those of WH5 their respective amended soils were not influenced by this difference. For example, WH5 soils amended with 30% GWC (amendment as received weight equivalent/soil dry weight) were found to have the highest pH values of 7.48 – 7.67 of all soils used in the pot trial.

The optimal pH for the growth of RCG has been found to be in the range of 7.7-8.2 (Usťak *et al.*, 2019). The lower pH of the soils amended with DWTR may have been a factor contributing to the lower RCG biomass production compared to plants grown in soils amended with GWC, for which the amended soils all fell into the optimal pH range.

6.4.6 Metal concentrations in biomass

The above ground biomass harvested from the pot trials was digested using microwave assisted aqua regia digestion as described in section 4.4.2. The digests were analysed for the total concentrations of PTE in the biomass using ICP-MS (Model 7700x, Agilent

Technology, UK). The ICP-MS was calibrated with a multi element standard at concentrations of 10,100,500,1000 and 5000 ug L^{-1}. The concentrations of the multi elemental standard were selected following a targeted literature review (separate to that discussed in chapter 2) of relevant RCG biomass concentrations (n=6 sets of results) that was conducted in order to determine the expected concentrations of PTE found in the RCG produced during the pot trial. This is shown in Table 31. All of literature selected concerned RCG grown in PTE contaminated soils, although none reported levels similar to the current study as discussed in section 2.16 of this study.

Table 31 Above ground biomass PTE concentrations in RCG from literature

PTE	**Lowest concentration mg/kg**	**Greatest concentration reported mg/kg**	**Reference**
Cd	0.04	0.312	(Rosikon *et al.*, 2016)
	0.08	0.56	(Polechońska and Klink, 2014)
	0.07	0.27	(Antonkiewicz *et al.*, 2016)
Cr	0.56	39.1	(Polechońska and Klink, 2014)
	0.9	1.7	(Antonkiewicz *et al.*, 2016)
Cu	1.2	24.3	(Polechońska and Klink, 2014)
	2.60	6.34	(Antonkiewicz *et al.*, 2016)
Ni	6.6	192	(Korzeniowska and Stanislawska-Glubiak, 2019)
	0.9	3.75	(Rosikon *et al.*, 2016)
	0.1	14.5	(Polechońska and Klink, 2014)
	1.04	2.24	(Antonkiewicz *et al.*, 2016)
Pb	1.8	13.7	(Unpublished MSc Dissertation Eccles, C 2018)
	0.1	7.5	(Polechońska and Klink, 2014)
	0.46	0.85	(Antonkiewicz *et al.*, 2016)
Zn	30.2	123	(Rosikon *et al.*, 2016)
	16.5	393	(Polechońska and Klink, 2014)
	26.8	48.3	(Antonkiewicz *et al.*, 2016)

6.4.7 Quality control

The quality of the analysis was assessed by analysing a certified reference material (European reference material (ERM) Rye grass - CD 281) in parallel with the samples. 0.1g with no replicates of ERM was analysed as is advised in the ERM certification report for the relevant elements (ERM CD281 Rye Grass).

The found element concentration values and the certified values for the ERM are presented in table 8. Comparable results were obtained for Cr, Ni and Pb when compared with the indicative values, indicating the good quality of the results. The slightly high % recovery of Cd, Cu and Zn may be attributed to low level of the analytes and the low limits of detection for those analytes when using an ICP-MS as discussed in the general methods chapter. The obtained value for As was in excess of the indicative value for the CRM and had a recovery discrepancy that is far higher than it is for any other element. Cross contamination maybe responsible for this as the CRM was digested with samples that were found to have As concentrations which ranged between 18.5 mg/kg and 1.1 mg/kg. Discrepancies in the obtained values for the CRM were taken into account when analysing the results of this digestion. The average moisture content of all biomass produced in pot trials was 86.9% ± 6.7% (n=30). Due to the limited sample amounts of dry biomass generated by the pot trials (average= 0.02g ±0.01g n=30) analysis was not carried out in triplicate but instead the total biomass collected for each factor was bulked (i.e. 5 or less plants from 3 pots of each experimental factor.

Table 32 Comparison of the obtained and certified pseudo today values of PTE concentrations (mg/kg), in dry weight ERM (Rye grass - CD 281) (n=1)

Element	Obtained values mg/kg (n=1)	Certified values mg/kg	Uncertainty of indicative value mg/kg	Recovery %
As	0.2	0.042	0.01	531
Cd	0.2	0.12	0.007	145
Cr	22.8	24.8	1.3	92
Cu	13.5	10.2	0.5	132
Ni	15.8	15.2	0.6	104
Pb	1.9	1.7	0.11	113
Zn	40.4	30.5	1.1	133
-	-	-	🟩	<25%
-	-	-	🟧	25% - 50%
-	-	-	🟥	>50%

The analysis of biomass sample WH3DWTR20% returned unreliable data as a whole that was not comparable to the other data provided by this analysis (except in the case of Cr and As) which is discussed in the following sections. It is for this reason that the results for WH3DWTR20% have been omitted from the following results and discussion. The results for WH3DWTR20% are shown in Table 33.

Table 33 Results from the analysis of biomass grown in pots containing WH3 mine soil and a target amendment rate of 30% DWTR. All results in mg/kg

	Pb	Zn	Cd	Cu	Cr	Ni	As
WH3DWTR20%	0.01	-1.03	0.25	0.07	0.18	-0.10	8.07

1.1.3 Arsenic - biomass uptake

Concentrations of As found within the pot trial biomass ranged from 18.5 mg/kg to 1.1 mg/kg and are shown in Figure 40.

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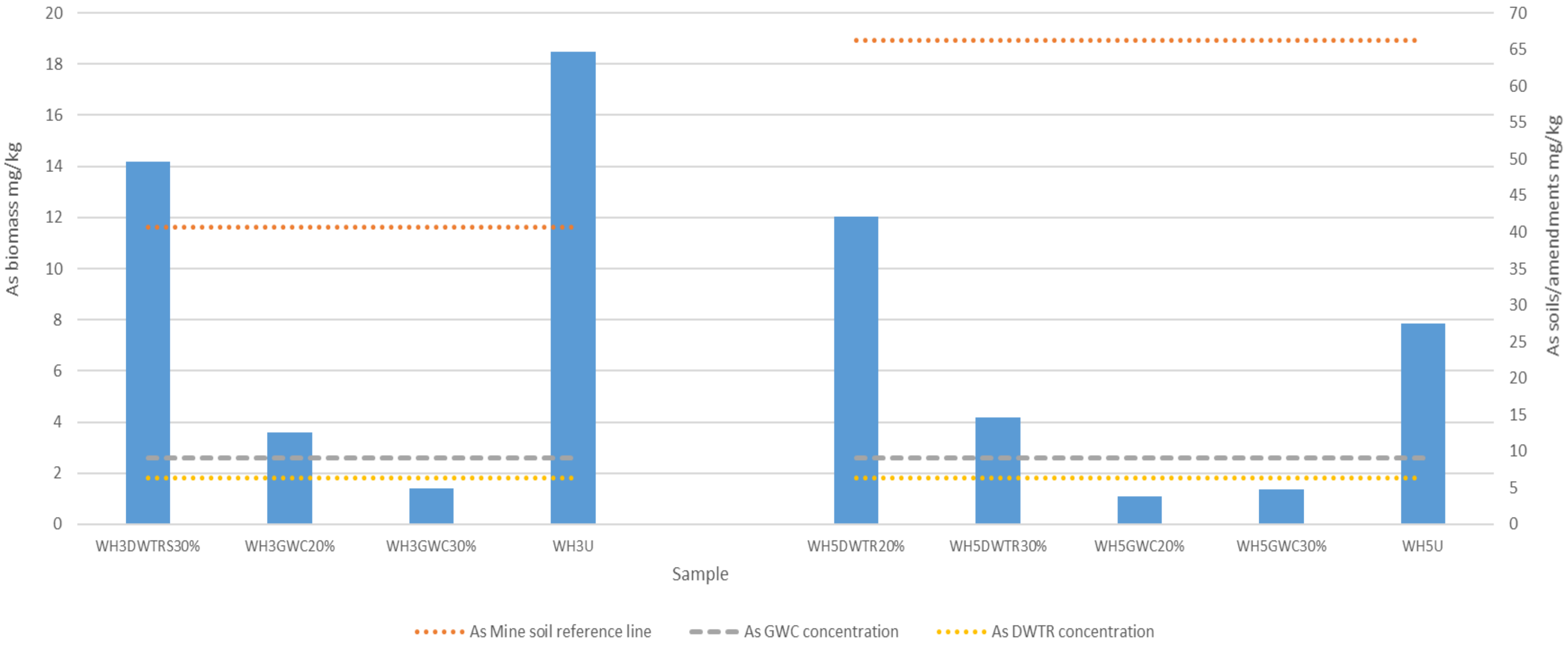

Figure 40 Bar chart showing As concentrations found in RCG biomass produced in a 10 week pot trial (one replicate due to low sample availability) as shown on the left hand y axes. Mine soil and amendment concentrations are also shown for reference on the right hand y axes

The greatest concentration of As was found in the biomass grown in unamended WH3 soil 18.5 mg/kg. Concentrations of As in the biomass grown in soils amended with GWC were lower than those found in biomass gown in DWTR. Concentrations of As found in GWC and DWTR were 9.03 mg/kg and 6.32 mg/kg respectively suggesting that it is not the concentration of As in the amendments that is the critical factor influencing concentrations in RCG biomass. Concentrations of As found in the biomass grown in WH5 soils amended with DWTR at 20% (amendment as received weight equivalent/soil dry weight) (12 mg/kg) were greater than those found in the unamended WH5 biomass (7.8 mg/kg). Biomass concentrations found in RCG biomass grown in unamended WH3 soils (18.5 mg/kg) were greater than those found in unamended WH5 soils (7.8 mg/kg) despite the mine soils from WH5 having greater soil concentrations of As than the mine soils from WH3, 66.2 mg/kg and 40.7 mg/kg respectively.

1.1.4 **Cadmium - biomass uptake**

Concentrations of Cd found in the RCG biomass produced by the pot trials ranged between 1.5 - 34.7 mg/kg and are shown in Figure 41

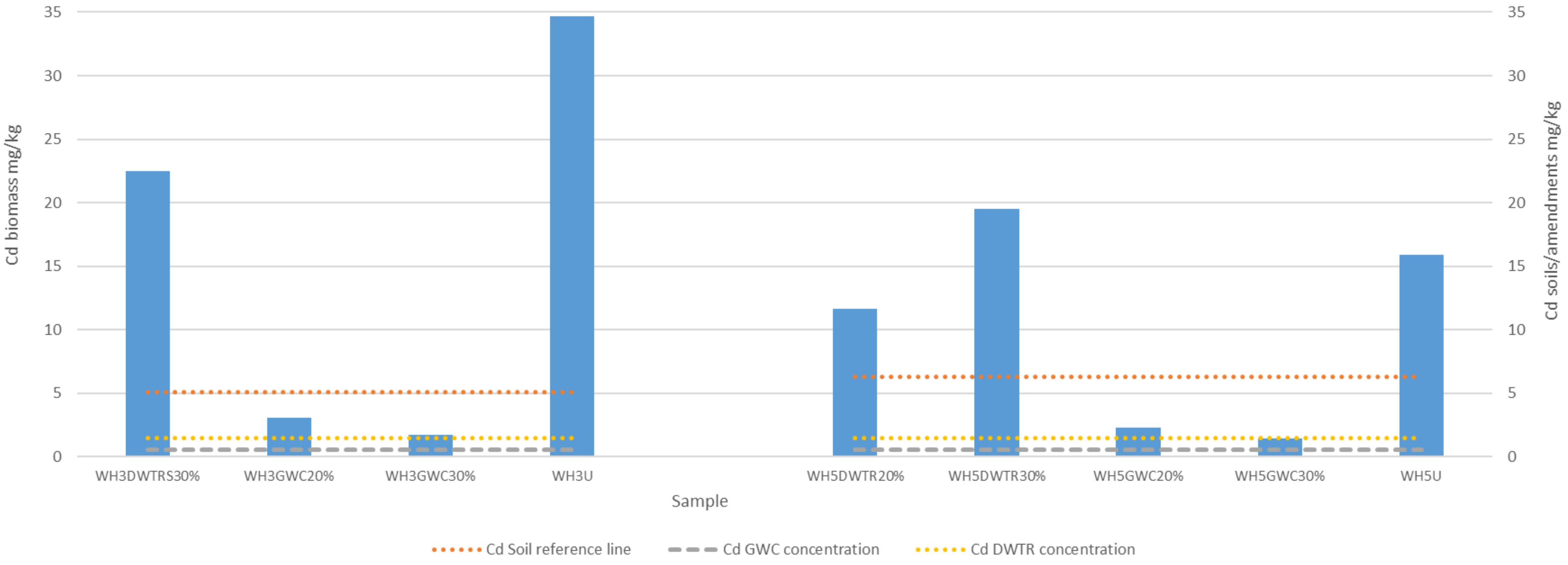

Figure 41 Cd concentrations found in RCG biomass produced in a 10 week pot trial (one replicate due to low sample availability) as shown on the left hand y axes. Mine soil and amendment concentrations are also shown for reference on the right hand y axes

The addition of GWC significantly reduced the Cd biomass concentrations in all biomass samples. The concentrations of Cd in the biomass produced in the unamended soils and those amended with DWTR was found to be higher than both the unamended soils (WH3 5.07 mg/kg and WH5 6.3 mg/kg) and the DWTR amendment (1.5 mg/kg). RCG is a known accumulator of Cd: for example, Antonkiewicz *et al*., found RCG biomass concentrations of 15.3 mg/kg Cd in amended soils containing less than 2.3 mg/kg Cd. Cadmium has been found to accumulates in leaves or herbaceous plant biomass in quantities 10 – 500 times higher at Pb-Zn mine sites when compared to plants grown in non-polluted environments (Yanqun *et al.*, 2005)

6.4.8 Chromium- biomass uptake

Concentrations of Cr found within the pot trial biomass ranged from 2.6 mg/kg to 0.2 mg/kg and are shown in Figure 42

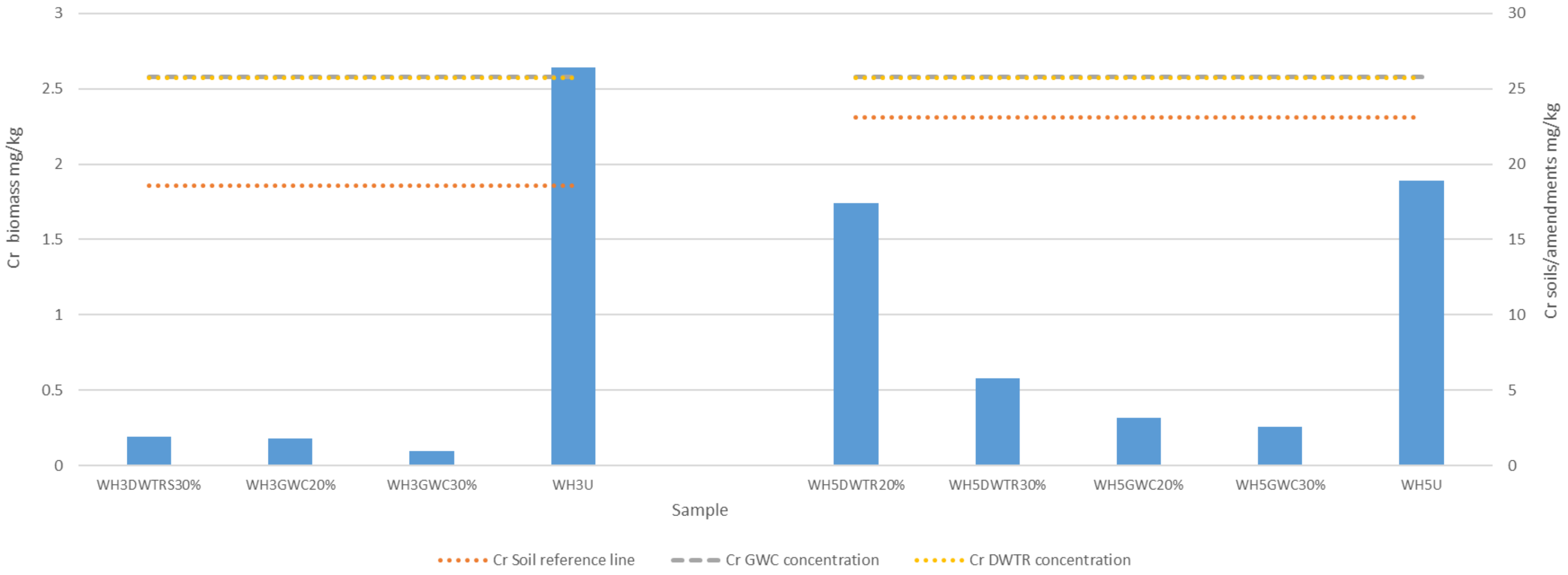

Figure 42 Cr concentrations found in RCG biomass produced in a 10 week pot trial (one replicate due to low sample availability) as shown on the left hand y axes. Mine soil and amendment concentrations are also shown for reference on the right hand y axes

The greatest concentration of Cr was found in the biomass grown in unamended WH3 soil, 2.6 mg/kg. Despite concentrations of Cr in mine soils and amendments ranging from 18.6 mg/kg to 25.8 mg/kg the results of the biomass analysis provided a relatively large range in results. Concentrations of Cr in the biomass were reduced by approximately 90% following the addition of both amendments to WH3 soils. The addition of GWC had the most significant effect on Cr biomass concentrations for WH5 mine soil. The addition of DWTR had less of an impact with 20% DWTR only resulting in a reduction of 0.9 mg/kg Cr when compared to the unamended WH5 mine soil.

6.4.9 Copper- biomass uptake

Concentrations of Cu found within the pot trial biomass ranged from 11.1 to 107 mg/kg and are shown in Figure 43.

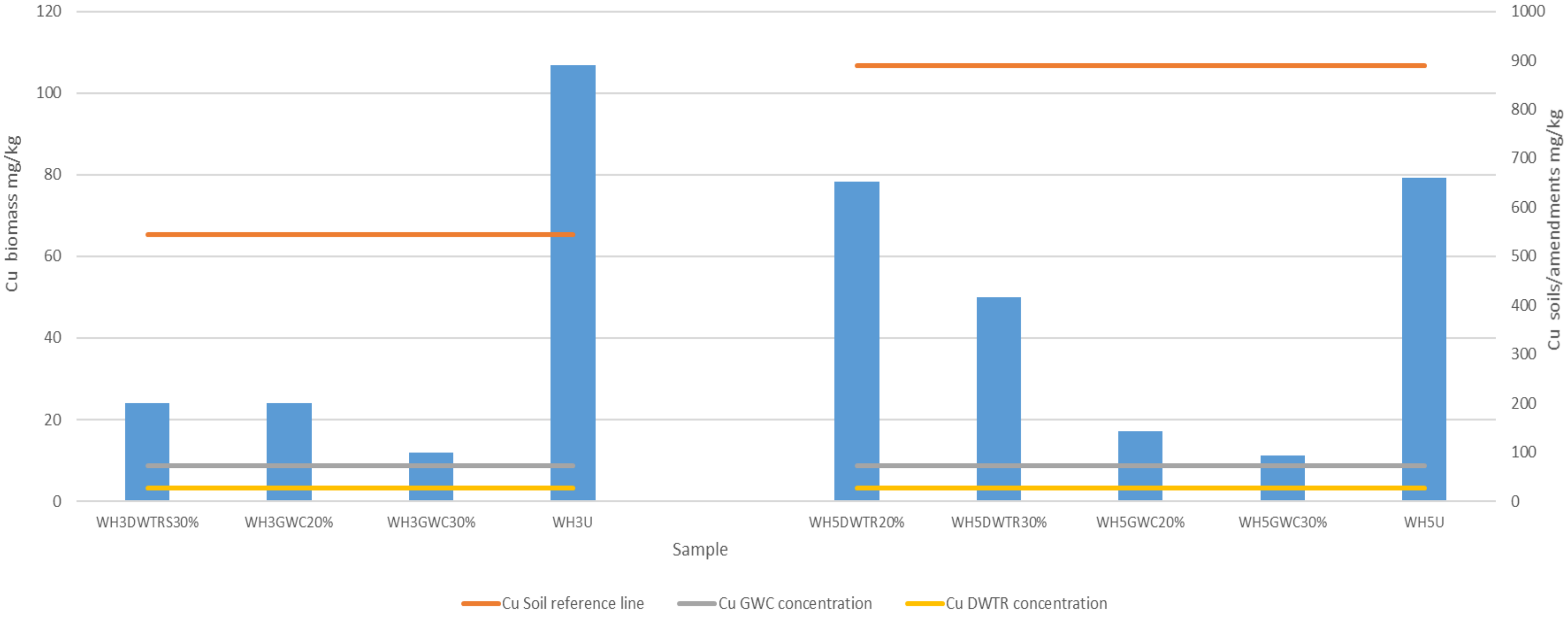

Figure 43 Cu concentrations found in RCG biomass produced in a 10 week pot trial (one replicate due to low sample availability) as shown on the left hand y axes. Mine soil and amendment concentrations are also shown for reference on the right hand y axes

The greatest concentration of Cu was found in the biomass produced in unamended WH3 mine soils. The lowest concentrations of Cu were all found in soils amended with GWC. Analysis of the amendments found greater concentrations of Cu in the GWC (72.1 mg/kg) than the DWTR (27.8 mg/kg). Li *et al*., found that the addition of 5% GWC to a contaminated soil reduced the concentrations of plant available Cu by a significant amount. This effect was attributed to increasing the pH level and complexation with humic acids found within the GWC (Li *et al.*, 2021).

6.4.10 Nickel- biomass uptake

The concentrations of Ni found within the pot trial biomass ranged from 7.1 mg/kg to 23.8 mg/kg and are shown in Figure 44.

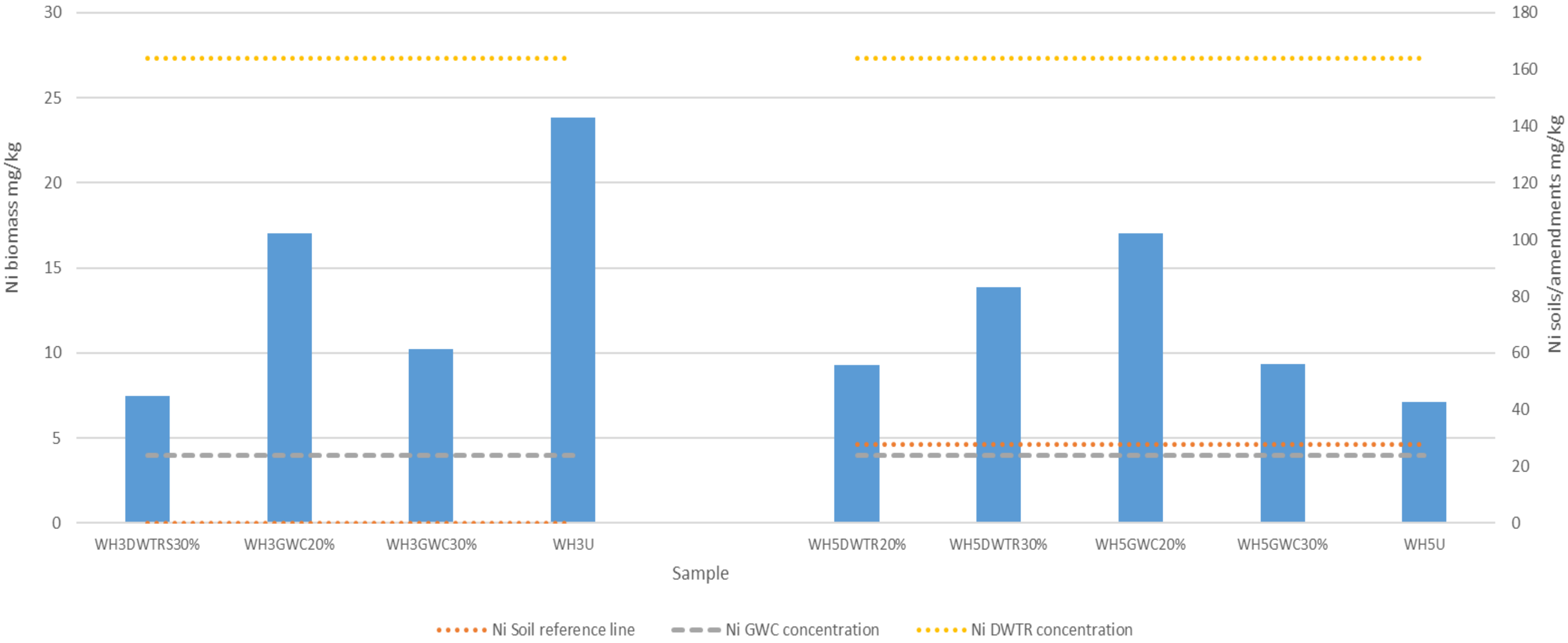

Figure 44 Ni concentrations found in RCG biomass produced in a 10 week pot trial (one replicate due to low sample availability) as shown on the left hand y axes. Mine soil and amendment concentrations are also shown for reference on the right hand y axes

The greatest concentration of Ni was found in the biomass grown in the unamended WH3 mine soil (23.8 mg/kg). Unlike other results the concentrations in the unamended WH5 soil were not similarly high, in fact biomass grown in the unamended WH5 mine soil had the lowest concentration (7.1 mg/kg). Analysis of the WH5 soil itself has shown that this mine soil has greater concentrations of Ni at 27.6 mg/kg than WH3 for which the result was below the limit of detection for the instrument. Soils amended with GWC produced biomass that had greater concentrations of Ni than soils amended with DWTR. Analysis of the amendment has shown that DWTR has far greater concentrations of Ni than GWC 164 mg/kg and 23.8 mg/kg respectively. Analysis of the CRM provided very good recoveries for Ni (104%) leaving little doubt that this analysis is accurate. It may be the case that the greater concentrations of Ni in the biomass grown in WH3 soils are a result of greater bioavailability and mobility of Ni within that soil.

Within the published literature, Ni concentrations found in RCG are very similar to the ones reported here which all lie within the range of those found following a comprehensive search i.e. 0.1 mg/kg – 31.6 mg/kg (see section 3.4 , n=15 articles). Greater concentrations of Ni in the soil does not seem to influence the concentrations within RCG biomass to the extent that can be seen in other metals. For example, Rosikon *et al.*, found that soil taken from a historic steel works and amended with sewage sludge contained 118 mg/kg Ni but only 3.76 mg/kg was found in the RCG biomass (Rosikon *et al.*, 2016). Nickel is the element that is by far most commonly phytominned, typically at biomass accumulation rates of approximately 10 g/kg (Zhang *et al.*, 2014). These results suggest that RCG would not by a suitable Ni phytomining species.

6.4.11 Lead - biomass uptake

The concentrations of Pb found in the pot trial biomass ranged from 151 mg/kg to 958 mg/kg and are shown in Figure 45.

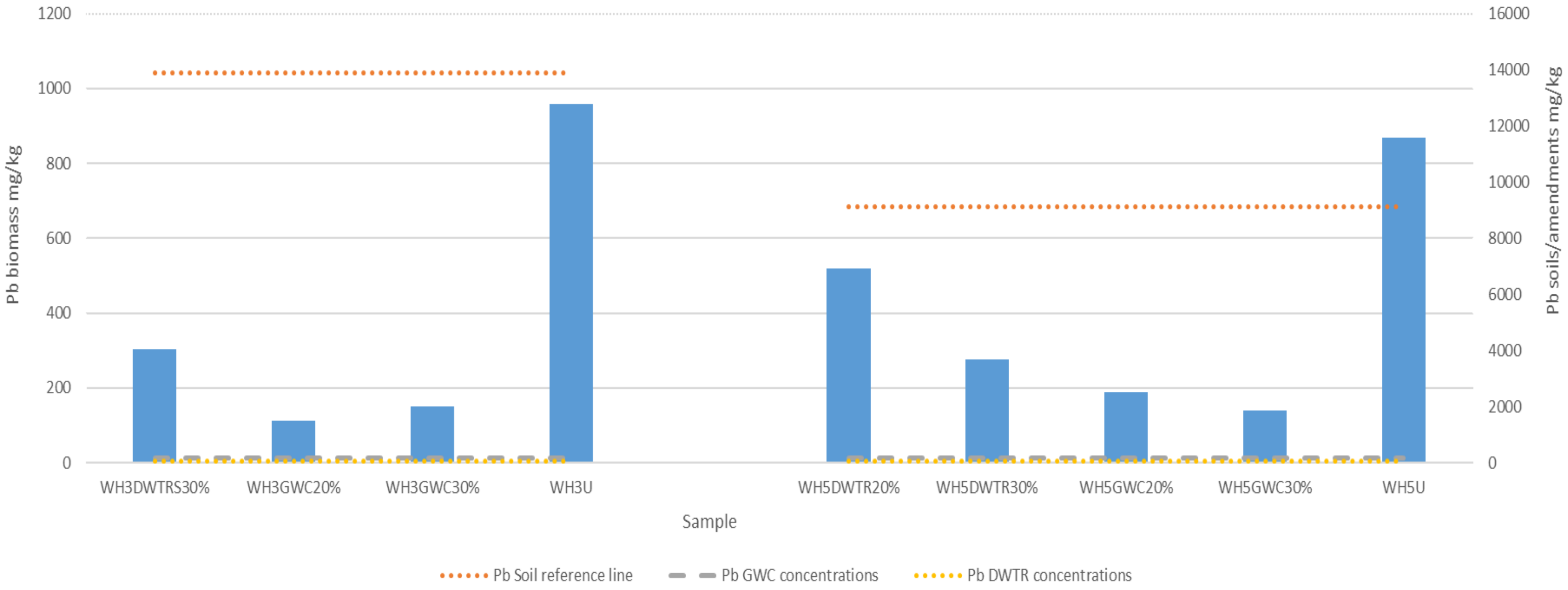

Figure 45 Pb concentrations found in RCG biomass produced in a 10 week pot trial (one replicate due to low sample availability) as shown on the left hand y axes. Mine soil and amendment concentrations are also shown for reference on the right hand y axes

RCG grown in unamended soils contained by far the greatest concentrations of Pb at 958 mg/kg for WH3U and 869 for WH5U. The lowest concentration of Pb was found in the biomass grown in soils from WH3 with 20% GWC. The four lowest concentrations of Pb were all found in pots amended with GWC and were all below 200 mg/kg. The addition of 20% and 30% DWTR clearly also had an impact on the biomass concentrations of Pb with concentrations ranging from 277 to 520 mg/kg.

The concentrations of Pb found within the biomass here are far greater than any in the published literature. The highest reported Pb concentrations found on similar studies was 64.25 mg/kg (Badmos *et al.*, 2015). The soils used for growing RCG in the study conducted by Badmos *et al* however held far lower concentrations of Pb at 69.2 mg/kg. None of the published literature however had soil concentrations that are similar to the concentrations mentioned in the current i.e. 9100 mg/kg – 13,900 mg/kg. For example, the greatest soil Pb concentrations used in an experiment with RCG found was 283 mg/kg and produced RCG biomass with a Pb concentration of 6 mg/kg (Mayerová *et al.*, 2017).

Levels of Pb in RCG biomass similar to the current study were presented recently, in conference proceedings, in plants that were grown on mine soils with Pb concentrations of 2000 – 61,200 mg/kg (Jensen, 2019). Jensen reported Pb concentrations of up to 4070 mg/kg in RCG biomass following a two-year field trial on a historic Pb mine in Wales.

6.4.12 Zinc biomass uptake

The concentrations of Zn found in the pot trial biomass ranged from 670 to 11400 mg/kg and are shown in Figure 46.

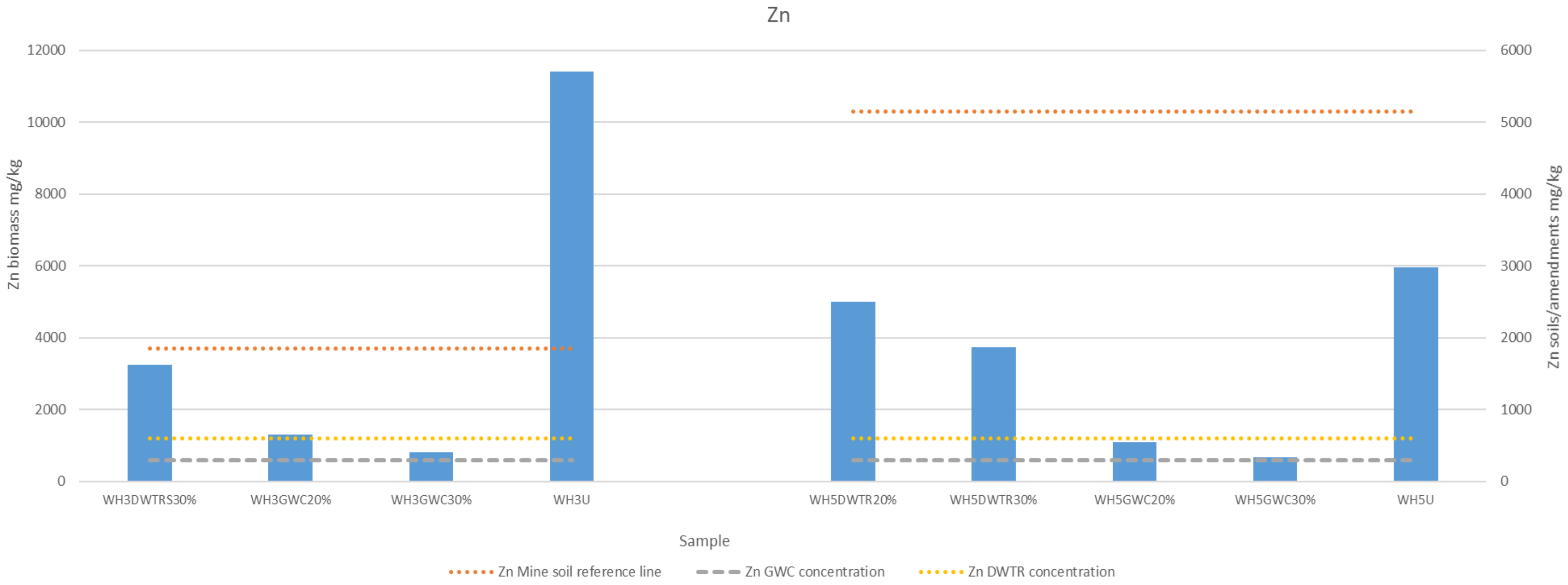

Figure 46 Zn concentrations found in RCG biomass produced in a 10 week pot trial (one replicate due to low sample availability) as shown on the left hand y axes. Mine soil and amendment concentrations are also shown for reference on the right hand y axes

RCG grown in the unamended soils contained by far the most Zn with concentrations of 11400 mg/kg and 5940 mg/kg in WH3 and WH5 respectively. The lowest concentrations of Zn were found in the soils amended with GWC with WH5GWC30% having the least at 670 mg/kg. The addition of DWTR had a less significant impact on the Zn biomass concentrations which ranged from 5000 mg/kg to 3200 mg/kg. Concentrations of Zn within RCG biomass were found to be greater than those found in the mine soils and amendments. For example, the Zn concentration of soils taken from WH3 is 1852 mg/kg and the biomass concentrations of RCG grown in those soils is 11,413 mg/kg. This effect is not uncommon and is reported in the literature although not the same extent. For example, Strasil, found that RCG grown in soils containing 19.6 mg/kg and 22 mg/kg Zn contained 47.4 mg/kg and 24.7 mg/kg Zn within the biomass, respectively (Strašil, 2012). Similarly, Badmos *et al.*, found concentrations of 96.38 mg/kg Zn within RCG biomass grown in soils containing 82.8 mg/kg Zn (Badmos *et al.*, 2015). However, such results are uncommon and the majority of the literature suggests that RCG contains on average 49% (n=14 articles) of the Zn found within the soil it is growing in. No work study has been published in which RCG was grown in soils with Zn concentrations of the magnitude of the current study.

6.5 Biological accumulation coefficient

The biological accumulation coefficient (BAC) as descried by Brooks was used to evaluate the impact that the amendments had on the mobility of PTE (Brooks, 1998). The BAC is also referred to as the enrichment coefficient and the bio accumulation/bio concentration factor within the relevant literature. According to Baker (Baker, 1981) the two basic types of tolerance strategy for plants growing in soils contaminated with PTE are accumulation and exclusion. Baker suggests that as excluder and accumulator plants could grow together in the same environment, the relationships between the soil and plant metal concentrations should be tested for each plant species separately. According to the criteria described by Brooks to define "hyperaccumulators" (plants with the ability to grow on metalliferous soils and to accumulate extraordinarily high amounts of heavy metals in the aerial organs), the bioaccumulation coefficient (ratio of the concentrations of PTE in plant to soil) is greater than 1 (Baker, 1981; Brooks, 1998). Several researchers have pointed out that the term 'hyperaccumulator' is often applied ambiguously and that the scientific community needs to adopt standard terminology and methods for confirming the reliability of analytical data in relation to hyperaccumulation of PTE. (van der Ent *et al.*, 2013; Reeves *et al.*, 2018).

When BAC values are below 1 the plant is deemed more suitable for phytostabilisation (the ability of some plants to 'stabilise' metals in the soil) as net accumulation occurs in the biomass below ground which is not harvestable (Brooks, 1998; Polechońska and Klink, 2014). The BAC was defined as the concentration of PTE in plant shoots divided by the concentrations in the soil/amended soil (Yanqun *et al.*, 2005; Melo *et al.*, 2009).

The equation for the BAC is shown in Equation 10.

$$\text{BAC} = \frac{metal\ concentration\ in\ biomass\ mg\ kg\ {-1}}{metal\ concentration\ in\ soil/amendment\ \ mg\ kg{-1}}$$

Equation 10 The biological accumulation coefficient

Unfortunately, due to time constraints it was not possible to analyse all the amended soils following the pot trials for their PTE concentrations. Soils from the pots filled amended WH5 soils at 30% target amendment rate were analysed as part of the sequential extraction procedure described in the following chapter and so have been included. The PTE concentrations for the remaining treatments were estimated by using Equation 11. The results of the application of this equation are shown in Table 34

$$\text{X} = \text{ Unamended soil concentration} - \left(\frac{\text{Unamended soil concentration}}{\text{\% amendment rate}}\right) + \left(\frac{Amendment\ concentration}{\%\ amendment\ rate}\right)$$

Equation 11 estimation of PTE concentrations in amended soils using in pot trials

Table 34 Pot trial soil and amendments estimates (mg/kg)

Pot trial treatments	Cd	Cr	Cu	Ni	Pb	Zn
WH5DWTR20%	4.28	26.0	746	84.6	9549	4097
WH3GWC20%	4.17	20.0	450	12.8	11130	1540
WH3GWC30%	3.71	20.8	403	14.1	9759	1384
WH5DWTR30%	3.94	26.0	657	94.5	8361	3659
WH5GWC20%	4.09	26.0	755	56.6	9570	4037
WH3DWTR20%	4.36	20.0	442	40.8	11109	1601
WH3DWTR30%	4.00	20.7	390	56.2	9726	1475

The results of the application of the BAC to the biomass and soil concentrations found in the analysis of the pot trial materials are shown in Table 35.

Table 35 showing the biological accumulation coefficient (BAC) for reed canary grass plants following a 10 week pot trial using green waste compost and drinking water treatment residue to amend soils. Results shown in red indicate that the amended soil concentrations were estimated using known soil and amendment concentrations.

Element	Cd	Cr	Cu	Ni	Pb	Zn
WH3U	6.8	0.1	0.2	2.4	0.1	6.2
WH3GWC30%	0.5	0.0	0.0	0.7	0.0	0.6
WH3GWC20%	0.7	0.0	0.1	1.3	0.0	0.8
WH3DWTR30%	5.6	0.0	0.1	0.1	0.0	2.2
WH5U	3.2	0.1	0.1	0.1	0.1	1.2
WH5GWC20%	0.6	0.0	0.0	0.3	0.0	0.3
WH5GWC30%	0.3	0.0	0.0	0.1	0.0	0.1
WH5DWTR20%	2.7	0.1	0.1	0.1	0.1	1.2
WH5DWTR30%	5.0	0.0	0.1	0.1	0.0	1.0

The BAC for the biomass analysed following the pot trials gives a clear result for the effect of the amendments on the mobility of Cd. The unamended soils have greater BAC (WH3U -6.8 WH5U- 3.2) values than those amended with GWC which range from 0.3 – 0.7. This suggests that the application of GWC has reduced the accumulation of Cd in the RCG plant biomass. The DWTR amendment did not have as clear an effect on the BAC for Cd and the values ranged from 2.7-5.6. For the WH5 soils the addition of DWTR increased the BAC by 1.8. This result suggests that RCG can act as an accumulator or excluder for Cd under the conditions described in the pot trial.

The BAC for Cr is consistently ≤0.1 for all of the growing media used in the pot trials. This gives a clear indication that RCG did not accumulate Cr to a great extent under the conditions described in this pot trial. Almost all of the amended soil (except WH5DWTR20%) had a Cr BAC of 0.0 suggesting that RCG grown in amended soils would not accumulate Cr in its above ground biomass. Further research would need to be conducted to confirm this finding.

The BAC for Cu is similarly low across all growing media used within the pot trial ranging from 0.0 – 0.2. The BAC is reduced by the amendment of the soils in every circumstance and this effect is seen more times with soils amended with GWC and less with soils amended with DWTR. These results suggest that RCG is able to exclude Cu from its above ground biomass despite the elevated concentrations in the mine soils (WH3 543 mg/kg and WH5 890 mg/kg)

The BAC for Ni breaks the trend in that it is greater in soils amended with GWC than in soils amended with DWTR. For the amended WH3 soils the BAC for WH3DWTR30% is 0.1 and for WH3GWC30% it is 1.3. This finding is less clear in the WH5 soils although WH5GWC20% provides the greatest BAC value of the set at 0.3. As discussed previously the DWTR amendment contained the greater concentrations of Ni (GWC 23.8 mg/kg DWTR 164 mg/kg) suggesting that the Ni in the GWC is either more plant available or the amendment effects the RCG in a way to make it accumulate more Ni. It could also be the case that DWTR has the effect of making the Ni less plant available. The greatest BAC for Ni is found for the plants grown in the unamended WH3 soils.

The BAC values for Pb are 0.1 for the unamended soils and 0.0 for the amended soils except WH5DWTR20% which is 0.1. This suggests that RCG is not accumulating the element in significant quantities compared to the high Pb soil concentrations, a similar result to that of Cr and Cu. As previously discussed Cu and Pb tend to act comparably in the presence of increased soil OM and this maybe the reason for their similar BAC values in the amended and unamended soils.

The BAC values for Zn range from 0.1 – 6.2. Both unamended mine soils have the greatest BAC values (WH3 6.2 WH5 1.2) however in the case of WH5 this is shared with WH5DWTR20% which is also 1.2. The amendment of the soil with GWC reduced the BAC values for Zn to a greater extent than that of DWTR at both target amendment rates. The BAC results for Zn correspond most with those for Cr, where a similar trend was observed.

The addition of both amendments to both mine soils reduced the BAC of all elements to RCG when compared to the control mine soils. It should be noted however, that the BAC does not fully account for the accumulation or stabilisation advantages as although plants grown in unamended soils had a high rate of BAC they produced far less biomass.

6.6 Chapter conclusions

This chapter considered the effect that two organic waste soil amendments had on the growth and PTE concentrations of reed canary grass biomass produced over a ten-week pot trial. It is clear that the different bulk densities of the two mine soils and moisture contents of the as received amendments affected the amount of amendment and thus nutrients addition/PTE dilution that each growth media received. The germination test showed that neither of these factors however acted as a limitation for seed germination which was probably more affected by soil to seed contact and growing media moisture content. Although germination tests are a common assessment of phytotoxicity, researchers have found similar results were the pollutant in the soil does not negatively impact seed germination (Bożym *et al.*, 2021). It is thought that is because the seed cover isolates the germplasm from negative external factors whereas roots for example, interact with PTE when assimilate nutrients from aqueous solutions (Gyuricza *et al.*, 2010).

The biomass production rate of each growing media gave a clear indication of the effect of the soil amendments. For example, soils amended with GWC at both target amendment rates produced greater biomass (DW) than those amended with DWTR. The greater biomass produced led to the GWC amended soils providing greater tolerance index values (8-23) when compared to those for the DWTR amended soils (2-5). The key factors for this difference are likely to be combination of the lower nutrient concentrations found in DWTR when compared to GWC, the lower amendment rate due to the moisture content difference of as received amendments and the impact that the amendments had on the pH's of the growing media. As discussed the amendment of the mine soils with GWC provided the different growing medias with pH's in the optimal range for RCG growth (7.7-8.2) whereas the DWTR lowered the pH below the optimum level (Usťak *et al.*, 2019).

Further indication of what factor may have had the greatest impact on biomass production in the test is revealed in the differences between plants mass grown in the WH3 and WH5 amended soils. The biomass produced in the WH3 soils was greater for each of the comparative growing media's than those for WH5 soils except DWTR30%. The WH3 amended soils did receive greater concentrations of amendment due to the amendment method and the greater bulk density of the soils when compared to WH5 soils. The WH3 soils were found to have greater concentrations of phytotoxic metals such as Pb and the amendment of the WH3 soils did not have as positive effect on the growing media pH. This suggests that the increase in nutrients and the dilution of PTE concentrations due to the increased amendment rate in the WH3 amended soils were the key factors for increasing biomass production of the RCG plants.

The concentrations of PTE found in the biomass produced during the pot trial provided clear trends. For example, the greatest concentrations of PTE were generally found in the biomass produced in the unamended soils, particularly the unamended WH3 mine soil. This was the case for Cr, Cu, Pb and Zn. However, this trend was not found for As and Cd where the concentrations in the biomass grown in WH5 soils amended with DWTR at 20% provided greater concentrations of the analytes than that grown in the unamended WH5 soils. The results for Ni also did not follow this trend, probably because the Ni concentrations in the amendments were similar or greater to that of the unamended mine soils. For example, the unamended WH5 soils produced biomass with less Ni than all of the other growing media's.

Biomass produced in the unamended WH3 soils had both the smallest average mass (DW n=5 plants in 3 pots) and greatest overall PTE concentrations. The analysis of biomass grown in pots with unamended WH3 soils also gave consistently higher concentrations of PTE than that of biomass produced in unamended WH5 soils, even for elements that WH5 had the greater soil concentrations. This suggests that either the WH3 plants went into shock and the PTE affected their normal physiological processes or that the PTE are absorbed in greater quantities at the beginning of growth and thus the concentrations were diluted in the plants that achieved greater biomass production.

The pH of the mine soils was altered considerably through the application of the amendments to the mine soils and this is likely a key factor in any changes in the PTE bioavailability. This and other factors that may have influenced the bioavailability of PTE in the pot trial soils will be explored in the following chapter. Green waste compost had a significantly better impact on biomass production than DWTR during a 10-week pot trial. This is potentially due to the greater concentration of nutrients and mass of amendment applied following dry matter calculations.

It is clear that RCG can grow in mine soils containing very high concentrations of PTE and low nutrients once amended with organic waste amendments but that more work is required to discover the ideal amendment rate and the potential impact the amendments are having on the mobility of PTE.

7 The effect of organic waste amendments on the fractionation of metals following pot trials

7.1 Aims and objectives

The aim of this chapter was to evaluate the impact of organic waste soil amendments and RCG growth on the fractionation of PTE found within historic mines soils used in the pot trials.

Specific objectives were the:

- Evaluation of the impact the addition of organic amendments had on the mobility of PTE within mine soils. This objective was met through the analysis of the changes in fractionation patterns of PTE found in pot trial soils through the application of the BCR sequential extraction procedure.
- Determination of whether the addition of organic waste amendments reduced the ecological risk of the PTE associated with the mine soils. This objective was met through the application of a risk assessment code used for calculating an element's ecological risk given its fractionation pattern.

7.2 Introduction

This chapter focuses on the results of a sequential extraction (SE) procedure which was carried out on some of the soils used in the pot trials, discussed in the previous chapter. As the understanding of the importance of the bioavailability and environmental mobility of PTE and their relationship with biological receptors increases, there has been a growing interest in assessing the speciation of elements in soils or sediments and their association with specific soil mineral phases. This analysis differs from the analysis of the total or pseudo total elemental concentrations of a sample and is achieved through the application of a sequential extraction procedure to infer an elements speciation.

Sequential extraction typically refers to the application of a series of reagents to the same sample with the aim of sub-dividing the total metal content. As the 'vigour' of the treatment increases through the steps of the procedure, from initial mild conditions (e.g. dilute acetic acid) to the use of harsher reagents (e.g. aqua regia), the PTE extracted in the initial stages of the process are generally those most weakly bound to the solid phase. Therefore it can be concluded that they have greater potential mobility and therefore pose greater risk to the environment, than those released later in the procedure (Bacon and Davidson, 2008).

Sequential chemical extraction is a method for finding the 'operational speciation' where it is the reagent that is used to extract the sample which defines the species, e.g. 'ammonium oxalate soluble' or 'moderately reducible' species rather than 'functional speciation' which refers to the observed role or behaviour of the element, e.g. plant available (Ure, 1991). A review by the International Union of Pure and Applied Chemistry (IUPAC) of the terms related to SE updated the term 'operational speciation' to 'fractionation' which was described as the "process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties (Templeton *et al.*, 2000). One of the most commonly used and accepted methods of SE was proposed by Tessier *et al*. in 1979 and is based on a series of five selective reagents to successively dissolve the mineralogical fractions responsible for retaining PTE (Tessier *et al.*, 1979). These are the exchangeable fraction, bound to carbonates, bound to Fe\\Mn oxides, bound to organic matter, and residual. As interest grew in the procedure new approaches and applications were designed eventually leading to a situation where it became difficult to draw meaningful comparisons between results obtained in different laboratories (Chang *et al.*, 1984; Gibson and Farmer, 1986; Bacon and Davidson, 2008). Another problem which hampered a good comparability of data derived from the difference procedures was the lack of a suitable reference material which lead to a lack in quality control (Rauret *et al.*, 1999).

7.2.1 The Bureau of Reference of the Commission sequential extraction procedure

Due to the need for standardization, the Community Bureau of Reference of the Commission of the European Communities (BCR) (later the Standards Measurement and Testing Programme) commissioned a project which led to a harmonised 3-steps sequential extraction procedure, and later development of a lake sediment certified reference material (CRM 601) for validation of the procedure in 1997 (Quevauviller *et al.*, 1997).

Difficulty in reproducing results led to further modifications of the method which now include the digestion of the residue of the sample following step 3 with aqua regia and the suggestion that the PT content of the sample should be analysed alongside the sequential extraction to serve as internal check for assessing the overall effectiveness of sequential extraction and element recoveries (Rauret *et al.*, 1999). The 'modified' BCR procedure also led to the development of a new lake sediment certified reference material (CRM701) (Pueyo *et al.*, 2001). The BCR sequential extraction is now widely used, a recently published paper using the procedure found that it has been featured in almost 70 publications every year over the past decade (Sagagi *et al.*, 2021)

7.2.2 Targeted phases of the modified BCR procedure

The targeted soil phases for each extraction step in the BCR sequential extraction procedure are described below. Section 4.4.3 in the general methods chapter describes in detail the reagent preparation and procedure used during the procedure.

7.2.2.1 Exchangeable phase

The first step in the BCR procedure targets the water soluble fraction, which is the soil solution, the exchangeable fraction and the acid soluble or carbonate bound fraction. This is done to simplify the method as the' exchangeable' fraction can thus represent the most labile targeted phases.

The soil solution contains the most mobile and plant available metal species which are generally found in very low concentrations requiring sensitive analytical instruments for detection such as ICP-MS. The water soluble fraction includes metals complexed with organic material and free ions and is normally liberated with a mechanical action such as centrifuge or displacement (Davidson, 2013).

Exchangeable metal ions and those in the soil solution are the metals which can be released most readily into the environment (Rao *et al.*, 2008). The metals corresponding to the exchangeable fraction usually represent a small portion of the total metal content in soil (Rauret, 1998), typically less than 2% of the total metals present in the sample (Rao *et al.*, 2008). The exchangeable fraction is made up metals that are absorbed weakly onto the surfaces of organic matter and inorganic (mineral) compounds. These metals are released by the action of cations, typically potassium, calcium, magnesium or ammonium, which break the electrostatic bonds (Rao *et al.*, 2008). The carbonate bound fraction can also be termed the 'acid soluble' fraction and is made up of metals that are precipitated or coprecipitate with carbonates. This fraction is very pH sensitive, and the metals can be liberated and go into solution at pH ≤ 5 by dissolving the carbonate minerals (Rauret, 1998).

7.2.2.2 Reducible

The reducible mineral phase is made up of iron and manganese oxyhydroxides (oxides) which act as 'scavengers' of metals and so have a great effect on the location, mobility and bioavailability of metals in soils (Gasparatos, 2013). Although oxides constitute only a small fraction of the total soil, their high sorption capacity based on their high surface area and high surface charge density, mean that a higher portion of metals are often found in this fraction. Typically, hydroxylammonium hydrochloride (a reducing agent in nitric acid medium) is used to target the reducible phase in sequential extraction and it is used in the BCR procedure. The reagent has been found to release some metals which are bound to organic matter leading to an overestimation of the reducible fraction and an underestimation of the following "oxidisable" fraction (Ahnstrom and Parker, 1999).

7.2.2.3 Oxidisable

The oxidisable phase is associated with dissolving organic matter and sulfides such as pyrite (Fe) and sphalerite (Zn) and thereby releasing the bound metals. Metals bound to organic matter tend to be less mobile and so remain longer in the soil but are mobilised by decomposition processes (Kennedy *et al.*, 1997). Organic matter is destroyed in the BCR procedure using hydrogen peroxide at 85^{o}C for several hours which liberates the associated metals into solution. However, a second reagent (ammonium acetate) is then required to retain the realised metals in solution preventing reabsorption or precipitation prior to analysis (Ure *et al.*, 1995).

7.2.2.4 Residual

As discussed previously the Rauet *et al* 1999 paper on an improvement to the sequential extraction suggested the introduction of a fourth step which involved the analysis of the residual material in order to improve the validity of results (Rauret *et al.*, 1999). The fourth step, much like a pseudo total analysis, involves the use of aqua regia under pressure to dissolve nearly all of the remaining material. The complete dissolution of the remaining material would require hydrofluoric acid (HF) but studies of a nature that requires sequential extraction test rarely warrant the high risk associated with the handling of HF. The digestion and analysis of the residual material in the extracted sample provides values which can be added to a mass balance of the values found in the previous extracts and then compared to the values from the digestion of a separate sample (Rauret *et al.*, 1999). Although the BCR procedure was originally developed for fluvial bottom sediments it has since been used for a wide range of analysis. Table 36 provides some recent examples of how the BCR procedure has been deployed.

Table 36 Recent examples of the application of the BCR sequential extraction procedure found in published literature that are relevant to this study.

Reference	Matrix	Analytes	Summary of relevant findings
(Liu *et al.*, 2021)	Landfill refuse	Cd, Cr, Cu, Pb, Zn	• Pb and Cr were not easily combined by ferromanganese minerals, while Cu was easily bound with organic matter. • Zn and Ni have high potential migration capacity,
(Rizwan *et al.*, 2021)	Arable spiked soils	Cu, Pb	• Incorporation of Biochar significantly immobilized Pb and Cu in spiked soils and reduced concentrations in biomass (tomato plants)
(Park *et al.*, 2021)	Coal ash	Rare earth elements; Ce, Dy, Er, Gd, La Pr, Nd, Sc, Sm, Y Gd,	• Tessier sequential extraction revealed that 85 % of rare earth elements remained in the residual fraction • With the exception of La, Nd, and Ce, the contents of several REEs in the residual fraction obtained using BCR sequential extraction were, in the range of 60–70 %.
(Medyńska-Juraszek *et al.*, 2020)	Copper smelter soil	Cd, Cr, Cu, Ni Pb, Zn	• Organic amendments noticeably reduced the uptake of heavy metals by various leafy vegetables but can also have the opposite effect in certain plants making plant selection important

7.2.3 Limitations of sequential extraction

There are several inherent limitations to SE approaches including; non-selectivity of reagents for target phase, incomplete extraction, re-adsorption and re-distribution of analytes among phases during the extraction process and the formation of artefacts (new mineral phases). In order to counter-act these limitations Davidson and Bacon suggest in their 2008 review of SE that the following steps are taken when applying a sequential extraction procedure;

I. follow standard protocols precisely;
II. use quality control measures such as a CRM and mass balance calculations;
III. report results in terms of operationally defined fractions rather than specific minerals phases such as iron oxide bound;
IV. present data in absolute concentrations rather than the potentially misleading fractionation percentage;
V. use SE in comparative studies such as unamended and amended soils;
VI. Be cautious in drawing conclusions regarding bioavailability without corroboratory evidence such as biomass PTE concentrations. (Bacon and Davidson, 2008)

Despite these limitations, sequential extraction has proved to be an important and widely applied tool for gaining information on the potential mobility and hence bioavailability and toxicity of PTEs in the environment.

7.2.4 Presentation of Sequential extraction results

The Sequential extraction procedure can generate a relatively large quantity of data, especially when compared to single extractions. In the modified BCR procedure, each sample that is analysed generates five results for each element (including the separate AQ digestion), which is then tripled if the extraction and analysis is carried out in triplicate as is typical in academic analysis. In their paper “is there a future for sequential chemical extraction?” Bacon and Davidson highlight the difficulty in presenting a sequential chemical extraction dataset in a way that is clear and understandable(Bacon and Davidson, 2008). Their paper suggests making use of bar charts which are colour coded to match the different extractions e.g. the colour red to represent the oxidisable fraction. They also highlight the issue of presenting the data as a percentage rather than an absolute number, a common problem in the field. The issue here is that a percentage can be misleading unless the absolute levels of metals are also provided as values. It is for this reason that within the current study both the percentages and absolute values are presented side by side as is exemplified by Gonneea and Paytan in their study of barium in sediments using a five-step sequential extraction procedure (Gonneea and Paytan, 2006).

As the current study is also concerned with amended and unamended soils it was also important to analyse the amendments in the same way as the samples to ensure that any effect realised concerning chemical mobility by amending the soil was not in fact due to a dilution of the soil and introduction of a new ‘material’ with a different fractionation pattern. The results of the analysis of the amendment and the unamended soils were then mass balanced based on amendment rate in order to provide a ‘calculated’ sample which could be used to support or disprove any hypothesis on the effects of the amendments on the chemical mobility of the elements in the unamended soils. The mass balance equation for the 'calculated' sample is show in Equation 12.

$$c = (u * r) + (a * r)$$

Where;
c is the calculated sample value,
u is the unamended soil value,
a is the amended soil value and
r is the amendment rate % as a decimal e.g. 30% = 0.3.

Equation 12 mass balance equation for the calculated sample value used in the presentation of SE results.

The AQ extractable value of a separate analysis from each sample is also shown in the results to provide further validation of the analysis and clues as to what may be taking place during soil amendment and the pot trial process in terms of chemical mobility.

7.3 Experimental

All soil from pots used in pot trials described in the previous chapter was air dried in a fume cupboard for 3 weeks at approximately 20 °C. Soils were then sieved with a 2 mm sieve to remove organic debris including roots, placed in sample bags and refrigerated at 4 °C in preparation for the BCR modified sequential extraction procedure. The procedure for the sequential extractions is outlined in the general methods chapter.

7.3.1 Pseudototal digestion

The pot trial soils were digested using microwave assisted aqua regia digestion described In section 4.4.1 of the general experimental procedures chapter. The digests were then analysed for the pseudototal concentrations of PTE in the sediments using ICP-OES as described in section 4.5 of the general experimental procedures chapter.

7.3.2 BCR sequential extraction procedure

The different fractions of the PTE in the pot trial soils were extracted using the BCR sequential extraction procedure as described in section 4.4.3 of the general procedures chapter. The extracts were analysed for PTE concentrations using ICP-OES as described in section 4.5 of the general experimental procedures chapter.

7.3.3 Quality control

The quality of the results from the sequential extraction procedure was assessed by analysing a certified reference material (CRM 701) in parallel with the samples. The results and the certified values for the CRM 701 are presented in Table 37. Procedural blank and reagent blank solutions were also analysed at regular intervals during the analysis for quality checks. Due to the lack of a centrifuge that could take 100 ml tubes the standard modified BCR method (Rauret *et al.*, 1999) was miniaturised for this analysis. Both the reagent volume and the sample mass was halved throughout the four steps of the procedure, this has previously been demonstrated to have no effect on the results obtained (Sagagi *et al.*, 2021). Unfortunately, an error in the analytical process resulted in As not being measured and therefore the analyte is not mentioned further as part of these results.

Table 37 Comparison of the obtained and certified values of mean PTE fractions (n=3) (mg/kg) standard deviation (SD; ± mg/kg) in dry weight CRM 701 using the BCR sequential extraction scheme.

Element	Step	Obtained values mg/kg (n=3)	Standard Deviation ±	Certified values mg/kg (n= 9-14)	Recovery %
Cd	1	7.7	0.0	7.3	105
Cr	1	2.6	0.0	2.3	114
Cu	1	49.4	0.4	49	100
Ni	1	16.3	0.4	15	106
Pb	1	3.9	0.1	3.2	123
Zn	1	215	0.9	205	105
Cd	2	4.3	0.1	3.8	113
Cr	2	47.5	1.8	46	104
Cu	2	110	4.2	124	89
Ni	2	28.5	1.2	26.6	107
Pb	2	128	4.0	126	102
Zn	2	118	3.4	114	104
Cd	3	0.3	0.0	0.3	120
Cr	3	144	3.7	143	101
Cu	3	53.7	1.0	55.0	98
Ni	3	13.8	0.3	15.3	90

Pb	3	9.3	0.3	9.3	100
Zn	3	42.1	0.9	46.0	91
Cd	4	0.2	0.0	0.1	157
Cr	4	77.0	1.1	62.5	123
Cu	4	40.1	1.3	38.5	104
Ni	4	40.2	2.0	41.4	97
Pb	4	12.2	0.3	11.0	111
Zn	4	107	4.3	95.0	113
Cd	Sum of 1-4	12.5	-	11.5	109
Cr	Sum of 1-4	271	-	254	107
Cu	Sum of 1-4	253	-	267	95
Ni	Sum of 1-4	98.8	-	98.3	101
Pb	Sum of 1-4	153	-	150	103
Zn	Sum of 1-4	482	-	460	105
Cd	Aqua regia extractable	14.4	0.4	11.7	123
Cr	Aqua regia extractable	280	8.7	272	103
Cu	Aqua regia extractable	264	10.0	275	96
Ni	Aqua regia extractable	100	3.3	103	97
Pb	Aqua regia extractable	147	4.2	143	103
Zn	Aqua regia extractable	487	15.4	454	107
-	-	-	-	🟩	± <25%
-	-	-	-	🟧	± 25 - 50%
-	-	-	-	🟥	± >50%

The concentrations (shown in Table 37), obtained when analysing the extracts from CRM 701 for Cd, Cr, Cu, Ni, Pb and Zn were comparable to the CRM certified values providing confidence in further results. The high % recovery of Cd in the residual fraction may be attributed to low level of the analyte and the low LOD for Cd when analysed using the ICP-OES. The majority of results are within ±25% of the certified values validating the accuracy of the applied analytical method(Wise, 2018).

A mass balance test was conducted on the of the sum of step 1-4 and the pseudo total values using Equation 13. This test shows the recovery percentage of each analyte and the results are shown in

Table 38.

$$\frac{sum\ of\ results\ from\ step\ 1-4}{Aqua\ regia\ extractable\ value} \times 100$$

Equation 13 Mass balance equation used for CRM analysis

The mass balance test (results shown in

Table 38) is used to find the recovery percentage of each analyte. A low percentage of recovery would suggest that some of an analyte had been lost during the sequential extraction procedure.

Table 38 Results of the mass balance test for the Squetnaial extraction procedure

Element	Mass balance (%)
Cd	87
Cr	97
Cu	96
Ni	99
Pb	104
Zn	99

Table 38 shows that only Cd was potentially lost during the procedure (87% recovered). This is likely connected with the high recovery of Cd during the CRM analysis over all steps of the SE procedure as shown in Table 37. However, this may also be connected with the analytical instrument difficulty in detecting Cd as previously discussed.

7.4 Results from the BCR sequential extraction procedure on soils amended with drinking water treatment residue

7.4.1 Cadmium

The Cd results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 47. Consistently low variability between triplicate results obtained for each step of the sequential extraction as indicated by the small size of the error bars, provides confidence in the results. Aqua regia extractable concentrations of Cd found in all samples was lower (approx. 50-60%) than the sum of the 4 separate extracts for all three samples. This may be a result of the low sensitivity of the instrument (ICP-OES) for Cd as discussed in the methods chapter. The concentrations of Cd found in both the sum of all extracts and in the AQ extractable were highest in the DWTR. This result is inconsistent with previous analyses of the material which gave concentrations of Cd at 1.5 mg/kg. Previous analysis of the unamended WH5 soil showed concentrations of Cd at 6.3 mg/kg which is consistent with this analysis. CRM recoveries for Cd were slightly high (maximum =120%) and the mass balance was lower than the other analytes (86%) but not to the extent that would explain such an increase in the DWTR. In the unamended soil Cd was found to be predominantly in the exchangeable fraction which is of concern given the toxicity of the analyte. Concentrations of Cd found within the liable fractions of the unamended mine soil present an ecological risk. The distribution of Cd in the operationally defined fractions of the unamended mine soil was comparable with similar studies of soils taken from historic mine sites (Ahmadipour et al., 2014; Medyńska-Juraszek et al., 2020).

The pseudo total concentrations of Cd and mass balance of the operationally defined fractions fell within the range of those reported in a similar study (Rodríguez et al., 2009).

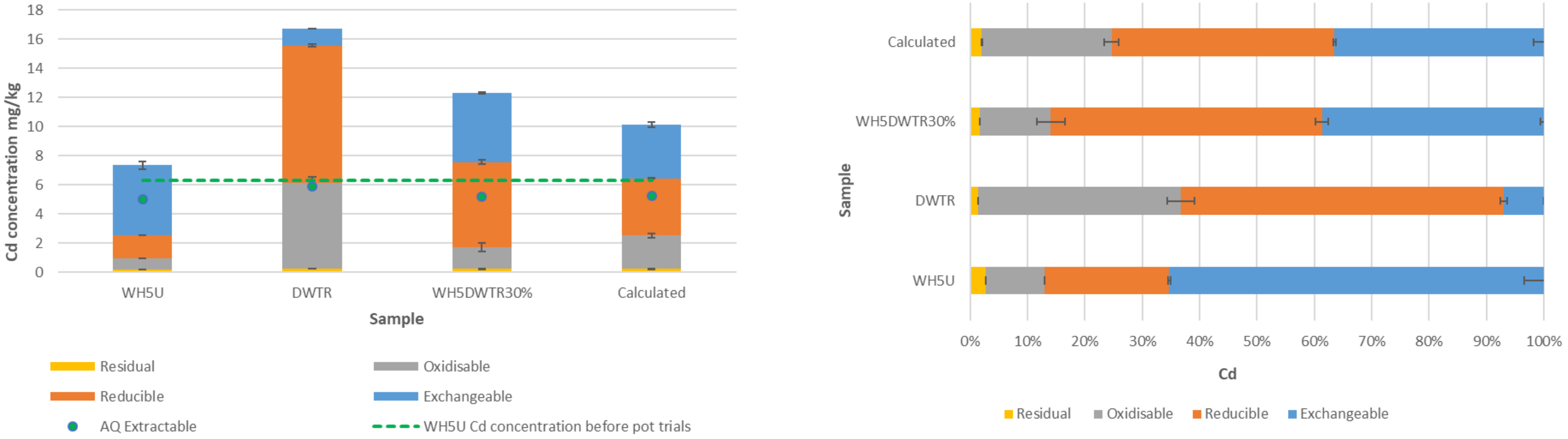

Figure 47 The distribution of Cd (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (DWTR), the amended mine soil WH5DWTR30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the DWTR. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fraction

The Cd in the DWTR was found to be predominantly in the reducible and oxidisable fractions. This finding is supported by the previously discussed work of Abo-El-Enein *et al*., who found that DWTR is an efficient adsorbent for Cd^{2+} (Abo-El-Enein *et al.*, 2017). The results for the DWTR amended soil shows a movement of concentrations of Cd from the oxidisable fraction towards the exchangeable. Statistically significant differences (see appendix 10.3) between the amended soil and the calculated concentrations suggest that the addition of DWTR to the mine soil increases the mobility of Cd. However, this is likely to be connected to the already greater concentrations of Cd in the exchangeable fraction rather than a chemical reaction. The results suggest that the addition of DWTR has had no obvious effect on the mobility of Cd. Elkhatib *et al*., found that following the addition of novel nanoparticles of DWTR to a solution spiked with Cd(II), the Cd sorbed to the nanoparticles at a high rate (98.4%). However, this effect was supressed in a multi ion solution that included Cu and Pb where these elements formed more stable monohydroxy complexes (Elkhatib *et al.*, 2016). This finding suggests that inter-element competition may have reduced the potential impact DWTR could have had on the mobility of Cd.

7.4.2 Chromium

The Cr results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 48.

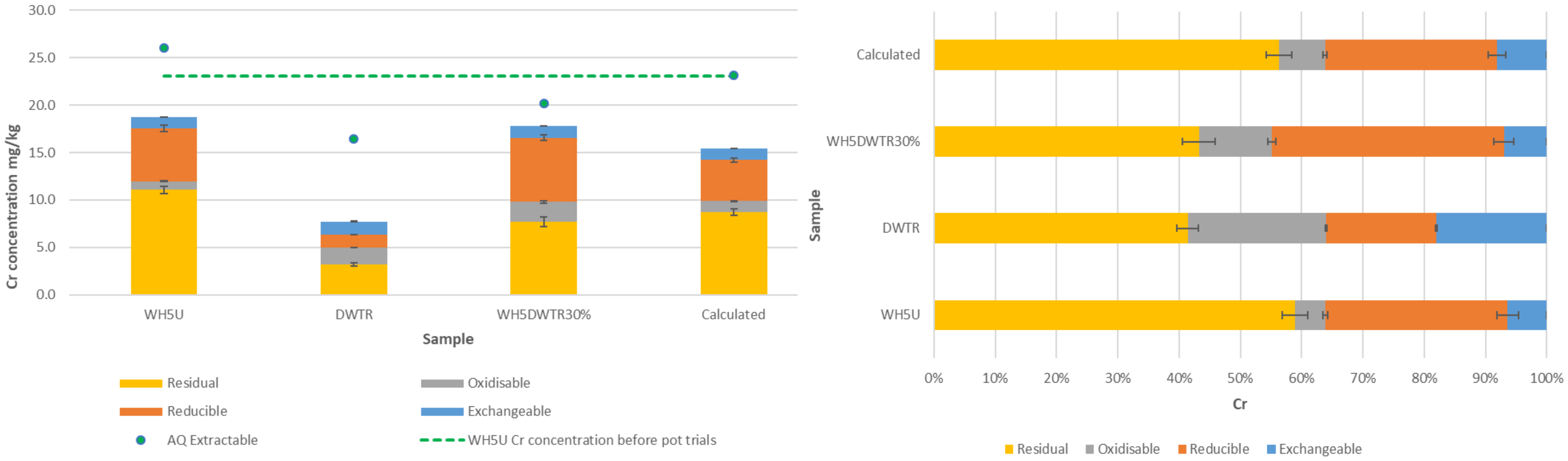

Figure 48 The distribution of Cr (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (DWTR), the amended mine soil WH5DWTR30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the DWTR. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

Consistently low variability between triplicate results obtained for each step of the sequential extraction as indicated by the small size of the error bars, provides confidence in the results. *Aqua regia* extractable results were consistently higher than the sum of the four extracts and were closer to previous analysis on soils and amendment (23.1 and 25.7 mg/kg respectively) samples that had not been used in the pot trial process. *Aqua regia* extractable results are similar to that of the previous analysis on WH5U (23.1 mg/kg) and DWTR (25.7 mg/kg) samples that had not been taken through the pot trial process. This suggests that some of the Cr may have not been extracted by the BCR procedure.

The majority of Cr in the mine soil was found within the residual fraction suggesting that its source is not anthropogenic. The most common form of naturally occurring Cr is chromium (III) and is typically held strongly in soil by cation exchange (Ertani *et al.*, 2017). Another indicator that the source of the Cr is not anthropogenic is that the concentrations do not exceed the mean average rural soil concentrations of Cr found by the UK soil and herbage pollutant survey (33.4 mg/kg n=183) (Creaser *et al.*, 2007). The majority of the Cr found in the DWTR was also in the residual fraction with an almost even distribution found in the other three operationally defined fractions. The explanation for this similarity may be that the Cr found in the DWTR has originated from the same locality as the mine soils and so is similarly influenced by naturally occurring concentrations. The mobility of naturally occurring Cr (III) has been found to increase in the presence of acidic conditions (Ertani *et al.*, 2017). The application of DWTR (pH 5.2) resulted in the reduction of the pH value in the amended soil from pH 6.5 (WH5U) to pH 5.8 (WH5DWTR30%). A statically significant movement from Cr in the residual phase (see appendix 10.3) to the oxidisable and reducible phases was detected suggesting that the lowering of pH may have increased the mobility of Cr.

7.4.3 Copper

The Cu results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 49.

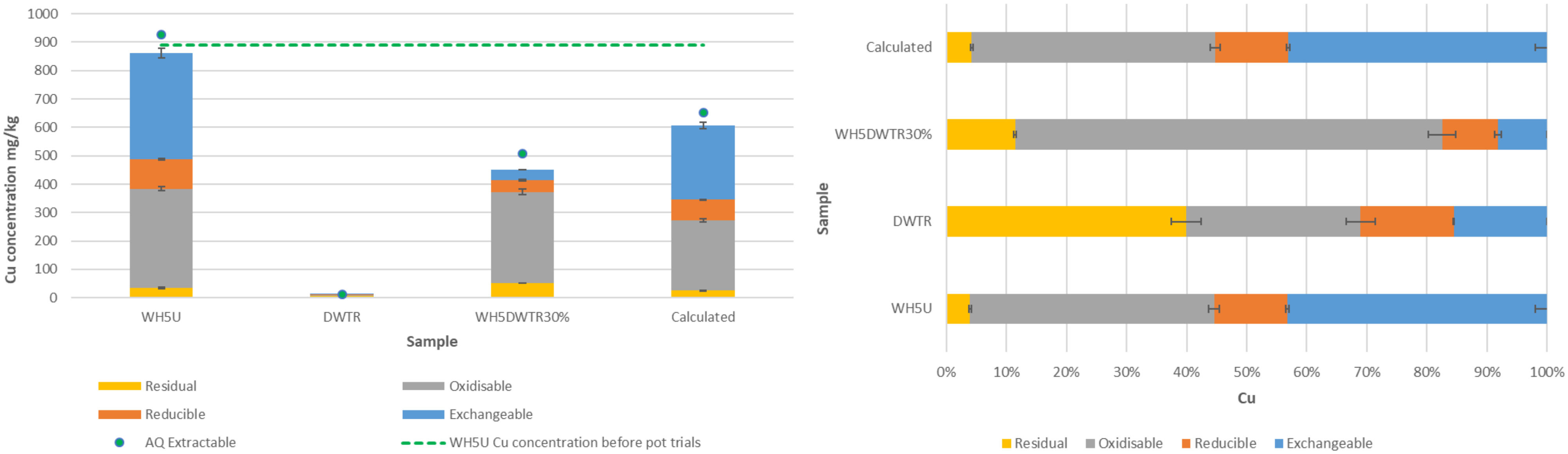

Figure 49 The distribution of Cu (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (DWTR), the amended mine soil WH5DWTR30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the DWTR. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

Consistently low variability between triplicate results obtained for each step of the sequential extraction as indicated by the small size of the error bars, provides confidence in the results. *Aqua regia* extractable concentrations provide further reliability to the results as they are very similar to the sum of the 4 separate extracts (see figure 17). Copper was found mainly in the exchangeable and oxidisable fractions of the mine soil. It is typical to find Cu in the oxidisable fraction when applying the BCR procedure as Cu has strong affinity for organic matter (Oorts, 2013). The pseudo total Cu concentrations and mass balance for the mine soil were found to be very similar to the previous analysis of mine soil WH5 (890 mg/kg).

A comparatively low concentration of Cu of the DWTR (15 mg/kg) is consistent with previous analysis (27.8 mg/kg) allowing for a clear identification of the impact that DWTR may be having on the fractionation of Cu.

A significant increase (see appendix 10.3) in the oxidisable fraction of the amended soil when compared to the calculated may be connected to the high concentrations of OM (30%) found in the DWTR. The addition of DWTR also reduced the biomass concentrations from 100 mg/kg in the unamended soil to 50 mg/kg in that amended with 30% (amendment as received weight equivalent/soil dry weight). This effect was more apparent in the WH3 soil where the addition of DWTR 30% (amendment as received weight equivalent/soil dry weight) reduced the biomass concentrations from 100 mg/kg in the unamended to 20 mg/kg. These findings suggest that the DWTR is reducing the mobility of a portion of the Cu. Many studies, using similar extraction procedures have also confirmed the ability of DWTR to reduce the mobility and plant availability of Cu (Mahdy and Fathi, 2008; Moharem *et al.*, 2013).

7.4.4 Nickel

The Ni results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 50

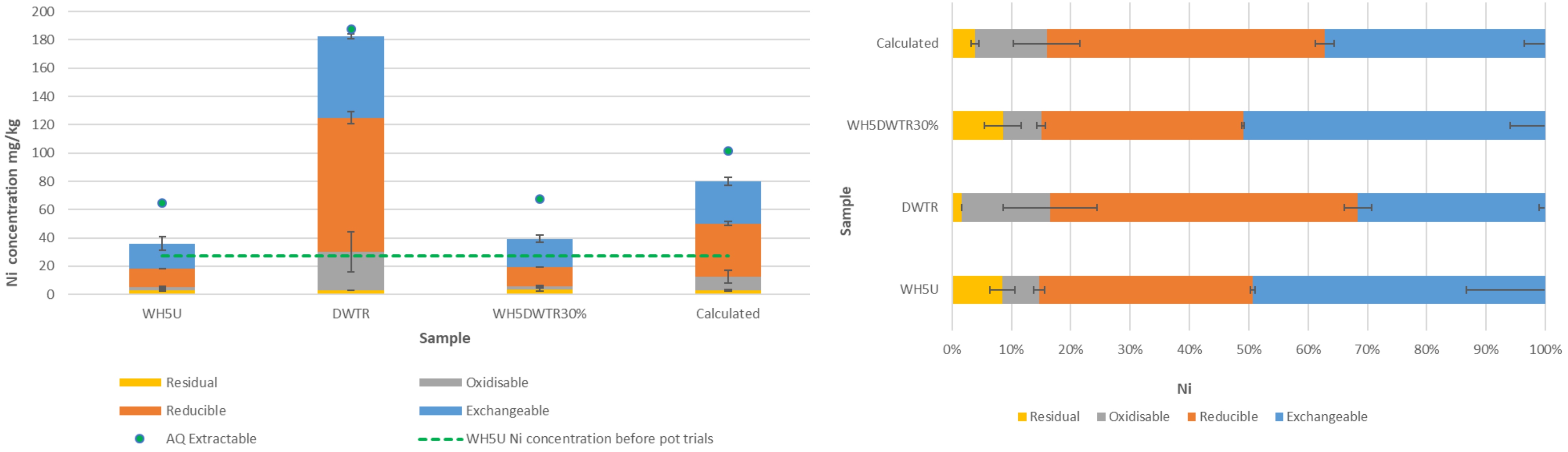

Figure 50 The distribution of Ni (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (DWTR), the amended mine soil WH5DWTR30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the DWTR. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions

The results for Ni had greater variability than all other results from the SE procedure but not to the extent as to effect the interpretation of their environmental significance. All AQ extractable concentrations were found to be slightly above the sum of the 4 extracts. The results are consistent with separate pseudo total analysis performed on samples not used in pot trials for WH5 and DWTR (27.6 and 164 mg/kg respectively). The Ni was found mainly in the in the exchangeable and reducible fractions in the unamended mine soil. Similar studies, show that Ni concentrations in mine soils are typically found in greater concentrations in the residual and oxidisable fractions and suggest that mining had limited impact on the analyte status (García-Ordiales *et al.*, 2016; Trifi *et al.*, 2018). For example, Trifi *et al*., found that 88% of the Ni in abandoned mine waste was only extractable by heated aqua regia. As the soil OM content of WH5 is just 2.2% the difference between the results of this study and others may be connected to a lack of OM for Ni to form organic complexes with coupled with, elemental competition for bonding sites with Cu, which is found in greater concentrations (Gonnelli and Renella, 2013). Concentrations of Ni found in DWTR sample were far higher than those found in the unamended mine soil. The distribution of Ni in the operationally defined fractions was found to be similar to the unamended mine soil i.e. the majority was found in in the liable fractions. This effect may be a result of the low pH of the DWTR (pH 5.2) as Ni solubility is increased at pH values below 6 (Gonnelli and Renella, 2013). Similarities between the unamended and amended both in terms of fractionation patterns and PT concentrations could suggest that the concentrations of Ni within the amended soil were not as homogeneous as that of other elements. The significant differences between the calculated and amended soil results are impacted by the far greater concentrations found in the amendment and so do not indicate a change in Ni mobility.

7.4.5 Lead

The Pb results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 48.

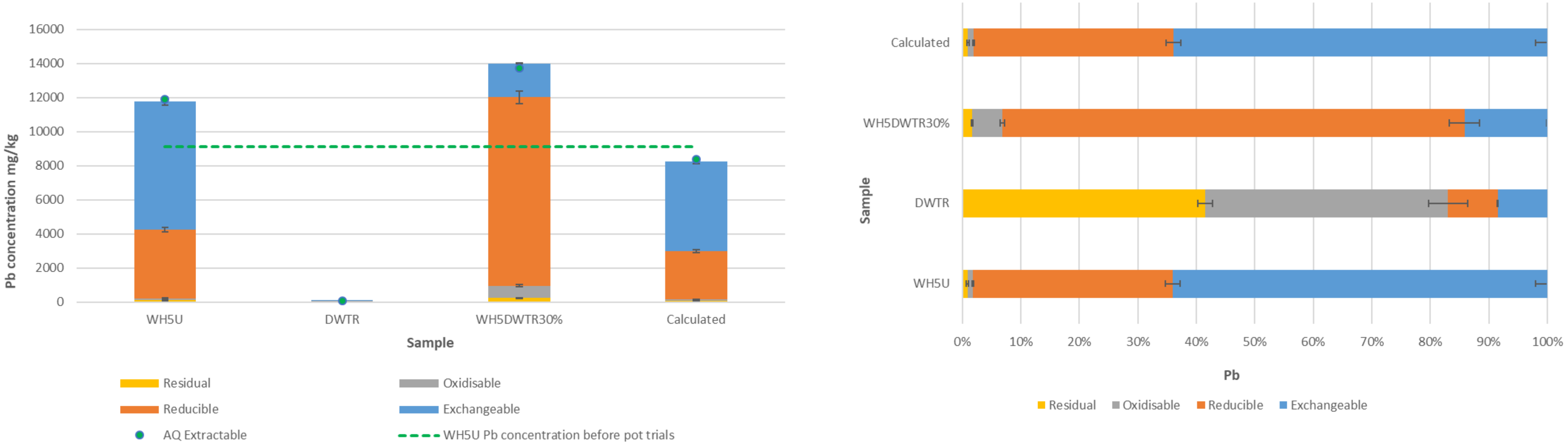

Figure 51 The distribution of Pb (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (DWTR), the amended mine soil WH5DWTR30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the DWTR. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

Consistently low variability between triplicate results obtained for each step of the sequential extraction as indicated by the small size of the error bars provides confidence in the results. *Aqua regia* extractable concentrations provide further reliability to the results as they are very similar to the mass balance sum of the 4 separate extracts. In the unamended WH5 soil Pb was found to be predominantly in the exchangeable fraction which indicates that it is highly mobile. This is of considerable concern given the high toxicity of the analyte. This is also unusual as previous studies of PTE fractionation patterns of historic mine soils have found that Pb is typically associated with the reducible fraction when sequential extraction procedures are applied (Clevenger, 1990; Rodríguez *et al.*, 2009).

This is the case in research conducted by Rodriguez *et al*., which studied mine soil with comparable characteristics to the current work which are important in Pb speciation such as OM (2.49%) and pH (5.7) (Rodríguez *et al.*, 2009). The unamended soil from plot WH5 of the current study has a OM content of 2.2% and a pH 6.5. A recent study suggested that the behaviour of Pb is unique to each specific mining waste environment due to the many different physio-chemical properties, making comparisons between two mine sites difficult (Miler *et al.*, 2022).

The high concentrations of Pb found in the exchangeable fraction of the mine soil made Pb the most mobile of all the PTE studied in this work. Whilst Pb in 'fresh' mine tailings can often be found in sulfide bound mineralogy (e.g Galena PbS), mine tailings that have been weathered over time can contain high concentrations of carbonate bound Pb (e.g Cerussite $PbCO_3$). A recent study of historic mine tailings in Belgium found that the dissolution of Cerussite was a controlling factor in the release of Pb acidic, alkaline and neutral soil conditions (Helser and Cappuyns, 2021). It is clear that a majority of the Pb in the WH5 soils is connected a highly mobile mineral species, most likely Cerussite and therefore was liberated by step one of the BCR extraction procedure.

The DWTR was found to have smaller concentrations of Pb (56.2 mg/kg) which were similar to previous analysis (50.5 mg/kg). The concentrations found in the operationally defined fractions differed from the unamended WH5 mine soils significantly and indicate that the Pb in the amendment in the residual fraction is most likely geogenic.

In the WH5 sample amended with 30% DWTR (amendment as received weight equivalent/soil dry weight) the Pb is found predominantly in the reducible fraction which indicates that Pb was occurring in a form bound to Fe/Mn oxides. The calculated fractionation patterns indicate that there should have been a drop in the total concentrations of Pb and no movement towards the reducible phase. Statistically significant (see appendix 10.3) changes in the in the operational speciation of the amended soil were found when compared to the unamended and calculated concentrations, suggesting that a process has taken place that alters the fractionation of Pb.

This process is likely to be the result of the introduction Fe oxides in the DWTR amendment. The high complexation and sorption potential of Pb in the presence of Fe oxides reported in the literature (Yiacoumi and Tien, 1995; Finlay, 2015). A reduction in the mobility of Pb through the addition of DWTR is a key finding of the current study and one that suggests a need for further study on the reuse of this material for remediation purposes.

The low concentrations of Pb in the DWTR had little effect on the concentrations within the amended soil alone. Greater concentrations of Pb were found in the amended soil when compared to the unamended. This suggests that Pb may have leached from of the unamended soil during the pot trial process. Concentrations of 869 mg/kg Pb were found in the biomass grown in unamended WH5 soils and only 277 mg/kg in the amended soil. The combination of metal uptake into biomass and leaching through regular watering over the course of 10 weeks where the majority of the Pb is in the liable fractions may therefore be responsible for the unamended soil Pb reduction. Previous analysis of the WH5 bulk sample taken before the pot trial process is unable to support this however as it provided a much lower concentration of Pb (9110 mg/kg) than mass balance and the AQ extractable results do for the unamended and amended pot trial soils.

7.4.6 Zinc

The Zn results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 52.

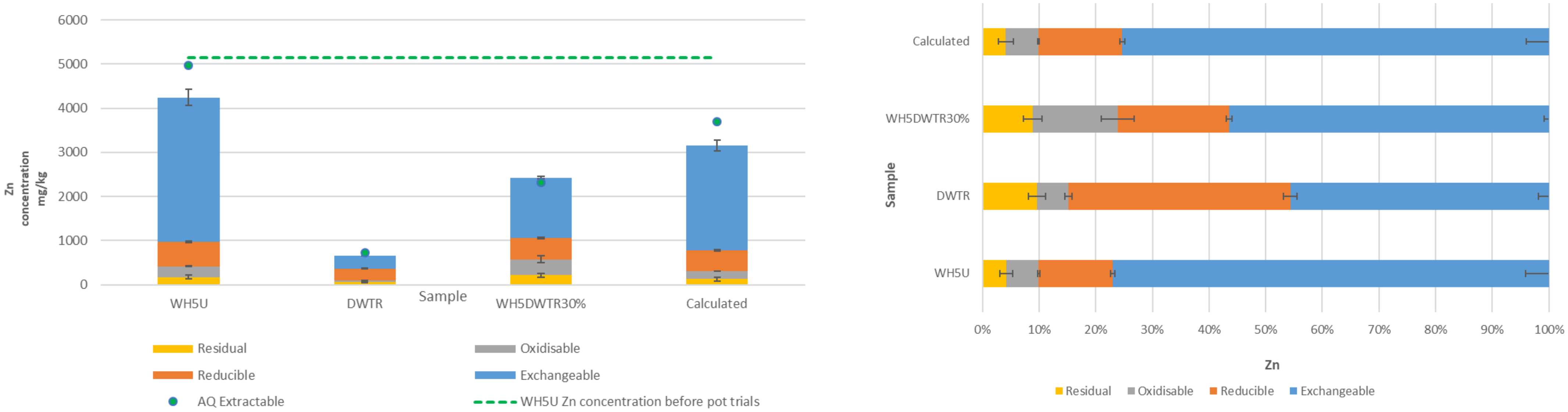

Figure 52 The distribution of Zn (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (DWTR), the amended mine soil WH5DWTR30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the DWTR. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

Low variability within the distribution of Zn provides good reliability for the results. The AQ extractable concentrations are generally close to the sum of the Zn concentrations found in the four extracts analysed during the BCR procedure. The majority of the Zn found in the unamended mine soil (WH5U) is in the exchangeable fraction. Rodriguez *et al*., reported a similar finding when applying the BCR method to mine tailings in Spain which had comparable pseudo total concentrations (Rodríguez *et al.*, 2009). The Zn pseudo total concentrations found in the DWTR (727 mg/kg) were slightly higher but comparable to previous analysis (595 mg/kg).

The addition of 30% DWTR does not seem to have a considerable effect on the solubility of Zn as the majority of the Zn in the amended soil remains in the exchangeable fraction. However, a considerable portion of the total concentration of Zn is lost in the amended soil when compared to the unamended. Concentrations in the biomass do not account for this. A mass balance calculation shows that the biomass produced in WH5 soils amended with DWTR at 30% with a dry weight of 0.012g (n=3) and a Zn concentration of 3740 mg/kg would have only removed 0.045 mg of Zn. The total Zn for WH5U is 4240 mg/kg and the total Zn for the amended soil is 2430 mg/kg a difference of 1810 mg/kg. The difference between the two for the exchangeable fraction is 1897 mg/kg which means that almost all the Zn that has been lost was bound in the most mobile fraction and has therefore likely been leached out during the pot trial process. It is only this loss of the exchangeable fraction that results in a statistically significant change (appendix 10.3) between the unamended and calculated with the amended soil.

The Zn concentration obtained in analysis of the unamended WH5 bulk sample before the pot trial process took pace was found to be 5150 mg/kg as shown as a reference line in figure 20. As this result is only slightly greater than the AQ extractable result obtained following the completion of the pot trial 4980 mg/kg it may be a combination of the pot trial process and the incomplete removal of Zn from the solid substrate using AQ digestion that is responsible for the lower concentrations in the sum of all the sequential extraction extracts.

7.5 Results from the BCR sequential extraction procedure on soils amended with green waste compost

7.5.1 Cadmium

The Cd results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 53. Consistently low variability between triplicate results obtained for each step of the sequential extraction as indicated by the small size of the error bars provides confidence in the results. The AQ extractable concentrations of Cd found in all samples was lower (approx. 30-40%) than the sum of the 4 separate extracts. This may be a result of the low sensitivity of the instrument (ICP-OES) for Cd: this effect has been seen in all Cd results obtained during this particular analysis.

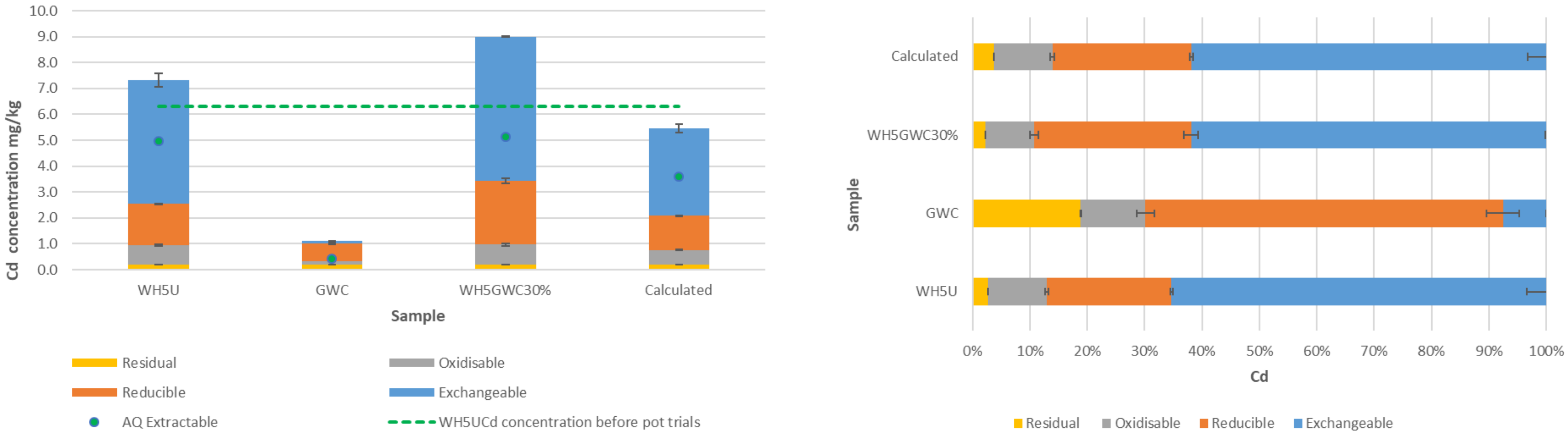

Figure 53 The distribution of Cd (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (GWC), the amended mine soil WH5GWC30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the GWC. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

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The Cd in the GWC was found to predominantly in the reducible fraction (66%). This may be due to the presence of both a high pH in the amendment (8.2) and the presence of oxyhydroxides such as Fe and Mn minerals that can influence the solubility of Cd in soils (Smolders and Mertens, 2013b).

The mine soil amended with GWC exhibits slightly greater concentrations in the more labile fractions particularly the reducible when compared with the fractionation patterns found in the unamended soil. This increase is significantly different from both the unamended soil and that of the calculated concentrations (see appendix 10.4). Li et at., found that the addition of 10% (amendment as received weight equivalent/soil dry weight) GWC was able to reduce the mobility of Cd in an originally uncontaminated soil that was spiked with Cd (Li *et al.*, 2020). The Cd biomass concentrations from RCG grown in the pot trials show that far less Cd is taken up into the biomass following the addition of GWC at both 20 and 30% (amendment as received weight equivalent/soil dry weight). For WH3 soils the Cd biomass concentrations fall from 35 to 3 mg/kg in the amended soil and in WH5 from 15 to 3 mg/kg. The greater uptake of Cd into biomass in the unamended soil may be the cause of the lower concentrations in the soils when compared to the amended. *Aqua regia* extractable results were consistently higher than the sum of the four extracts and were closer to previous analysis on the mine soil and amendment (23.1 and 25.8 mg/kg respectively) samples that had not been used in the pot trial process. This suggests that the pot trial process is not the cause of the greater AQ extractable concentrations.

7.5.2 Chromium

The Cr results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 51.

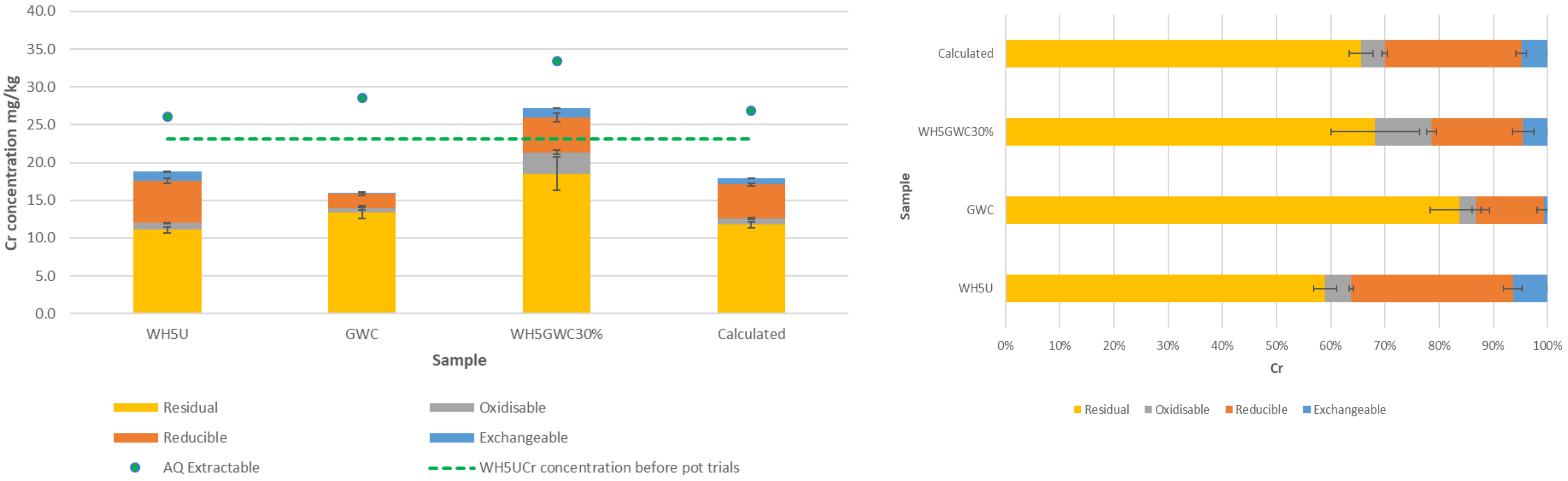

Figure 54 The distribution of Cr (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (GWC), the amended mine soil WH5GWC30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the GWC. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

The majority of results for Cr had low standard deviation with the exception of the residual fraction of the amended soil. It is this fraction that is being impacted by the addition of GWC and is significantly different to both the amended and calculated (see appendix 10.4). The lack of uniformity of the samples for the impact on the residual fraction could suggest that a reaction is taking place within the sample due to the addition of the amendment but that this reaction is not happening in a uniform way. This effect was also observed to a lesser extent in the soils amended with DWTR. The Cr in the GWC was found to be predominantly in the residual fraction following digestion with heated aqua regia (84%).

A similar study using 10% (v/w) GWC as a contaminated soil amendment found that the mobility of Cr increased following a pot trial (Medyńska-Juraszek *et al.*, 2020). Yimeng *et al*., applied the BCR procedure to a Cr-contaminated soil and found that concentrations increased in the residual fraction following co-pyrolysis with 20% rice straw at 300 C° (Yimeng *et al.*, 2020). Although this experiment differs considerably to the current study there is little published evidence of Cr concentrations found in greater quantities in the residual fraction following amendment. The mobility of Chromium (III) has been found to decrease in the presence of alkali conditions (Xu *et al.*, 2020). Xu *et al*., found that this effect increases over time and that whilst initially the Cr will move into the oxidisable phase (associated with organic matter bonding) at pH 8 , after 15 days Cr will eventually more into the residual phase (Xu *et al.*, 2020). The application of GWC (pH 8.2) resulted in the increase of the pH value in the amended soil from pH 6.5 (WH5U) to pH 7.2 (WH5GWC30%). It is therefore likely that the increase in pH over the 10-week pot trial period is responsible for the described change in Cr fractionation.

7.5.3 Copper

The Cu results from the analysis of the pot trial soils following the BCR procedure are shown in ***Figure 55***

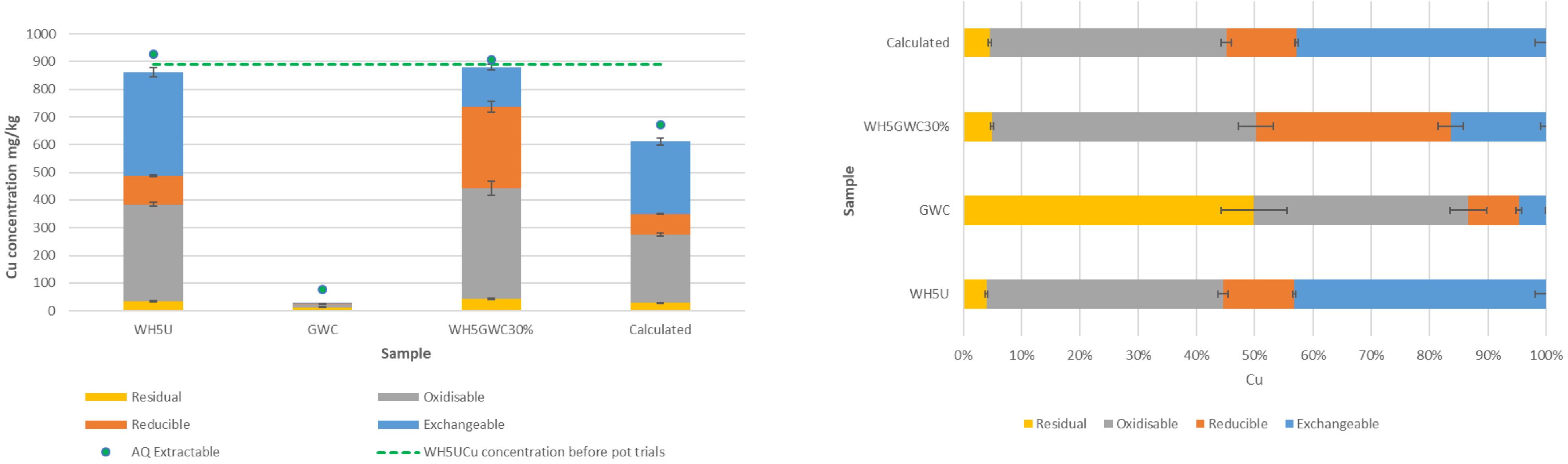

Figure 55 The distribution of Cu (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (GWC), the amended mine soil WH5GWC30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the GWC. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

Consistently low variability between triplicate results obtained for each step of the sequential extraction as indicated by the small size of the error bars provides confidence in the results. The AQ extractable results were also found to be consistent with the sum of the extractions allowing for confidence in the results. The low concentration of Cu in the GWC sample is consistent with previous analysis of the amendment (72.1 mg/kg) allowing for a clear identification of the impact that GWC may be having on the fractionation of Cu.

The soils amended with GWC show greater concentrations in the reducible fraction and lower concentrations in the exchangeable fraction when compared to the unamended soils. This is unlike in the pot trial soils amended with DWTR where Cu concentrations were found predominantly in the oxidisable fraction leaving very little in the reducible. This change in fractionation of Cu is significant when compared with that in both the unamended mine soil and calculated concentrations (see appendix 10.4). Although Li *et al.,* attribute this effect to complexation with humic acids found in the GWC, Clemente and Bernal have found that humic acids can mobilise Cu due to complexation with soluble organic matter (Clemente and Bernal, 2006).

The addition of GWC reduced the biomass concentrations from 100 mg/kg in the unamended soil to 11.1 mg/kg in that amended with 30% (amendment as received weight equivalent/soil dry weight). A decrease in biomass Cu concentrations and in Cu concentrations found in the exchangeable fraction once the BCR sequential extraction procedure is applied indicates that this method has provided consistent information on the effects of GWC in pot trials and metal fractions in the soil–metal concentrations uptake by the plant, a finding that has been confirmed by similar studies (Sungur *et al.*, 2014).

7.5.4 Nickel

The Ni results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 53.

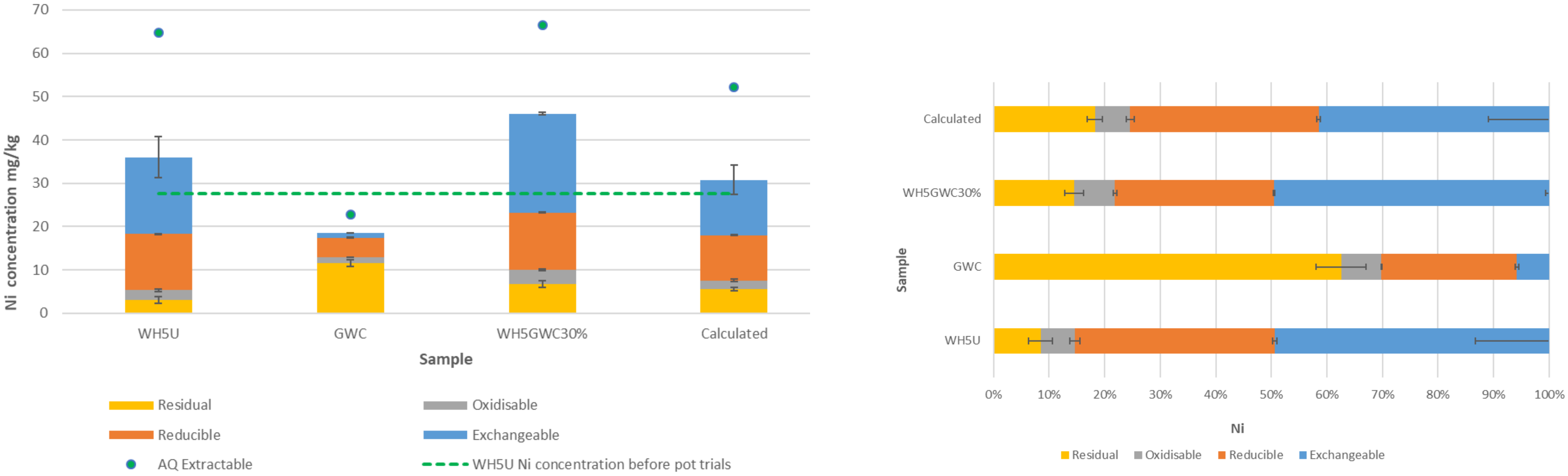

Figure 56 The distribution of Ni (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (GWC), the amended mine soil WH5GWC30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the GWC. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

Results from the SE procedure generally had low variability except for the exchangeable fraction of the unamended soil (this is also seen in the calculated concentrations as an artefact). *Aqua regia* extractable results were found to be greater than the sum of the concentrations found from the SE procedure but are consistent with separate pseudo total analysis performed samples not used in pot trials for WH5 and GWC (27.6 and 23.8 mg/kg respectively).

The majority of the Ni in the GWC was found in the residual fraction (64%). Several studies have reported similar fractionation patterns for Ni in mature GWC (He *et al.*, 1995; Greenway and Jun Song, 2002; Greenway and Song, 2002; Tandy *et al.*, 2009). Tandy *et al*., found that, in a fresh compost, the available fractions of Ni that were extracted increased due to labile organic matter breakdown coupled with a drop in pH, which occurs during the heating phase of the composting process (Tandy *et al.*, 2009). Once the GWC was over 22 weeks old however, a sharp rise in Ni in the residual fraction (88%) was discovered with very little in the available fraction (7%). The authors could not attribute this to any particular condition (Tandy *et al.*, 2009). Similar to the Ni fractionation patterns found in the soils amended with DWTR, no significant effect of the addition of GWC on the fractionation of Ni was found (see appendix 10.4). This result matches the biomass concentrations for Ni in which no discernible pattern was detected in the unamended and amended soils. Nickel (II) has a very low solubility in soils with pH above 7 and forms inner sphere complexes with soil solid phases the binding of which is very reversible (Gonnelli and Renella, 2013). This may be the reason why little change in fractionation has been observed following the addition of GWC.

7.5.5 Lead

The Pb results from the analysis of the pot trial soils following the BCR procedure are shown in

Figure 57

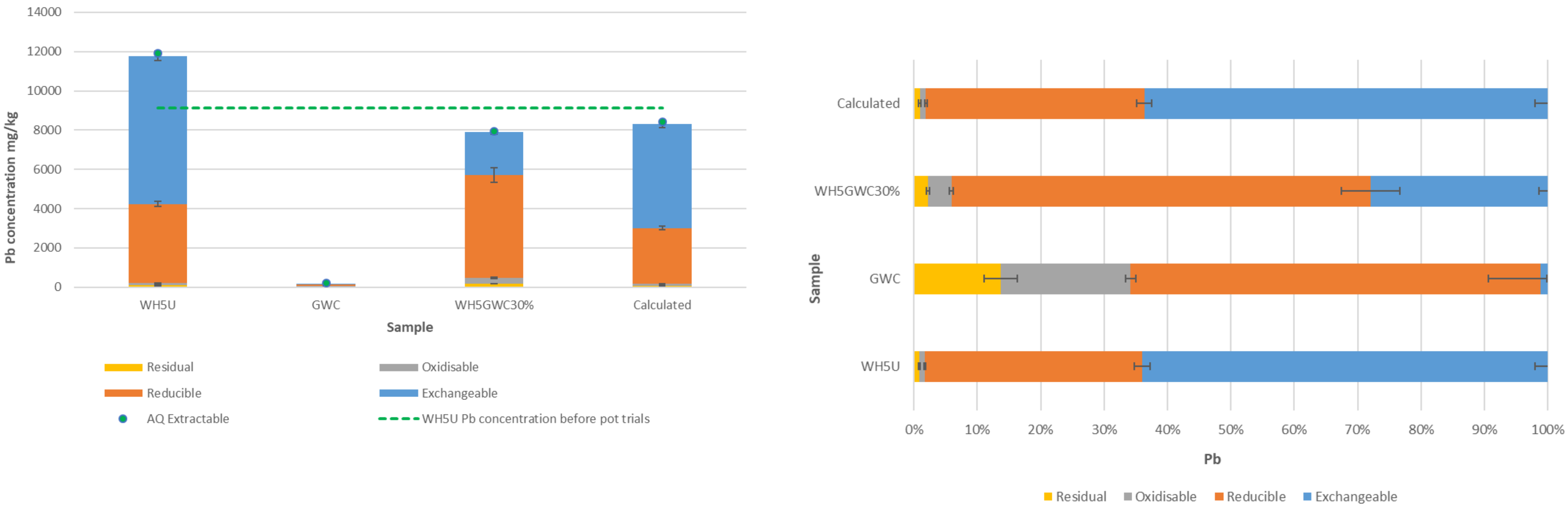

Figure 57 The distribution of Pb (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (GWC), the amended mine soil WH5GWC30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the GWC. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

The low variability between triplicate results obtained for each step of the sequential extraction as indicated by the small size of the error bars means that the results can be considered reliable. *Aqua regia* extractable concentrations provide further reliability to the results as they are very similar to the mass balance sum of the 4 separate extracts. Relatively low concentrations of Pb were found in the amendment sample which suggest that Pb concentrations alone are unlikely to have an effect on fractionation patterns.

The majority of Pb found in the GWC was associated with reducible fractions (63%), such as Fe oxide bound. Several authors have found that Pb in mature GWC is typically associated with the organically bound fractions as the analyte is re-adsorbed onto humic-like substances which usually make up a large portion of the organic matter by the end of composting process (Hsu and Lo, 2001; Tandy *et al.*, 2009). Although some of the Pb in the analysed GWC sample is associated with the oxidisable fraction (20%), this finding suggests that the compost is possibly not as mature as others that have been studied.

The sample amended with GWC has significantly different Pb concentrations (see appendix 10.4) in the exchangeable and reducible fractions when compared to the unamended soil and the calculated concentrations. It is clear that the addition of 30% GWC (amendment as received weight equivalent/soil dry weight) is having an impact on the speciation of Pb in the amended sample and is reducing overall mobility of the analyte. It is likely that there are three factors involved in the decreasing the extraction efficiency of Pb by acetic acid (the reagent use as the exchangeable fraction indicator of BCR procedure). The effects of both organic matter and an increase of pH are well understood factors in decreasing the solubility of Pb (Alvarenga *et al.*, 2008; Li *et al.*, 2021). The addition of GWC with an organic matter content of 26.4% resulting in an increase from pH 6.5 (WH5U) to pH 7.67 (WH%GWC30%). The high complexation and sorption potential of Pb in the presence of humic material is well reported in the literature (Bradl, 2004; Palansooriya *et al.*, 2020). It is also conceivable that the high P (2174 mg/kg) content of the GWC could have provided enough soluble phosphate to promote Pb phosphate precipitation (Cao *et al.*, 2003; Bosso *et al.*, 2008; Fleming *et al.*, 2013) .Cao *et al*., found that the addition of H_3PO_4 to Pb contaminated soils resulted in the decrease of Pb concentrations in the liable fractions by 53% when the Tessier sequential extraction method was applied (Tessier *et al.*, 1979; Cao *et al.*, 2003). This was attributed to the formation of a insoluble pyromorphite-like mineral in the surface and subsurface of the soil. The addition of P to the soil also enhanced metal uptake in the roots of St. Augustine grass (*Stenotaphrum secundatum*) whilst reducing Pb translocation from root to shoot via the formation of a pyromorphite-like mineral on the membrane surface of the root (Cao *et al.*, 2003). This finding suggest that a phytostabilisation strategy for the remediation of Pb mine soils should involve the use of an amendment with high P concentrations such as GWC.

7.5.6 Zinc

The Zn results from the analysis of the pot trial soils following the BCR procedure are shown in Figure 58.

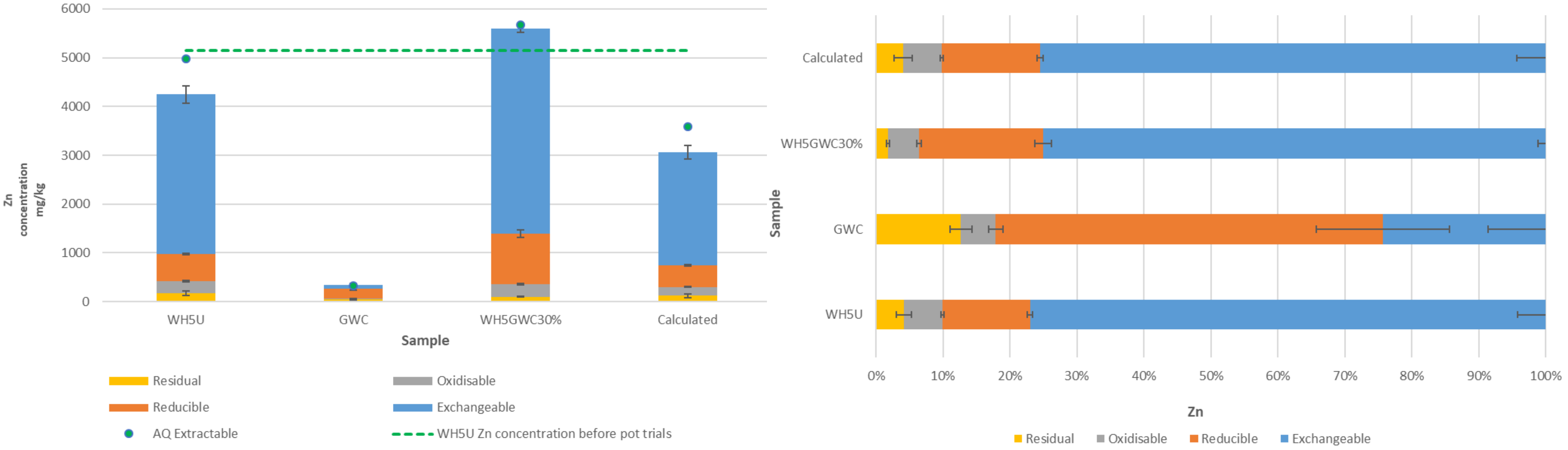

Figure 58 The distribution of Zn (mg/kg) in the operationally defined fractions of the unamended mine soil (WH5U) the amendment (GWC), the amended mine soil WH5GWC30% amended at 30% (amendment as received weight equivalent/soil dry weight) and a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the GWC. The error bar represents the mean ± standard deviation (n = 3). A separate sample was digested in aqua regia (AQ) to compare with the sum of the four operationally defined fractions.

Low variability between triplicate results obtained for each step of the sequential extraction, as indicated by the small size of the error bars provide reliability for the precision in the results. Relatively low concentrations of Zn were found in the GWC amendment sample which suggest that Zn concentrations alone are unlikely to have an effect on fractionation patterns. The majority of Zn found in the GWC was associated with the most available fractions (85%). The availability of Zn has been reported to increase as part of the composting process connected to humic substances and changes in pH (Amir *et al.*, 2005; Tandy *et al.*, 2009).

The pot trial soil amended with 30% GWC (amendment as received weight equivalent/soil dry weight) contains significantly greater concentrations (see Table 52) of Zn in the liable fractions when compared to the unamended soil. This suggests that Zn may have leached from the unamended soil during the pot trial process as previously discussed in section 7.4.6. The significantly greater biomass (p=0.05) produced in the WH5GWC30% (0.040g ± 0.03) DW) soils compared to that produced by the unamended WH5U (0.004g ± 0.002) soils may have resulted in less leaching of Zn. Shrestha et at., noticed this effect in unvegetated soils during a pot trial which analysed the impact of compost based amendments on the phytoremediation potential of switch grass (*Panicum virgatum*) (Shrestha *et al.*, 2019).

7.6 Summary of pot trial sequential extraction results

Table 39 shows the statistical significance of the changes in speciation of the PTE found in the pot trial soils when compared to the unamended (but planted) soil. These changes are the result the of following or a combination of the following; the amendment-soil concentration differences, the plant effects (uptake or root exudates), the chemical interaction between soil and amendment and also leaching effects (possibly also differential effects from interactions with some of the above). A one-way ANOVA with a Bonferroni post hoc test at 0.05 significance level was used to test for significance.

There were 8 and 10 significant changes in the operational speciation of PTE in soils amended with DWTR and GWC respectively compared with the unamended soils. The operational speciation of Cu and Pb was the most prone to change in soils of both amendments (4 changes each). For these two analytes both the amendments were found to reduce their mobility as greater concentrations were found in the reducible fraction and less in the exchangeable following soil amendment. Neither amendment had a significant change on the operational speciation of Ni. Both amendments increased the mobility of Cd, most noticeably GWC. Both amendments increased the amount of Cr that was found in the residual fraction following the BCR procedure. DWTR decreased the mobility of Zn whereas GWC increased it, although this is thought to be connected to the leaching of Zn throughout the pot trial process.

Table 39 Summary of the statistical significance of the changes in speciation of the PTE speciation following the 10 week pot trial in amended soils. A one-way ANOVA with a Bonferroni post hoc test at 0.05 significance level was used for PTE concentration (mg/kg; n = 3) in step 1 - 4 of the BCR sequential extraction scheme for pot trial soils amended compared to an unamended control. An l or m indicates that the result is significantly less or more than then respective results found in the unamended soil.

Sample	**Changes in operational speciation of PTE in pot trial soils comparing amended soils with the unamended soils after plant growth.**					
WH5DWTR30%	Cd	Cr	Cu	Ni	Pb	Zn
Exchangeable	↔ 1.000	↔ 1.000	↓ 0.000l	↔ 1.000	↓ 0.000l	↓ 0.000l
Reducible	↑ 0.000m	↔ 1.000	↓ 0.000l	↔ 1.000	↑ 0.000m	↔ 1.000
Oxidisable	↑ 0.000m	↔ 1.000	↔ 0.653	↔ 1.000	↔ 0.160	↔ 1.000
Residual	↔ 1.000	↑ 0.043m	↔ 1.000	↔ 1.000	↔ 1.000	↔ 1.000
WH5GWC30%						
Exchangeable	↑ 0.000m	↔ 1.000	↓ 0.000l	↔ 1.000	↓ 0.000l	↑ 0.000m
Reducible	↑ 0.000m	↔ 1.000	↑ 0.000m	↔ 1.000	↑ 0.000m	↑ 0.000m
Oxidisable	↔ 1.000	↔ 1.000	↑ 0.000m	↔ 1.000	↔ 1.000	↔ 1.000
Residual	↔ 1.000	↑ 0.000m	↔ 1.000	↔ 1.000	↔ 1.000	↔ 1.000

m = more than – up ↑

l = less than – down ↓

No significant change ↔

Table 40 shows the statistical significance of the changes in speciation of the PTE found in the pot trial soils when compared to a calculated sample based on the outcome of replacing the results for 30% of the unamended soil with 30% of the amendment. As an introductionary comment on the Table 40, It is important to also compare the calculated or mass balance of an amended soils fractionation patterns as this decreases the chance that a 'significant' result is actually just based on changes in concentration of an analyte rather than change in fractionation. The changes found here may be the result of; the plant effects (uptake or root exudates), the chemical interaction between soil and amendment and also leaching effects. The following is a description of where the unamended soils differ from the calculated when compared to the amended. A One-way ANOVA with a Bonferroni post hoc test at 0.05 significance level was used to test for significance.

For Cd the key statistical difference occurred in the soils amended with DWTR. Here an increase in the available Cd was observed as there is a significant change in the exchangeable fraction of the amended soil when compared to that of the mass balance calculation. This result is coupled with a significant reduction in Cd found in the oxidisable fraction. No change between the statistical tests using either the calculated (Table 4) or amended soil concentrations (Table 3) was noted for the soils amended with GWC suggesting that the observation that the amended is increasing the availability of the Cd is potentially correct.

Table 40 Summery of statistically significant changes in PTE speciation following a 10 week pot trial in amended soils. A one-way ANOVA with a Bonferroni post hoc test at 0.05 significance level was used for PTE concentration (mg/kg; n = 3) in step 1 - 4 of the BCR sequential extraction scheme for pot trial soils amended compared to calculated soil concentrations. An l or m indicates that the result is significantly less or more than then respective results found in the amended soil.

Sample	**Changes in operational speciation of PTE in pot trial soils in amended soils compared to equivalent calculated mixtures of end-members after plant growth**					
WH5DWTR30%	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Exchangeable	↑ 0.000m	↔ 1.000	↓ 0. 000l	↔ 0.289	↓ 0.000l	↓ 0.000l
Reducible	↑ 0.000m	↔ 1.000	↔ 0.183	↓ 0.000l	↓ 0.000l	↔ 1.000
Oxidisable	↓ 0.000L	↔ 1.000	↑ 0.000m	↔ 1.000	↑ 0.009m	↔ 0.859
Residual	↔ 1.000	↔ 1.000	↔ 1.000	↔ 1.000	↔ 1.000	↔ 1.000
WH5GWC30%	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Exchangeable	↑ 0.000m	↔ 1.000	↓ 0.000l	↔ 0.169	↓ 0.000l	↑ 0.000m
Reducible	↑ 0.000m	↔ 1.000	↑ 0.000m	↔ 1.000	↑ 0.000m	↑ 0.000m
Oxidisable	↔ 1.000	↔ 1.000	↑ 0.000m	↔ 1.000	↔ 1.000	↔ 1.000
Residual	↔ 1.000	↑ 0.000m	↔ 1.000	↔ 1.000	↔ 1.000	↔ 1.000
m = more than – up ↑	l = less than – down ↓	No significant change ↔				

The comparison of the amended soil concentrations (Table 39) with the calculated concentrations (Table 40) shows that the significant result found when comparing the unamended soil to the DWTR amended soil for the increase in residual Cr is most likely a result of the summation of the concentrations found in both soils rather than a chemical reaction resulting in a change in fractionation. This shown in Table 40 where the concentrations of Cr in soils amended with DWTR do not significantly change in any of the operational fractions. However, for the GWC amended soil the significant movement away from the residual fraction is found to be consistent between the two tables.

The comparison of the Cu results between the unamended and calculated concentrations when compared to the DWTR amended allow for the observation that the significant decrease in Cu mobility is connected with an increase in the oxidisable or organic matter bound fraction. However, for the GWC amended soils the significant decrease in Cu mobility remains attributed to increases in both the reducible and oxidisable fractions.

Nickel in the soils amended with DWTR have significantly less Ni in the reducible fraction of the calculated mass balance. This is not the case when comparing concentrations in the unamended soil suggesting that a chemical reaction is taking place.

Lead in the soils amended with DWTR have significantly greater concentrations in the oxidisable fraction and lowering in the exchangeable and reducible fractions when compared to the calculated mass balance in table 12. This finding may be one of the most substantial of the study given the toxicity and quantity of the analyte and the difference in the risks to human health associated with a change in the availability of Pb. This effect is not apparent when comparing the amended soil with the unamended where it would appear that the Pb is 'moving' into the reducible fraction. The results for GWC and Pb do not differ when compared to a calculated mass balance but do confirm the positive results of the increase in reducible Pb and decrease in exchangeable Pb.

The statistical comparison with the mass balance calculation do not differ with the unamended soil for Zn. This may confirm the hypothesis that the significant results for Zn are connected to the leaching of the analyte throughout the pot trial process.

7.7 Risk assessment code

The risk assessment code (RAC) is used to assess risk and mobility of the most labile fractions of the PTE in question. The RAC has been used on a range materials including soil and sewage sludge (Asmoay *et al.*, 2019; Tytła, 2019) and was originally developed for sediments (Perin *et al.*, 1985). There is some debate as to whether the RAC should be applied to sequential extraction results in this way, although it does currently remain in popular use within the field (Basheeru *et al.*, 2021; Kumar *et al.*, 2021; Matabane *et al.*, 2021; Sungur and Isler, 2021). A RAC value of 1% is considered to present no risk to the aquatic environment but, if that 1% is part of a very high total PTE concentration, then there may still be a risk (Bacon and Davidson, 2008). In the case of this study the RAC is applied to the 'exchangeable' fraction and is calculated as the concentration of each PTE in the exchangeable fraction divided by the sum of the fractions (including the residual). This is shown in Equation 14.

$$RAC = \frac{exchangeable}{exchangeable + reducible + oxidisable + residual} \times 100$$

Equation 14 risk assessment code (RAC) calculation

The effects of the addition of both DWTR and GWC at 30% (amendment as received weight equivalent/soil dry weight) to pot trial soils on ecological risk assessment using the RAC are listed in Table 41.

Table 41 The effects of the addition of either DWTR or GWC at 30% (amendment as received weight equivalent/soil dry weight) to pot trial soils on ecological risk of PTE using the risk assessment code (RAC)

Sample	Risk assessment code (RAC) (%)						Category	
	Cd	Cr	Cu	Ni	Pb	Zn	1% < RAC ≤ 10%	Low risk (LR)
WH5U	65	6	43	49	64	77	10% < RAC ≤ 30%	Medium risk (MR)
WH5GWC30%	62	4	16	50	28	75	30% < RAC ≤ 50%	High risk (HR)
WH5DWTR30%	39	7	8	51	14	56	50% > RAC	Very high risk (VHR)
🟩	Movement into lower risk category							
🟥	Movement into higher risk category							

Both amendments lowered the RAC value of almost elements apart from Ni and the Cr found in the soil amended with DWTR, this was not very pronounced (an increase of 1 or 2% RAC value). Both DWTR and GWC raised the RAC value for Ni by 2% and 1% respectively. Although the addition of both amendments did move Ni into a higher risk category (VHR) it is not a significant enough change to justify highlighting further or drawing conclusions from.

One of this issues with the using this form of risk assessment following sequential extraction is that it focuses on the exchangeable fraction and not the exchangeable and reducible even though the reducible fraction also represents labile PTE. It is for this reason that the Cd concentrations here seem to be falling in risk when from looking at Table 41 it is clear that the analyte is becoming more mobile following soil amendment. The concentrations shown in Figure 47 and Figure 53 indicate that Cd is found in greater concentrations in the reducible fraction and less in the oxidisable following amendment with DWTR and to a lesser extent GWC. This is of concern given the toxicity of the analyte but would not be detected by a traditional RAC calculation following sequential extraction.

The most beneficial effects were seen for Cu and Pb for which the RAC was reduced by 2 or 3 risk categories in both amended soils. The statistical analysis of the results (shown in Table 39 and Table 40) is further evidence for this change in risk. This result is particularly interesting given the toxicity of the two analytes and their high concentrations in the mine soil. Although a beneficial effect can be noted in the reduction of availability of two PTE following the application of both amendments, there considerable concentrations and toxicity mean they remain a risk to human health, the issue raised in the previously mentioned review (Bacon and Davidson, 2008).

7.8 Chapter conclusions

The application of the sequential extraction procedure on unamended and amended WH5 pot trial soils allowed for detailed observations on the effect that these amendments can have on the mobility of PTE within soils. The sequential extraction results were achieved following a miniaturisation of the standard method. The successful recovery for the analytes measured in the CRM suggests that the alternations to the method did not impact the results and therefore could become common place. Further research is required to confirm this finding. Almost all of the sequential extraction results for each analyte had consistently low variability between triplicate results for each step of the sequential extraction as indicated by the small size of the error bars and AQ extractable concentrations that were very similar to the mass balance sum of the 4 separate extracts providing confidence in the results. No result had a level of variability between triplicates to the extent as to cause a reduction in reliability.

In the unamended WH5 soil Cd was found to be predominantly in the exchangeable fraction which is of concern given the toxicity of the analyte. The addition of DWTR was found to have an effect increasing the mobility of Cd but this was likely a result of the amendment process rather than a chemical reaction due to the higher concentrations found in the DWTR. The addition of GWC also resulted in greater concentrations in the more labile fractions when compared to the unamended soils, although this was less pronounced than for the DWTR. A combination of inter-element competition for sorption sites and greater Cd uptake in biomass in unamended soils was hypothesised as reasons for these results.

The majority of Cr in the mine soil was found within the residual fraction and also did not exceed the average rural soil concentrations of Cr found by the UK soil and herbage pollutant survey suggesting that its source is not anthropogenic (Creaser *et al.*, 2007). The Cr in both the GWC and DWTR was found to be predominantly in the least mobile, residual fraction. The addition of the DWTR lowered the pH of the amended soil and is thought to be responsible for the slight increase in Cr mobility found in the amended soil when compared to the unamended. The addition of GWC slightly lowered the mobility of Cr and it is thought, based on the findings of similar studies that a change of pH following soil amendment may also have played a role in this change.

The Cu in the mine soil was found mainly in the exchangeable and oxidisable fractions, which is typical when applying the BCR procedure as Cu has strong affinity for organic matter. The low concentrations of Cu found in the amendments had a relatively even distribution of fractionation which made drawing conclusions about the effect on the mine soil Cu more simple when compared to Cd and Cr. Significant increases in the oxidisable fraction and decreases in the exchangeable fraction of the DWTR amended soil when compared to the unamended soil led to the conclusion that the high OM content of the amendment led to a reduction in the mobility of Cu, this effect has been seen in many similar studies. For the soils amended with GWC it was the reducible fraction that increased when the exchangeable fraction decreases when compared to the unamended soils. The effect of the addition both amendments was to considerable lower the RAC value for Cu suggesting scope for further research into their effects on Cu contaminated soils.

The results for Ni had greater variability than all other results from the SE procedure but not to the extent as to effect the interpretation of their environmental significance. The Ni concentrations in the unamended soil were far lower than those in the DWTR amendment and similar to those in the GWC and were in a fractionation pattern unlike those associated with historic mining suggesting that the mine soils were not impacted by Ni. The addition of either amendment to the mine soil did not lead to any notable changes in Ni fractionation.

The high concentrations of Pb found in the exchangeable fraction of the WH5 mine soil made Pb the most mobile of all the PTE studied in this work. The fractionation pattern suggests that the Pb is most likely carbonate bound and therefore Cerussite. In the mine soils amended with DWTR the Pb was predominantly in the reducible fraction which indicates that Pb was occurring in a form bound to Fe/Mn oxides which are common in DWTR due to the water treatment process. This movement in Pb constituted a significate change in mobility and suggests that DWTR could be used to remediate Pb contaminated soils. The addition of GWC also resulted in significant increases in the reducible fraction and decrease in the exchangeable. This change was attributed to increases in OM content (humic materials) and pH deceasing the solubility of Pb. There is a possibility the high P content of the GWC also played a role in decreasing the mobility of Pb through the formation of an insoluble pyromorphite-like mineral. Further research on the effects of P when used in mine soil remediation should be conducted to confirm this finding. Both amendments had the effect of considerably lowering the RAC value for Pb from a very high risk to a medium risk. However, this would not be the case if the RAC was applied differently taking into account the reducible fraction as well.

The majority of the Zn found in the unamended mine soil was found to be in the exchangeable fraction a finding reported in other similar studies. The addition of DWTR does not seem to have a considerable effect on the solubility of Zn as the majority of the Zn in the amended soil remained in the exchangeable fraction. A combination of the pot trial process Zn leaching or biomass accumulation and/or a failure in the analysis has been suggested as reasons for the Zn concentrations of the DWTR amended soil being lower than that of the unamended. The Zn concentrations in the GWC amended soil were found to greater than those in the unamended soils and this was also attributed to the same potential effect. Neither amendment had a considerable effect on the mobility of Zn and the RAC value did not move into a lower risk category following amendment.

8 The results of a 1.5-year long field trial using organic waste soil amendments to promote the growth of reed canary grass at a historic mine site

8.1 Aims and objectives

The aim of this chapter was to evaluate the potential of reed canary grass grown in a field trial with organic waste soil amendments as a phytostabilising remediation option for historic mine soils.

The specific objectives were: -

1. To compare the survival, establishment rate and biomass yield of three varieties of RCG when grown in different organic waste soil amendments during a 1.5-year long field trial
2. To understand if the variety of RCG and the amendment of the soil has an effect on the translocation of PTE into biomass by analysis of the biomass for concentrations of PTE from the field trial,
3. To investigate further the nature of concentrations of PTE found within biomass analysis using XCT scans to detect levels of density.

8.2 Introduction

The following chapter focuses on the results of a field trial at Whiteheaps mine. The field trial involved two fenced areas (plots) divided into nine sections (blocks) which each contained 36 individual reed canary grass plants (plant) grown in 1 of 4 experimental soil amendment factors (sets). The nature of the historic mine plot, the mine soils and the amendments used in this experiment have been extensively discussed in chapter 4 of this study.

There has been little published work involving fields where reed canary grass is grown on soils containing PTE with the majority of the research confined to laboratory pot and micro plot trial studies (Neuschütz and Greger, 2010a; Badmos *et al.*, 2015; Korzeniowska and Stanislawska-Glubiak, 2019). Despite recent interest in the topic, there have also been few field scale trials that evaluate the ability of organic waste amendments to immobilise PTE and promote growth on similarly contaminated soils to this study, with the majority of published research involving pot trials in laboratory's (Mahdy and Fathi, 2008; Hartley *et al.*, 2009; Karami *et al.*, 2011; Bolan *et al.*, 2014; Finlay, 2015; McCann *et al.*, 2015). A recent review of organic waste amendment applications at metal mine impacted plots concluded that, whilst phytostabilisation using amendments is currently transitioning from laboratory scale towards field trial and full-scale use, a full valuation of the benefits of such efforts is yet to realised and so more field trial studies are required (Brown *et al.*, 2016).

Smith and Slater conducted a field trial involving reed canary grass grown in agricultural soils amended with chicken litter and sewage sludge cake in order to investigate the effects of organic and inorganic fertilizer applications to energy crops (Smith and Slater, 2010). Miscanthus and the perennial cane grass *Arundo donax* were also planted. The field trial involved sheep grazing fields which were sprayed with glyphosate herbicide, ploughed, rotivated and harrowed before being planted with RCG seed at a sowing rate of 7.5 kg/ha. Limed sewage cake, chicken litter and inorganic nitrogen were applied at 37.5 kg available N/ha (low application level), 50 kg available N/ ha (medium application level) and 87.5 kg available N/ha (high application level). The study found that whilst Miscanthus and *Arundo donax* exhibited increased growth in response to organic waste soil amendment, the RCG did not. The study hypothesised that RCG has either a delayed response to fertilizer application or that the levels applied were insufficient to warrant a response (Smith and Slater, 2010).

Strasil *et al*., tested the response of RCG to Nitrogen (N) fertilisation and in field experiments over the course of seven years (1996-2003) (Strašil *et al.*, 2005). They also evaluated yield response to different harvest times (July, November, March) (Strašil *et al.*, 2005). The field trial involved three application rates of N fertilisation (0, 30, 60 kg/ha) on three brownfield plots in the Czech Republic. The study found that RCG yields responded positively to increasing doses of nitrogen. The study also found that even though harvesting in July led to yields with significantly greater moisture contents when compared to the two winter harvests, the yield from a summer crop was still greater than the November and March harvests due to the far greater wet mass of the summer yield (Strašil *et al.*, 2005). Despite low concentrations of PTE in the soils, the study found the RCG concentrations did not exceeded the permissible values set in the Czech Republic for food and feed purposes.

Lord (2015) grew RCG at five one-hectare brownfield plot field trials in the North East of England. The study involved amending the soils using in-situ GWC incorporation at an amendment rate of 250, 500 and 750 t/ha. Miscanthus and short rotation coppice Willow were also planted. Lord found that RCG was the best suited energy crop to the challenging soil conditions found on non-agricultural land as it outperformed the other species involved in the experiment in terms of ease of establishment, cost, time to maturity and yield. The study also found that the RCG biomass showed limited uptake from the PTE in the brown field soils when compared to the other species (Lord, 2015a). The in situ incorporation of GWC at 500 t/ha was found to be a cost effective way of achieving a viable seed bed and shallow, water retentive growth medium for RCG. Antonkiewicz *et al*., grew the RCG 'Bamse' variety in a six year long field trial in soils amended with sewage sludge at amendment application rates of 0,10,20,40, and 60 mg/ha (Antonkiewicz *et al.*, 2016). Miscanthus was also grown. The study found that the sewage sludge increased yields with increasing amendment rates and that the PTE in the sludge were translocated into the RCG biomass at a much lower rate than to the Miscanthus, making RCG not suitable for the remediation (by phytoextraction) of sewage sludge but potentially suitable for phytostabilisation.

8.3 Experimental

8.3.1 RCG plant growth

Three genetic varieties of RCG were grown from seed in seed trays for 12 weeks in open conditions under grow lamps in a laboratory. A total of 648 seed tray spaces were planted with three seeds in each space to allow for germination failure, providing 216 plugs to plant out of each cultivar. Average temperature ranged from 18 – 22 °C with little variation and light intensity was maintained at 2000 Lumen/m^2. Varieties grown, origins and known cultivar traits are presented in Table 42.

Table 42 - Varieties grown, origins and known genetic traits of reed canary grass used in field trial

RCG variety	Cultivar traits	Seed origin
BS5237	Game cover crop Unknown heritage – originally obtained in England – most likely "Pedja" cultivar variety	Aberystwyth University Institute of Biological, Environmental and Rural Sciences - IBERS
SW RF5004	Selected for high biomass and seed production for use in the biofuel industry from crossings with origins in Switzerland, South Eastern Europe and Portugal.	Lantmännen Lantbruk, Sweden
SW RF5032	Bellevue cultivar bred for vigour, seed retention and low alkaloid concentrations as a forage crop crossed with SW RF5004 Bellevue was originally developed by the Plant Science Department at the Macdonald Campus of McGill University, St Anne de Bellewe, Quebec (Coulman, 1995)	Lantmännen Lantbruk, Sweden

8.3.2 Layout of field trial

Two 9 x 9 m areas fully enclosed with rabbit-proof fencing were constructed at plots WH3 and WH5. The fenced areas were then divided into nine square blocks providing planting space for plants from three cultivar varieties planted in four intended amendment factors (Un-amended, GWC 30%, DWTR 30 % and a mixture of both amendments at 15% of each) as outlined in Figure 59. The nomenclature for the field trial is shown in Figure 59 and is as follows;

1. Whole fenced area – Plot
2. One of nine areas within the plot by which the four amendment factors and/or seed varieties have each been replicated – Block
3. The area within a block for which the soil has been amended for 9 individual; plants in one of the four possible ways – Set
4. An individual RCG plant – plant. The resulting plant spacing was 0.5 m on a regular grid.
5. Group – The 3 sets of 9 plants of a seed variety with a particular amendment type
6. Planting site – the 9 locations in each set (36 in each plot or 324 in each plot) where a plug was inserted.
7. Categories – All plants of a certain variety in a single plot, or all plants with a certain amendment

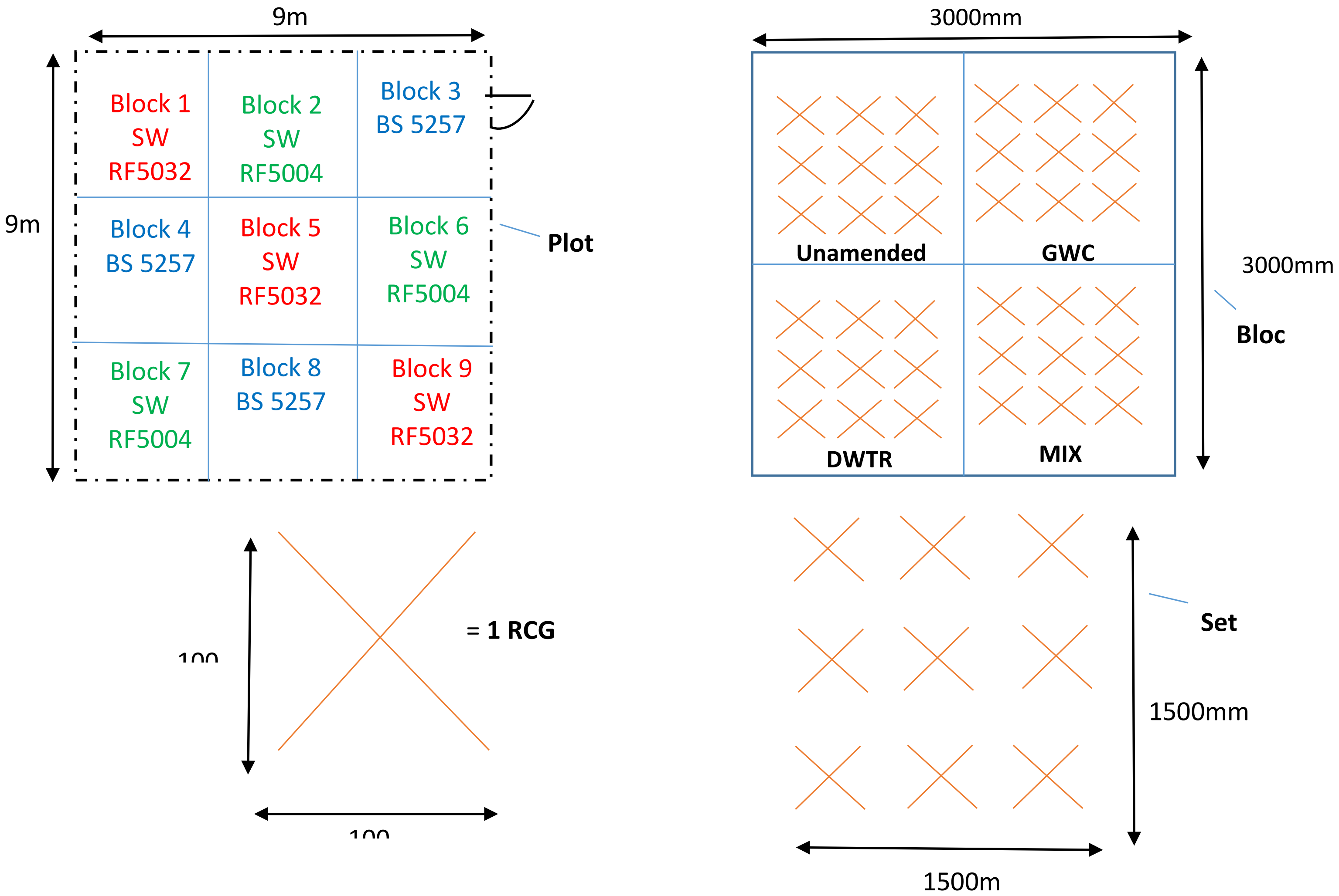

Figure 59 Amendment/planting plan for Whiteheaps phytostabilisation field trial for both WH3 and WH5.

8.4 Soil amendment method

8.4.1 WH3 – Volumetric method

The volume of the mine soil to be amended and amount of amendment used was determined by use of a 1 L plastic pot as follows: One litre of soil was removed from each planting space, which had been prepared by digging a 10 cm deep pit using a border spade, giving 9 L per set of 9 which was then weighed (approximately 10-12 kg wet mass) . Three litres of the required amendments were added and the mixture reweighed, allowing the as received (AR) amendment by mass to be calculated for each set. Due to the different densities, these are different for each amendment (Table 43). The mean amendment rates by mass were calculated by finding the average mass of amendment the 9 sets of each amendment type. The mass of amendment used in each set was calculated by the difference between the mass of the mine soil before amendment and the amended soil.

Table 43 Average application rates of amendments used in field trial at plot WH3 (n=9)

Amendment	Mean amendment application per set, kg (n=9)	Standard Deviation kg ±	Density, of amendment kg/L	Amendment/soil mass ratio (AR)	Amendment as % of total (AR)
GWC	1.9	0.05	0.63	0.17	14.2
DWTR	2.6	0.25	0.87	0.23	18.5
Mixture	2.27	0.24	0.76	0.19	16.2

Soil and amendment were homogenised for approximately five minutes in an electric 46 L cement mixture (300 W 230 V SIP Loughborough, UK) powered by a four stroke petrol generator (EX650 240 V, Honda, Shizuoka, Japan) on plot. One litre of the amended soil was then returned to each planting space and firmed in. The same homogenisation procedure was used on the unamended plots. The amended area for each of the nine plants was approximately 10 cm x 10 cm (the spades width) or $0.01m^2$ giving a total area within one set of $0.9m^2$ (i.e. 9 x $0.01m^2$). The calculated mean amendment mass and rates are shown in table 2. Amended soils were then left to naturally hydrate and equilibrate prior to planting for approximately one month.

8.4.2 WH5 Gravimetric method

Soils on plot WH5 were amended following a different method. One kilogram of soil was removed from each of the nine planting spaces using a spade and weighed in a plastic bucket on a spring balance connected to an A frame giving 9 kg. The amendment was then added to the soil at a rate of 3.7 kg to provide a mixture of 12.7 kg. The amended of the mine soil provided an amendment/soil (wet) mass ratio of 0.333 equal to 30% amendment weight/soil weight. The soil and amendment mixture was homogenised in a cement mixture using the aforementioned method. Approximately 1.4 kg of the amended soil mixture was returned to each planting space and firmed in, so all amended soil was used in this case. The amended area for each of the nine plants was 10cm X 10cm (the spades width) giving a total area within one plot of $0.9m^2$. The same procedure was used on the unamended plots. Amended soils were then left to naturally hydrate and equilibrate prior to planting for a maximum of three days.

8.4.3 Nutrient loadings in the amended field trial soils as per amendment rate

The nutrient loadings of the soils can be assessed in two ways. Firstly, given that the area amended is for each set of nine plants is 1.5 m x 1.5 m (2.25 m^2) then the average amendment rate can be found dividing the mass of the applied amendment (Kg) per set by the area of the set. This result is then converted into t/ha. For example, 1.9/2.25*10 gives 8.4 t/ha. This method for working out the nutrient loadings is appropriate for a regulatory perspective as it is concerned with the amendment applied to the soil for the plot as a whole.

The effective as received tonnes per hectare rate was found by dividing the known amendment weight by the actual amended area of a whole set of nine plants i.e. nine plants, each of which is planted in a space of 0.1m x 0.1m (0.09m^2) and multiplying that result by 10 to give t/ha. This method for working out the nutrient loadings is more appropriate for the perspective of growing plants as it is concerned with the effective amendment applied to each plant. The treatment rate of the amendments with the moisture content taken into account and the respective methods used are as is found in table 3. The dry weight (t/ha) application rate shown in Table 44 represents the dry matter content (%) of the as received rate.

Table 44 Application rate of amendments used in field trials by both average amendment rate to each set of nine plants (2.25m^2) and effective amendment rate for each plant (0.01m^2)

Amendment rate (%, wet mass)	**Dry matter content (%)**	**Amendment applied to each set of 9 plants (2.25m^2), kg**	**As received tonnes per hectare per set (t/ha)**	**Dry weight application rate per set (t/ha)**	**Amendment applied to each plant (0.01m^2), kg**	**As received tonnes per hectare per plant (t/ha)**	**Dry weight application rate per plant (t/ha)**
WH3							
GWC 14.2%	62.7	1.9	8.4	5.3	0.21	211	132
DWTR 18.5%	24	2.6	11.6	2.8	0.29	289	69.3
Mixture 16.2%	43.4	2.27	10.1	4.4	0.25	252	109
WH5							
GWC 30%	62.7	3.7	16.4	10.3	0.41	411	258
DWTR 30%	24	3.7	16.4	3.9	0.41	411	98.7
Mixture 30%	43.4	3.7	16.4	7.1	0.41	411	178

By looking at Table 44 it is clear that the different amendment methods and densities have resulted is very different application rates between both amendment types (GWC, DWTR and MIX) and the two plots (WH3 and WH5) once moisture content of the amendment is taken into account. This difference equates to almost double the as received amount of GWC being applied to the soil for plot WH5 when compared to plot WH3. The difference in moisture content between GWC and DWTR results in far greater quantities of dry GWC applied to the soil than DWTR. For example, at plot WH5 as DWTR has a dry matter content of 24 % and GWC 62.7 % the dry weight average application rate equated to 3.9 t/ha for DWTR and 10.3 t/ha for GWC. As the dry weight rates are so different from one another the nutrient loadings for all the types of amended growing media are therefore also different. The nutrient loadings for GWC, DWTR and MIX for both WH3 and WH5 soils can be found in tables 4, 5 and 6. Table 45, Table 46 and

Table 47 represent the calculated nutrient loadings in the amendments at the given application rates and are not an estimate of nutrient concentrations within the soils following amendment. As discussed in the previous chapter concentrations for available N and P are within the unamended mine soils were found to be below the limits of detection for the instrument (LOD = <2.5 mg/l for P and <1 mg/l for NO^{-}_{3} and NH^{+}_{4}). Concentrations of K for the mine soils were found to be very low (WH3 30 mg/l WH5 34 mg/l). Concentrations of total N for the mine soils were also found to be low (WH3 0.09 % w/w and WH5 0.14 % w/w). The nutrient concentrations of the two amendments and the mine soils are discussed in much greater depth in section 5.3.3 of this study.

The amendment nutrient loadings were calculated using the dry weight nutrient concentrations obtained through commercial analysis of the amendments. Further discussion of this can be found in chapter 4 of this study. Equation 15Equation 15 was used to calculate the amendment loading in each amendment option. The dry amendment rate per plant (0.01m^2) rather than average rate per set of plants (2.25m^2) was selected as a more representative metric for assessing the nutrients potentially available to the plants.

$$L = \frac{C * W}{1000}$$

Equation 15 Nutrient loadings for as received amendment rates

Where;
L is the final nutrient loading (kg/ha),
C is the dry weight nutrient concentration (mg/kg),
and W dry weight application rate per plant (t/ha).

Total nitrogen concentrations were initially provided as a percent and adjusted accordingly. The calculations for the mixture of the two amendments involved 50% of each from the GWC and DWTR analysis (e.g. dry matter content/ total Nitrogen etc.) and did not adjust these values for differences in dry matter content or bulk density or the amendments. The nutrient loadings are intended to provide an understanding of the potential differences between the amendment options rather than a precise breakdown of their nutrient concentrations.

Table 45 Nutrient loadings of GWC used for field trial at both plots WH3 and WH5. Zero values given for concentrations below the limits of detection of the instrument.

Determined	GWC analysis (mg/kg) dry	Nutrient loadings (kg/ha) per plant (0.01m^2) in plot WH3 at dry amendment application rate of 132t/ha	Nutrient loadings (kg/ha) per plant (0.01m^2) for plot WH5 at dry amendment application rate of 258 t/ha
Amendment rate (%)	-	14.2	30
Total nitrogen	12300	1624	3173
Ammonium nitrogen	23.8	3.14	6.14
Nitrate N	<10	0	0
Total P	2174	287	561
Water soluble P	62.5	8.25	16.1
Total K	6363	840	1642
Water soluble K	2629	347	678

Table 46 Nutrient loadings of DWTR used for field trial at both plots WH3 and WH5. Zero values given for concentrations below the limits of detection of the instrument.

Determined	DWTR analysis (mg/kg) dry	Nutrient loadings (kg/ha) per plant (0.01m^2) for plot WH3 at a dry application rate of 69.3t/ha	Nutrient loadings (kg/ha) per plant (0.01m^2) for plot WH5 at a dry application rate of 98.7t/ha
Amendment rate (%)	-	18.5	30
Total nitrogen	10700	742	1056
Ammonium nitrogen	<10	0	0
Nitrate N	<10	0	0
Total P	887	61.5	87.5
Water soluble P	0.01	0	0
Total K	369	25.6	36.4
Water soluble K	194	13.4	19.1

Table 47 Nutrient loadings of the mixture of GWC and DWTR used for field trial at both plots WH3 and WH5. Zero values given for concentrations below the limits of detection of the instrument.

Determined	**Mixture 50/50 dry (mg/kg)**	**Nutrient loadings (kg/ha) per plant ($0.01m^2$) for plot WH3 at a dry amendment rate of 109t/ha**	**Nutrient loadings (kg/ha) per plant ($0.01m^2$) for plot WH5 at a dry application rate of 178t/ha**
Amendment rate (%)	-	16.2	30
Total nitrogen	11500	1254	2047
Ammonium nitrogen	17	1.84	3.01
Nitrate N	<10	0	0
Total P	1531	167	272
Water soluble P	31	3.41	5.56
Total K	3366	367	599
Water soluble K	1412	154	251

Table 45, Table 46 and

Table 47 show that the levels of available nutrients in the amended soils were markedly lower in those amended with DWTR than with GWC. For example, on plot WH5 soils amended with GWC received an estimated 678 kg/ha K whereas soils amended with DWTR received 19.1 kg/ha K. This is due to far lower concentrations of water soluble and total nutrients in the DWTR when compared to GWC. This effect is increased due to the big differences in the dry matter content of the amendments, the GWC has 62.7% dry matter and the DWTR 24%.

The potential nutrient loadings within the mixture (MIX) (shown in table 6) of the two amendments are greater than half of the nutrients in the GWC as a result of the higher dry matter content of GWC when compared to DWTR. Although the two amendment have been found to contain similar total N (GWC 1.2% and DWTR 1.1%), the available N in the DWTR was found to be below the limit of detection. The GWC also contains greater concentrations of both P and K when compared to the DWTR. The lack of water soluble P in the DWTR is likely to be a result of the ability for the residue to bind P into less extractable forms (Dayton and Basta, 2001).

The lower concentrations of available nutrients in DWTR combined with the lower application rates (due to lower dry matter content) when compared to the GWC, means that loadings of essential elements in the DWTR amended growing media will be much lower than in the GWC amended growing media. The impact of the differences in the amendment rates due to the difference in amendment method for the two plots is also clear from Table 45, Table 46 and

Table 47 as the WH3 soils clearly received lower concentrations of N, P and K compared to the WH5 soils. The data in these tables suggest that soils amended with

GWC will produce greater biomass than soils amended with DWTR and that amended WH5 soils should produce greater biomass than amended WH5 soils. As the differences between the amendment application rates has now been made clear the nomenclature throughout this chapter for the amendments will be GWC, DWTR and MIX for all amended growing media with the relevant amendment rate stated.

8.4.4 Planting out RCG plugs

All 648 plugs were planted out using the following method. A seedling dibber was used to make a hole in the amended soil approximately 10cm deep with a diameter of 5cm. The plug containing 3 germinated seeds was then placed in the hole and firmed in. Due to a lack of surplus plugs, the protocol was not altered depending on whether all three seeds had germinated. No plug was planted without at least one healthy growing plant. Planting took place over two weeks in late July 2019.

8.4.5 Initial growth and survival rate recording

Monthly monitoring of field trial growth rate began in September 2019. Initial data collected on field trial establishment involved recording the height and number of tillers of each plant monthly. A 30cm ruler was used to recorded greatest sword height of entire plant in each planting site. All tillers associated with each plant were recorded. Survival rate was also recorded. A negative result for survival rate includes both dead and missing plants.

After 11 months of recording height and tiller number data the monthly recording method was altered in order to meet the travel restriction requirements of the coronavirus risk assessment. However, by comparing existing data for height and tiller numbers, the recording of height alone was found to show the same patterns and was quicker.

8.4.6 Harvesting and processing field trial biomass

In October 2020 all biomass was harvested from both WH3 and WH5 over the course of one week. Individual plants form each plot were cut with scissors at approximately 5cm above ground and placed in labelled zip lock bags. For example, the nine individual plants from set 1 of block 1 plot WH3 were placed in nine separate bags. Bagged plants were returned to the laboratory and any visible dirt and debris was removed. Plants were removed from bags and washed in UP DI water three times. Plants were then dried in their cleaned bags in an oven at 40 ^{O}C until at a constant weight. The dry samples were removed from the bags and the empty bags were weighed to find the weight by difference and therefore the dry mass of the individual plant. The Mann-Whitney U statically test was used to look for significance in the yields of biomass as the data showed skewed distributions (making parametric tests less powerful) with many extreme values which meant a test that was represented by the median rather than one that uses the mean (e.g. ANOVA or t-test) would be a better representation of significance. The statistical tests were carried out in SPSS (IBM) as previously discussed in the general methods chapter of this study.

Once the dry mass was recorded the plants were bulked by their variety, amendment and field trial plot before analysis. For example, WH3GWCSWRF5004 contained the 3 x 9 plants in that group. The bulking of the biomass in this way provided 12 biomass samples from each plot and allowed for comparisons between both the RCG varieties and the amendments to be made.

The bulked samples were washed a further three times in UP DI to make sure that all dirt and dust had been removed in order to prevent contamination of the biomass. The bulked samples were then homogenised in a GM 300 knife mill (Retch, Germany) with blade rotation speed of 4000rpm for three minutes.

8.4.7 Biomass analysis

The biomass analysis described in this chapter was completed commercially by ACME labs (BV minerals, Canada) using an ICP-MS. This analysis required 1g of washed, dried homogenised biomass which was then digested, first in HNO_3, then aqua regia. Sub samples from the described bulk samples were used for this analysis. The elemental concentration data was processed and statistically analysed using a standard student t-test with SPSS (IBM). The statistical tests were carried out as previously discussed in the general methods chapter of this study.

8.4.8 X-ray computed tomography

Reed canary grass samples were collected from plots WH3 and WH5 in July and August 2021 for analysis using X-ray computed tomography. At both plots samples were taken from the same block meaning that they were both the SWRF5004 variety grown in soils amended with DWTR. Grass samples were dried at 100 ºC until at a constant mass. The grass analysed in the first scan was unwashed but dried. The grass analysed in the second and third scans was washed with deionised water and dried. For the first scan the grass samples were placed in 50 mL centrifuge tubes and scanned at 60-micron resolution using apparatus described in section 4.6. The grass samples analysed in the second and third scans were placed in 15 mL centrifuge tubes and scanned first at 70-micron resolution (covering the whole length of the tube), then at 22-micron resolution (covering a 32.6 mm length along the tube). For all scans, the entire centrifuge tube diameter was reconstructed. The scan parameters were chosen to maximise contrast in the reconstructed image and minimise scan duration, these are outlined in the general methods section 4.6.2.

8.5 Results and discussion

8.5.1 Field trial meteorological conditions

Figure 60 shows the monthly mean average rainfall that was taken at the closest weather station to the Whiteheaps site (Durham). As indicated the start and end points of this field trial are Summer 2019 and October 2020. It is clear from the data that the Summer in which the field trial was begun was unusually wet when compared to the data for 2018, 2020 and 2021 in the same time period. This is however, unlikely to have hindered the growth of RCG which is a wetland plant other than to result in sheet flooding that may have swept away several young plants as shown in Figure 57.

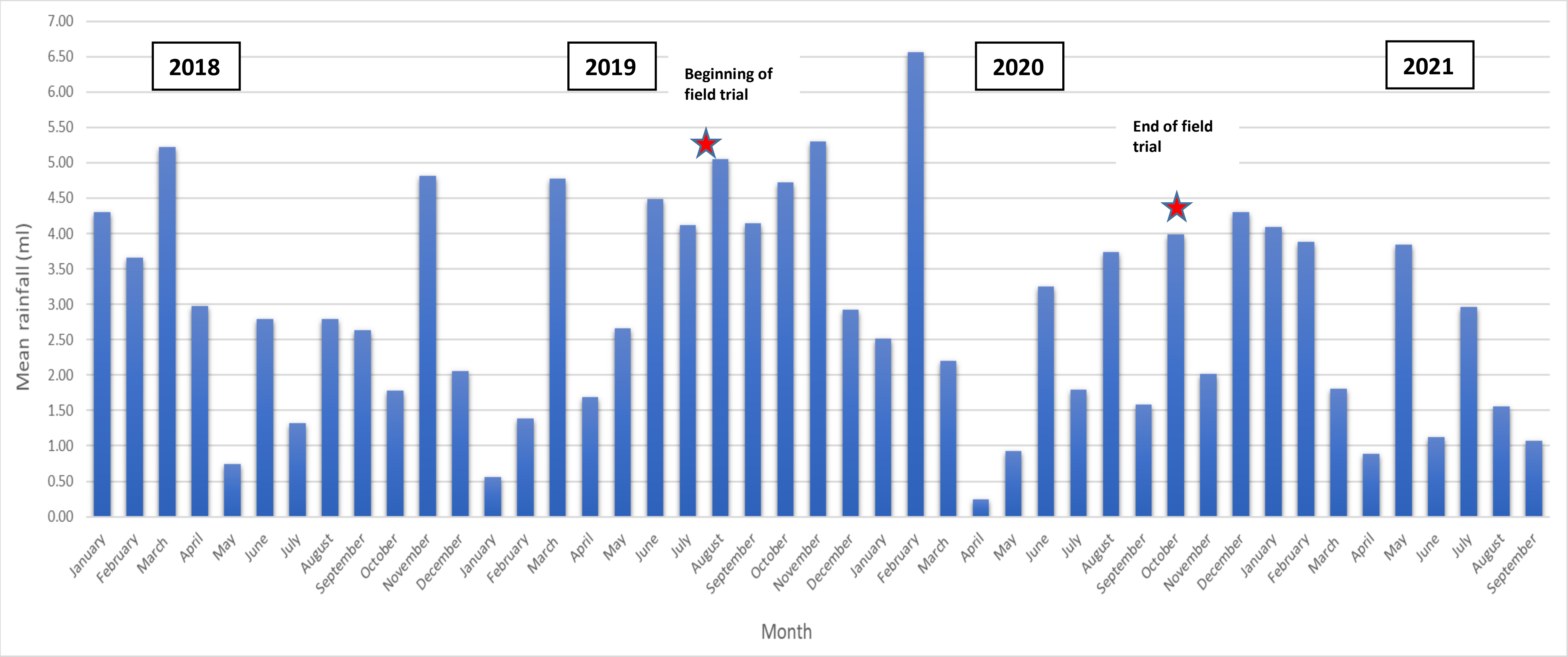

Figure 60 Mean average monthly rainfall for Whiteheaps with a star symbol to indicate the beginning and end of the trial. Obtained following data request from the Met Office, Durham weather station (Met office, 2021)

8.5.2 Field trial results

8.5.2.1 *Monthly monitoring*

The recording of just height rather than height and tiler number as a method for monitoring growth at the field trial was found to not impact the results in a significant way as the original monitoring metric (i.e. tiller number multiplied by maximum height) was found to give similar results to measuring just maximum height, as is shown in Figure 61.

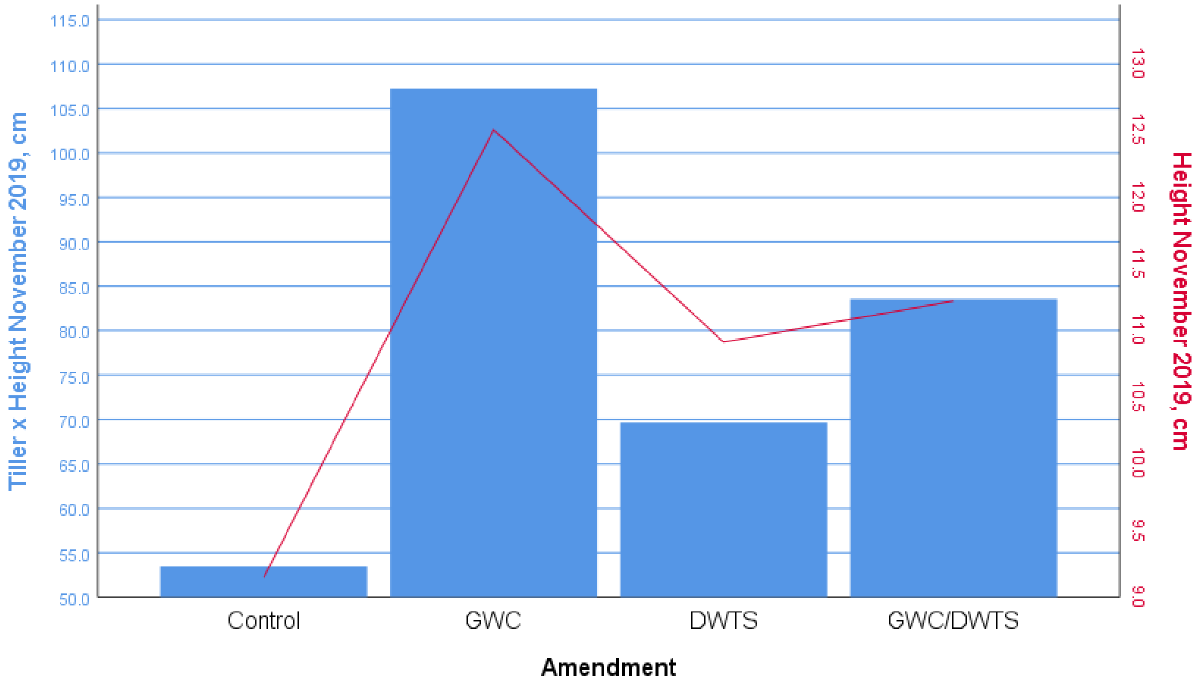

Figure 61 Dual axis chart showing just heights of RCG plants grown in amended soils compared with the growth monitoring metric of tiller number multiplied by height at plot WH5 recorded in November 2019.

8.5.2.2 Survival rates

The survival rates were recorded in October 2020 during the harvesting of the biomass and are shown below in Table 48. Both missing and dead plants were recorded as having not survived the trial.

Table 48 Survival rates of RCG plants grown at whiteheaps field trial. Rates recorded in October 2020 during harvesting of biomass. N= 81 for each of the 4 amendment factors and 108 for the each of the seed varieties

Factor	**WH3**	**WH5**
Missing/dead plants by amendment		
Unamended	15	4
GWC	11	2
DWTR	8	2
mixture	6	1
Missing/dead plants by variety		
SWRF 5004	12	3
SWRF 5032	11	1
BS5237	17	5
Total		
Missing/dead plants	40/324	9/324
Survival rate, %	87.7	92.7

High survival rates on both WH3 (87.7%) and WH5 (92.7%) show that RCG were not affected to by the conditions of the plot to the extent that they were unable to survive. The RCG grown in unamended soils had the lowest survival rate at both WH3 (87.9%)and WH5 (96.7%). The mixture of GWC and DWTR gave the best survival rates for both plots. The variety BS5237 had the lowest survival rate of all three RCG varieties both WH3 (81.6%) and WH5 (94.6%). Sheet flooding was observed at plot WH3 and this is likely the main cause of the lower plant survival rate found there. The effect of the sheet flooding is shown in Figure 62.

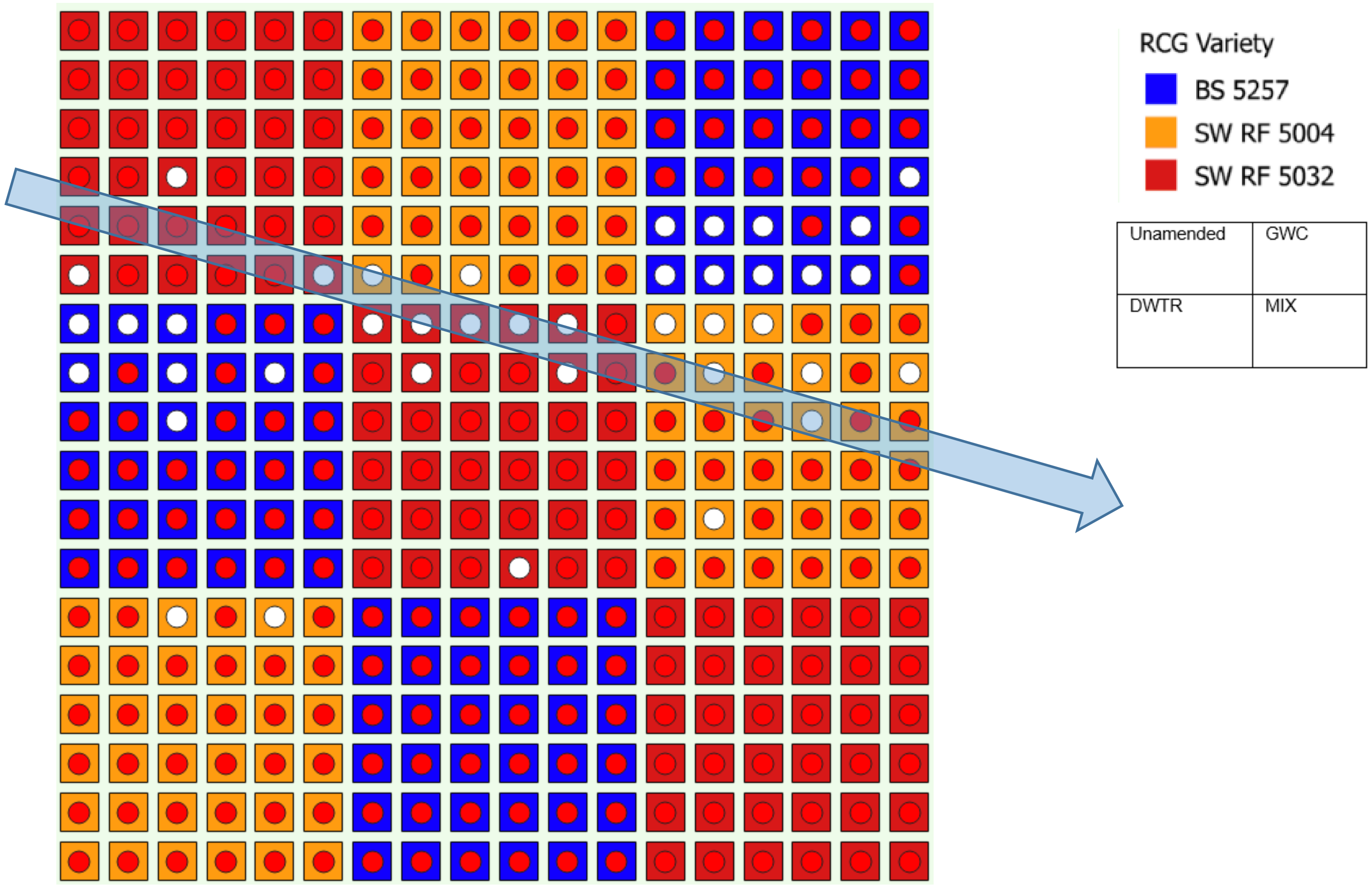

Figure 62 GIS map of plot WH3 showing dead or missing plants as white circles and living plants as red circles recorded in October 2020. The observed direction of the sheet flooding moving across the plot is shown as is the variety of RCG grown in each block.

8.5.3 Harvesting month selection

In order to allow the RCG plants to achieve their maximum growth during the field trial the growth of each plant was recorded monthly. By September 2020 it was clear that the maximum growth had been achieved during August 2020 and so the decision was made to harvest the plants in October during the beginning of their senescence period. This can be seen in Figure 63 where the maximum height for plants grown in each amendment type is found in August.

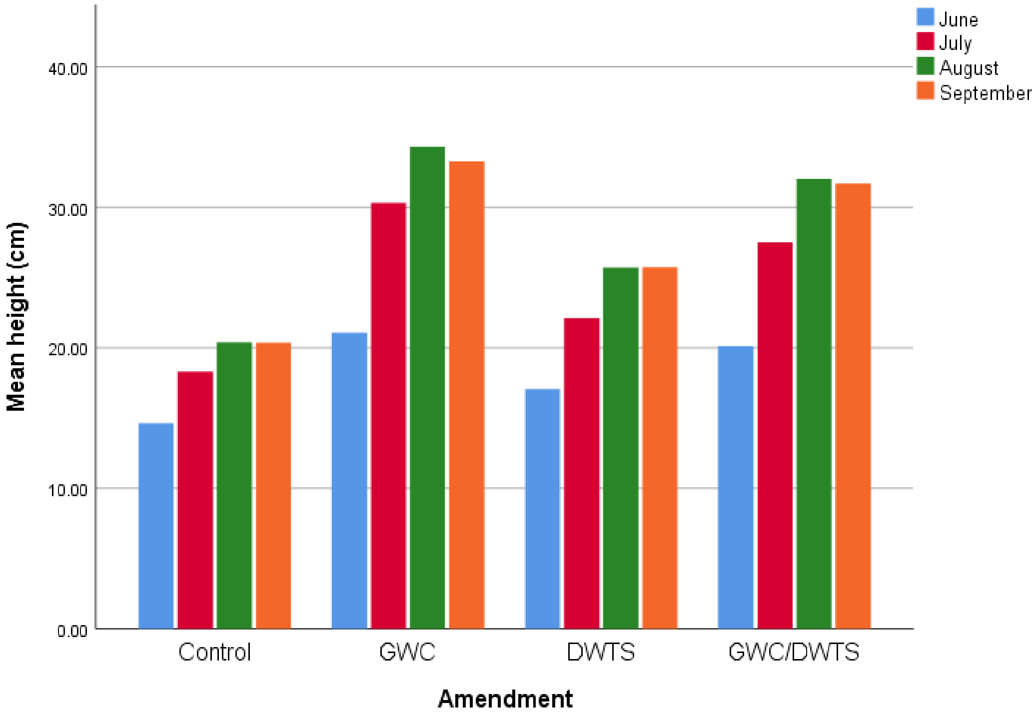

Figure 63 Cluster bar chart showing mean height of RCG plants recorded over four months in 2020 with soil amendments at plot WH5.

Due to predation by rabbits the plants at plot WH3 were found to be shortened over the month of July, this is shown in Figure 64. It is for this reason that the maximum height recordings for WH3 are taken from the July monthly plot monitoring data. As WH5 was unaffected by pests the maximum height recordings are from the August 2020 monthly plot monitoring data.

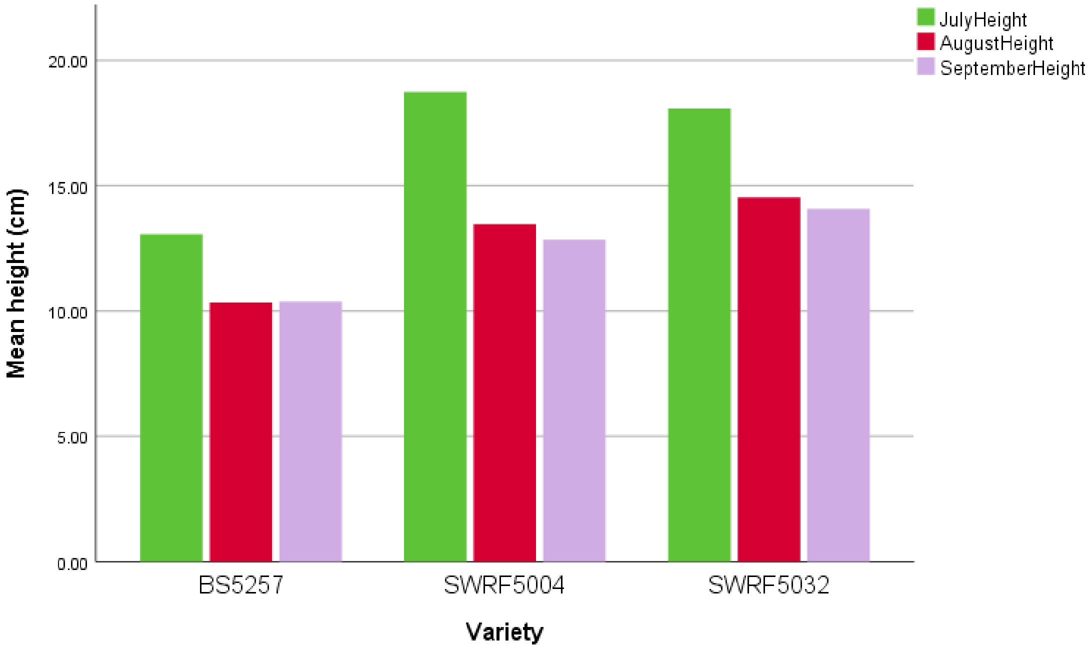

Figure 64 Cluster bar chart showing mean height of three varieties of RCG plants recorded over a period of three months in 2020 at field trial plot WH3

8.5.4 Field trial biomass

8.5.4.1 Plant heights at maximum extent WH3

The final maximum height recordings for plot WH3 were recorded in July 2020 and are shown in Figure 65 and Figure 66. All three types of amended soils produced RCG plants that had a median height which was significantly taller ($p< 0.05$) than those grown in the control soil. None of the three amended soils had a median height which was significantly taller ($p< 0.05$) from the others. However, the median height for plants grown in GWC amended soils appear to be greater than that of the other two amendment types. The plants grown in GWC (amendment rate 14.2 % AR) also provided the tallest outlier results and the greatest range.

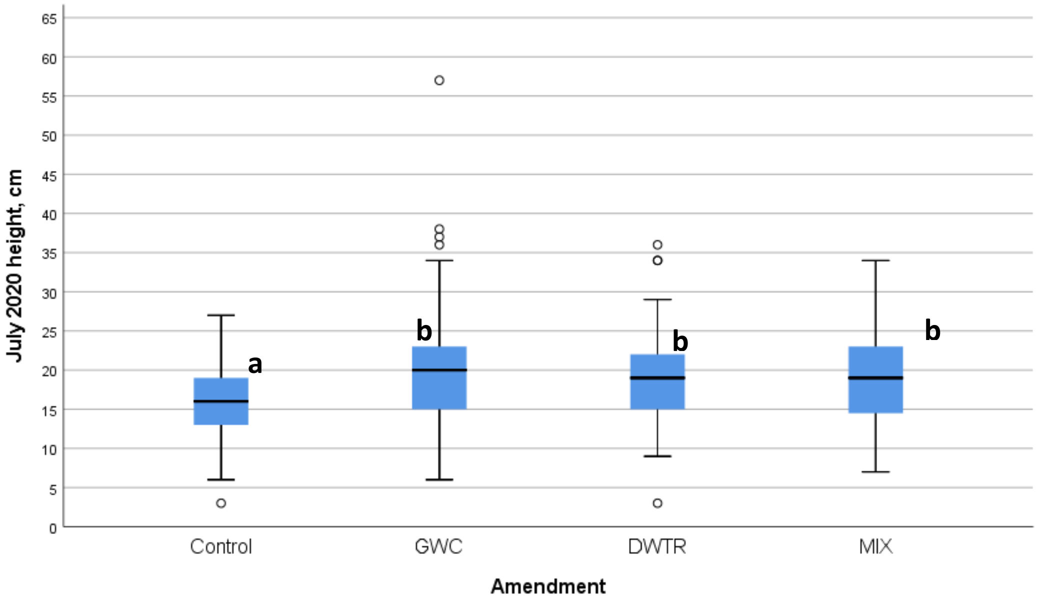

Figure 65 Box plot showing height of RCG grown in unamended mine soils and soils amended with green waste compost (GWC) (amendment rate 14.2 % AR) , drinking water treatment residue (amendment rate 18.4% AR) and a 50/50 mixture (mixture) of the two amendments by volume (amendment rate 16.1% AR) at plot WH3, recorded in July 2020. Outliers are represented with a circle, n=81 for each amendment minus any zero values. Box plots marked with the same letter indicate that the median value of that data does not differ significantly according to the Mann-Whitney U test (p < 0.05).

The more improved varieties of RCG (SWRF5004 and SWRF5032) produced plants with a median height which was significantly taller (p< 0.05) than the BS5237 variety. The median for the two SWRF varieties was not significantly different (p< 0.05). The variety developed as a biofuel crop (SWRF5004) appear to produce plants with a greater median average height and the tallest outliers when compared to the other two varieties. The interquartile range of all three varieties was approximately 21-23cm.

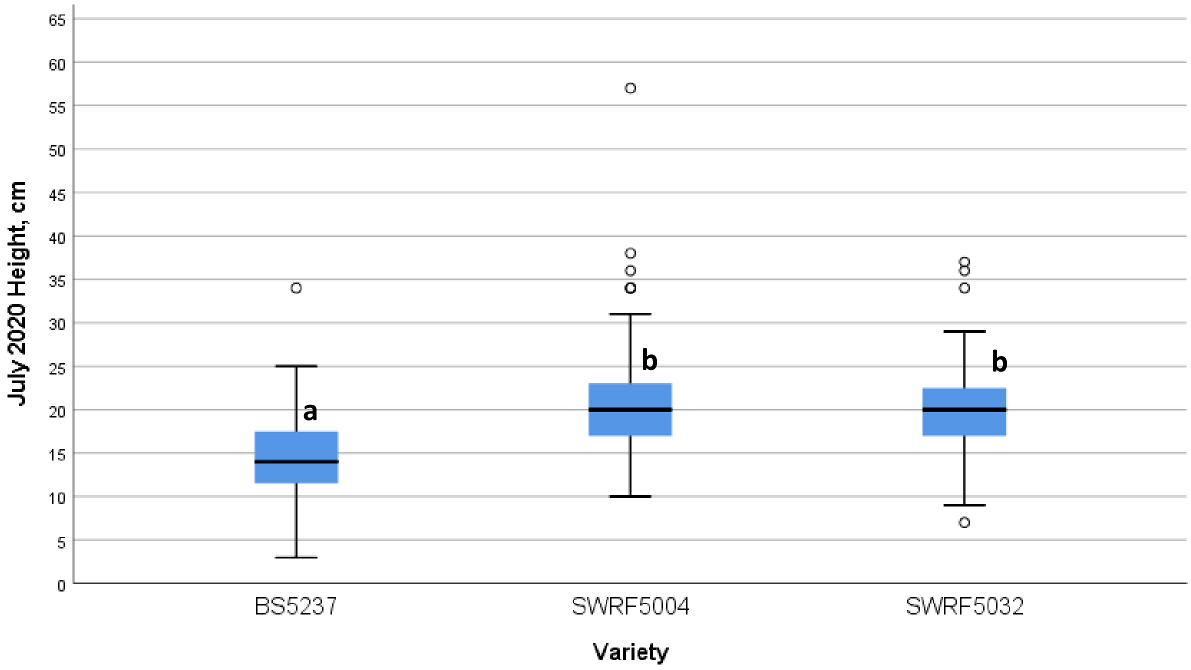

Figure 66 Box plot showing height of three varieties of RCG grown at plot WH3, recorded in July 2020. Outliers are represented with a circle, n=108 minus any zero values for each variety. Box plots marked with the same letter indicate that the median value of that data does not differ significantly according to the Mann-Whitney U test ($p < 0.05$).

8.5.4.2 Plant heights at maximum extent WH5

The final maximum height recordings for plot WH5 were recorded in August 2020 and are shown Figure 67 and Figure 68. The soils amended with GWC (amendment rate 25 % AR) and a 50/50 mixture (mixture) of the two amendments by mass (amendment rate 30% AR) produced RCG plants that had a median height which was significantly taller ($p< 0.05$) than those grown in the control soil. The median height of the plants grown in DWTR (amendment rate 30% AR) did not significantly differ from either those grown in the unamended control soils or the soils amended with GWC ($p < 0.05$). The median height for plants grown in the mixture amended soils was greater than that of the other amendment types. The plants grown in GWC provided the tallest outlier results and the greatest range. The three varieties of RCG did not produced plants with a median height that was significantly taller ($p < 0.05$) than each other. The variety developed as a biofuel crop (SWRF5004) produced plants with an apparently greater median average height and the tallest outliers when compared to the other two varieties. The interquartile range of all three varieties was approximately 40-45cm.

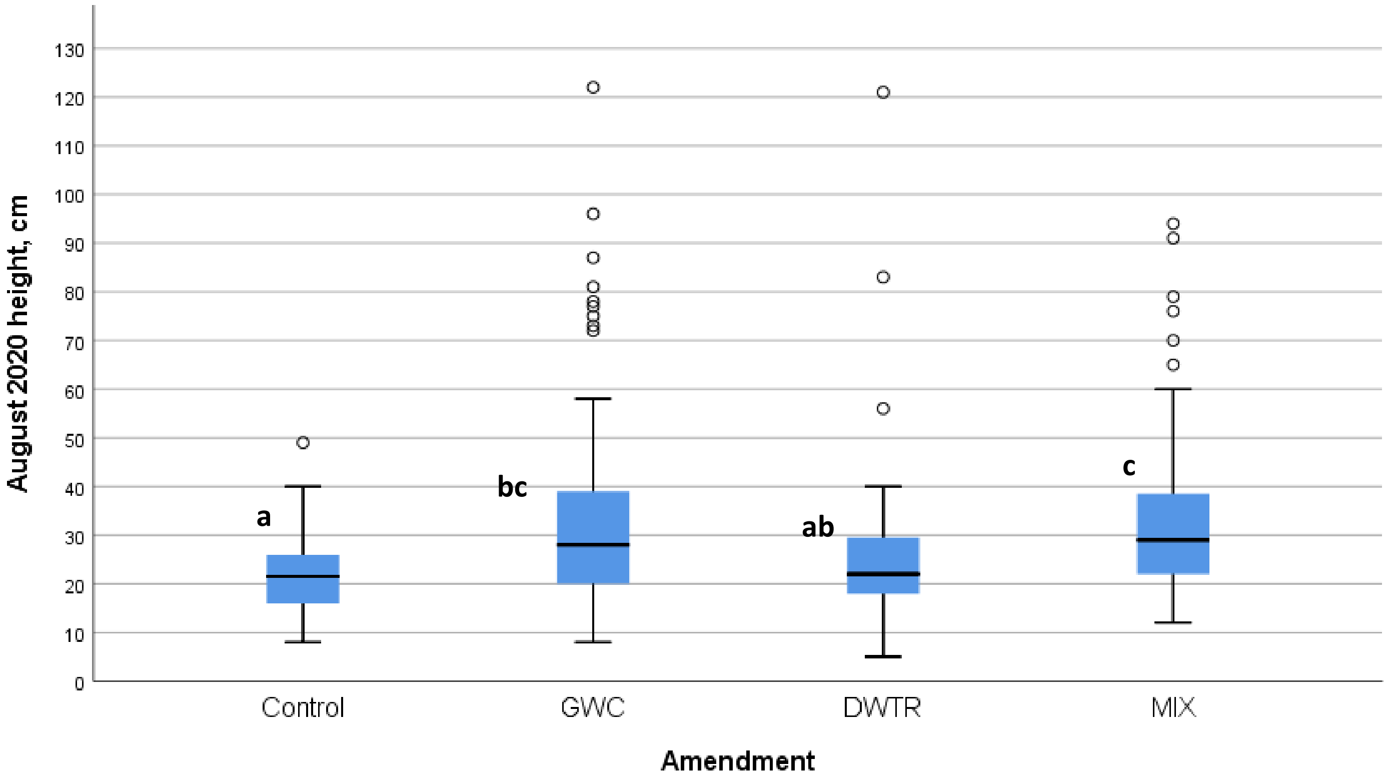

Figure 67 Box plot showing height of RCG grown in unamended mine soils and soils amended with green waste compost (GWC) (amendment rate 30 % AR) , drinking water treatment residue (DWTR) (amendment rate 30% AR) and a 50/50 mixture (mixture) of the two amendments by mass (amendment rate 30% AR) at plot WH5, recorded in August 2020. Outliers are represented with a circle, n=81 minus any zero values. Box plots marked with the same letter indicate that the median value of that data does not differ significantly according to the Mann-Whitney U test (p < 0.05).

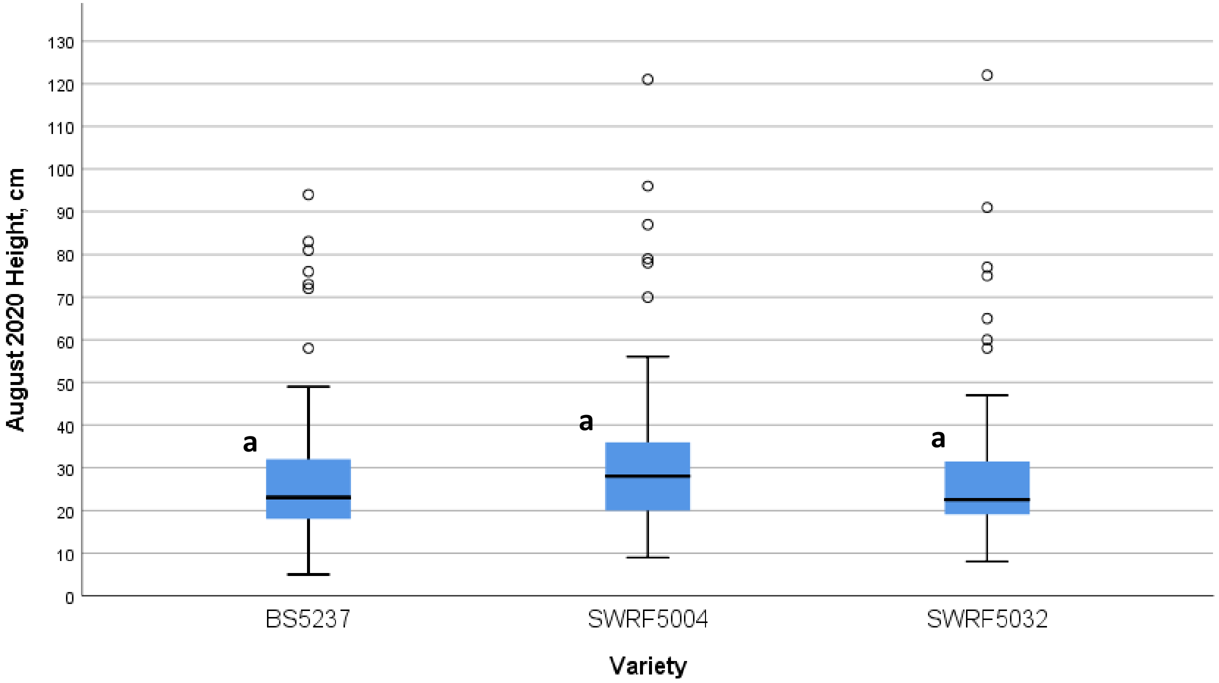

Figure 68 Box plot showing height of three varieties of RCG grown at plot WH5, recorded in August 2020. Outliers are represented with a circle, n=108 minus any zero values. Box plots marked with the same letter indicate that the median value of that data does not differ significantly according to the Mann-Whitney U test ($p < 0.05$).

8.5.5 Dry weight yield of biomass grown at Whiteheaps

8.5.6 WH3 - Amendment

The dry weight yield for plot WH3 results by amendment type are shown in Figure 69. The size of the whisker on the box plots indicates that the data points are spread out over a large range of values as is expected for a field trial of this nature where plot conditions can effect plants in a non-uniform way. All zero values (plants that did not survive) have been removed from the following data as predation from herbivores and damage from sheet flooding were not influenced by soil amendment or RCG variety in any meaningful way. The mean average dry weight of an individual RCG plants produced at WH3 across all experimental factors 0.56g (n=284). The RCG plants grown in the unamended control soil displayed the least variation and had the lowest mean value dry weight of less than 0.39g. The control soils did produce some plants with a dry mass of over 1g and these outliers are represented by circles in Figure 69.

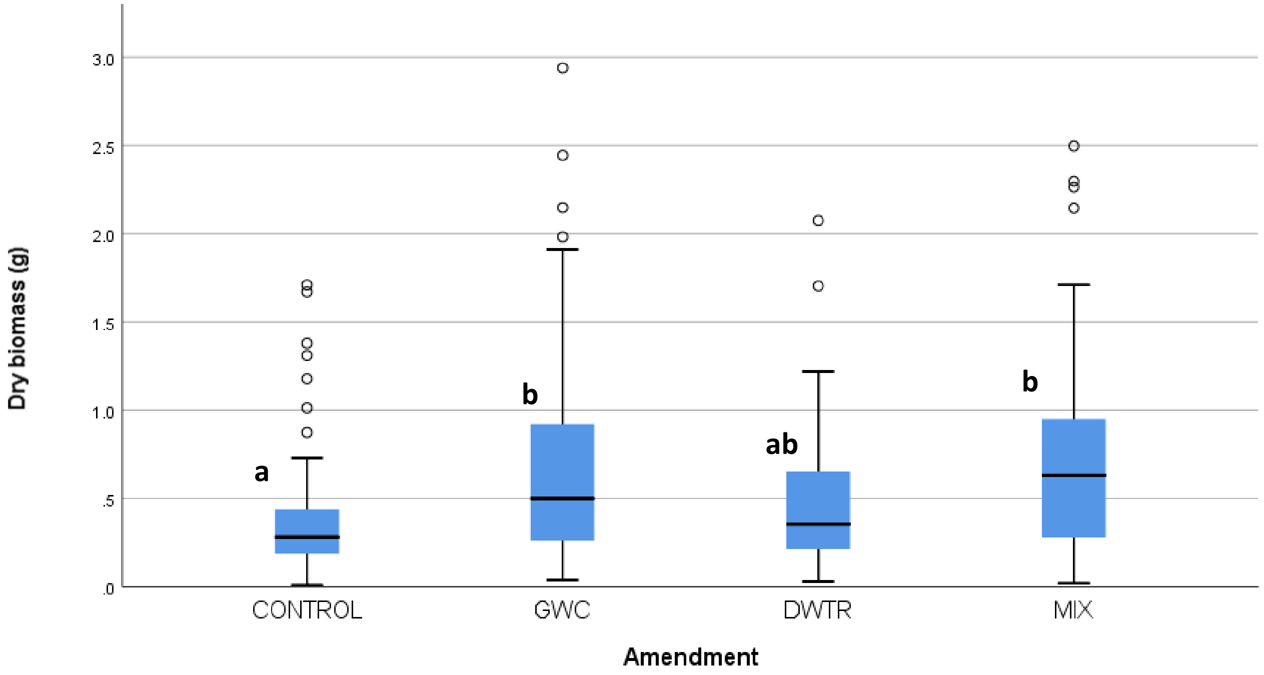

Figure 69 Box plot showing dry weight of RCG grown in unamended mine soils and soils amended with green waste compost (GWC) (amendment rate 14.2 % AR) , drinking water treatment residue (amendment rate 18.4% AR) and a 50/50 mixture (mixture) of the two amendments by volume (amendment rate 16.1% AR) at plot WH3 and sampled in October 2020. Outliers are represented with circle, n=81 minus any zero values. Box plots marked with the same letter indicate that the median value of that data does not differ significantly according to the Mann-Whitney U test ($p < 0.05$).

The RCG plants grown in the unamended control soil displayed the least variation and had the lowest mean value dry weight of less than 0.39g. The control soils did produce some plants with a dry mass of over 1g and these outliers are represented by circles in Figure 69.

The plants grown in the soils amended with GWC had significant yield improvements ($p<0.05$) when compared to both the unamended control and the DWTR, with an average mean value of 0.7g dry mass. These results had a greater spread than the control with the upper quartile maximum almost 3 times that of the unamended soils. Outliers for the plants grown in GWC amended soils ranged from approximately 2-3g.

The mean value for the dry biomass yield from soils amended with DWTR (0.46g) was neither significantly different from the unamended control or the amended soils. However, as shown in fig. 5 the skew of the data for DWTR is noticeably greater than that of the control.

The mean value for the dry biomass yield from soils amended with the mixture of GWC and DWTR (0.69g) was found to be significantly different from the unamended soils. The mean biomass yield from the mixture soil was slightly greater than that from the GWC soil.

8.5.7 WH3 - Variety

The dry weight yield for plot WH3 results by RCG variety are shown in Figure 70. The oven dried biomass yield of RCG from WH3 gave significant differences ($p<0.05$) between the less cultivated BS5237 variety and the two varieties which have been bred to a greater extent for certain properties.

The variety SWRF5004 produced the greatest yield with a mean oven dry weight of 0.69g (n=96 plants). This variety was developed for greater biomass and seed production for use in the biofuel industry by Svalof Weibull in Sweden and has been shown to outcompete other RCG cultivars in previous field trials including SWRF5032 and the BS5237 (IBERS) (See section 3.2.7). The BS5237 variety produced a far lower mean yield of 0.37g (n=91 plants,) a result which suggests scope for further research on how genotypes can be matched with and developed for the diverse environments found on contaminated land.

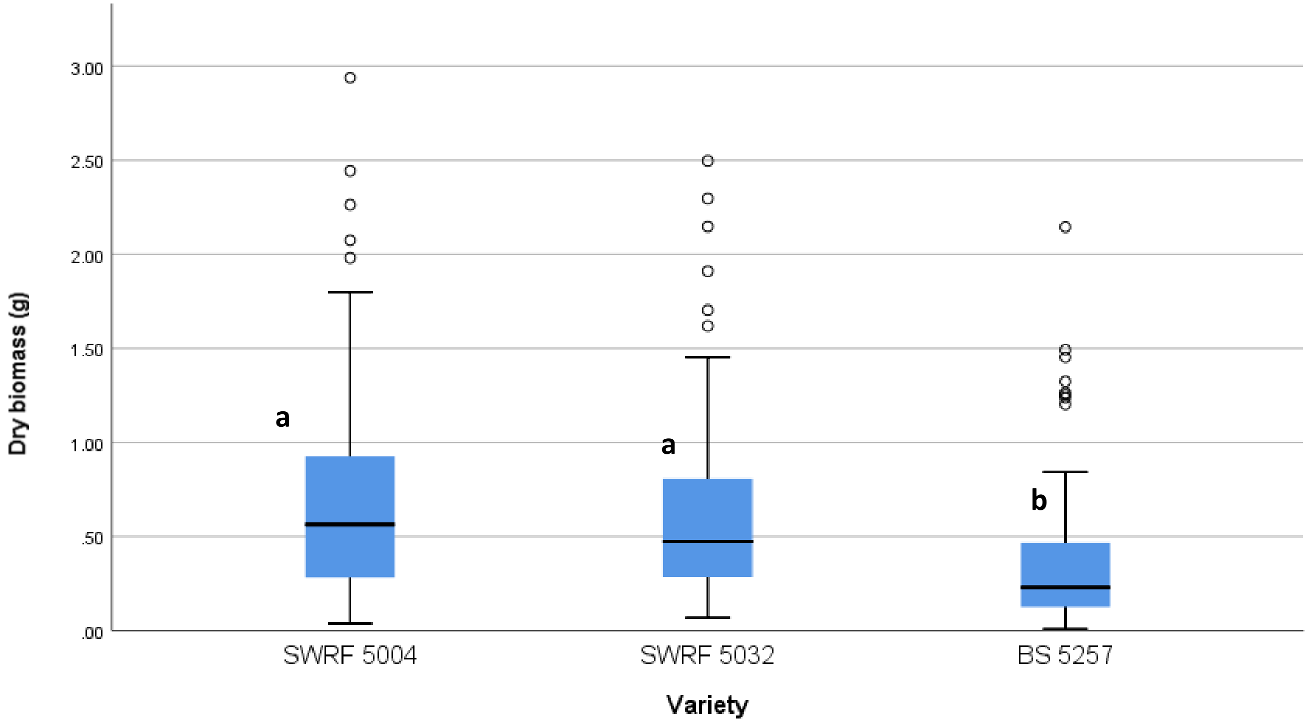

Figure 70 Box plot showing dry weight of three varieties of RCG grown at plot WH3, harvested in October 2020. The error bars represent ± standard deviation (n=108 minus any zero values). Box plots marked with the same letter indicate that the median value of that data does not differ significantly according to the Mann-Whitney U test ($p < 0.05$).

8.5.8 WH5 – Amendment

The dry weight yield for plot WH5 results by amendment type are shown in Figure 71.The RCG plants grown in the unamended control soil displayed the least variation and had the lowest mean value dry weight of less than 0.62g. The unamended control soils did produce a few plants with a dry mass of over 1g and these outliers are represented by circles in Figure 71.

The plants grown in the soils amended with GWC had significant yield improvements ($p<0.05$) when compared to both the unamended control and the DWTR, with an average mean value of 2.4g dry mass. These results had a greater spread than the control with the upper quartile maximum almost 3 times that of the unamended soils. Outliers for the plants grown in GWC amended soils ranged from approximately 8-18g. The mean value for the dry biomass yield from soils amended with DWTR (1.1g) was both significantly greater than that of the unamended control and less than the other amended soils. The mean value for the dry biomass yield from soils amended with the mixture of GWC and DWTR (1.9g) was found to be significantly greater than that of the unamended and DWTR soils.

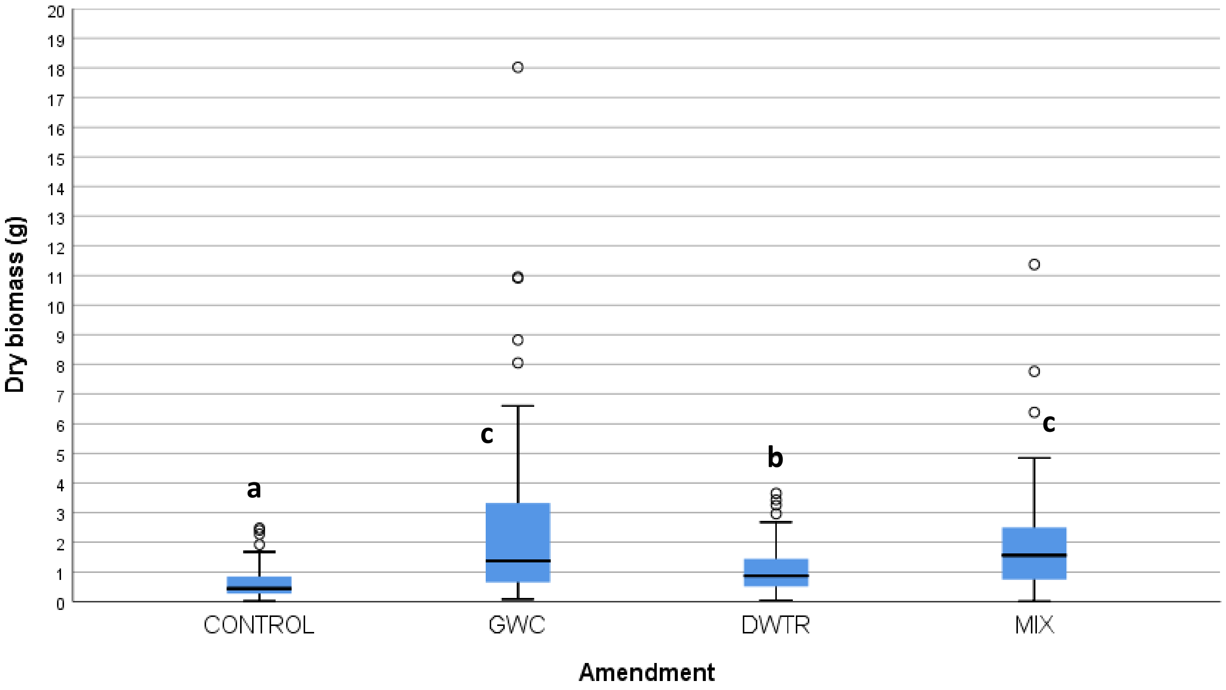

Figure 71 Box plot showing dry weight of RCG grown in unamended mine soils and soils amended with green waste compost (GWC) (amendment rate 25 % AR) , drinking water treatment residue (DWTR) (amendment rate 30% AR) and a 50/50 mixture (mixture) of the two amendments by mass (amendment rate 30% AR) at plot WH5. The error bars represent ± standard deviation (n=81, minus any zero values). Box plots marked with the same letter indicate that the median value of that data does not differ significantly according to the Mann-Whitney U test (p < 0.05).

8.5.9 WH5- Variety

The dry weight yield for plot WH5 results by RCG variety are shown in Figure 72. No significant differences were found in the oven dried biomass of RCG produced at WH5 between the three varieties The variety SWRF5004 produced the greatest yield as it did on plot WH3, with a mean oven dry weight of 1.58g (n=105 plants). The SWRF5032 and BS5237 varieties produced lower but similar mean average yields of 1.49g and 1.56g (n=103 plants) respectively.

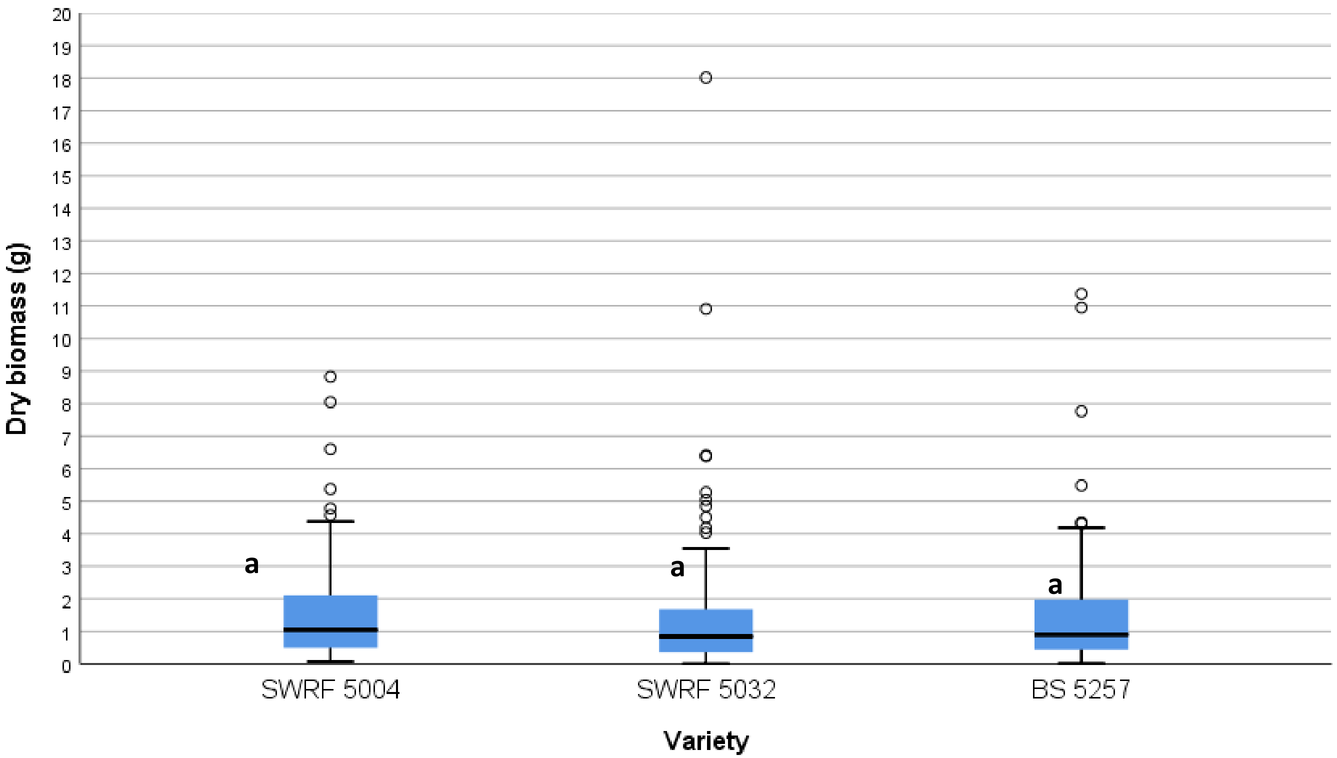

Figure 72 Box plot showing dry weight of three varieties of RCG grown at plot WH5. The error bars represent ± standard deviation (n=108, minus any zero values). Box plots marked with the same letter indicate that the median value of that data does not differ significantly according to the Mann-Whitney U test ($p < 0.05$).

8.5.10 Summary of maximum height and dry weight yield results

The overall results of the field trials in terms of the relative productivity of the four soil amendment factors give a clear indication of the benefits of the organic waste amendments. For both WH3 and WH5 soils amended with GWC produced significantly taller plants with a greater dry mass than that of the unamended control soils. Similar effects from GWC on plant growth on contaminated soils have been well reported (Hartley *et al.*, 2009; Karami *et al.*, 2011; Badmos *et al.*, 2015; Lord, 2015a; Li *et al.*, 2020, 2021; Medyńska-Juraszek *et al.*, 2020). Badmos *et al*., found that a GWC application rate of 180 t/ha resulted in an increase in 130 - 337% compared to an unamended contaminated soil control. Karmai *et al*., found similarly high increases in biomass yield following the application of 30% (v/v) GWC on contaminated soils (Karami *et al.*, 2011). On plot WH5 the increase in dry biomass yield for plants grown in soils amended with GWC when compared to the unamended control was 387%.

The DWTR had significant effects on the height of the plants at plot WH3 and the dry biomass produced at WH5 when compared to the unamended control soils. Observable increases in both height and mass were recorded elsewhere but were not found to be statistically significant. The use of DWTR as a soil amendment for increasing plant growth has not been studied as much as GWC although some published works are available (Mahdy *et al.*, 2007; Badmos *et al.*, 2015; Rodríguez *et al.*, 2016). On plot WH5 the increase in dry biomass yield for plants grown in soils amended with DWTR when compared to the unamended control was 177%. This compares well to the results of Rodriguez *et al*., 2016 who found statistically significant improvements in the biomass production of *Lupin albus* grown in metal mine soils following the addition of DWTR at 15% (w/w) (Rodríguez *et al.*, 2016). The mixture of GWC and DWTR had significant effects on the height and dry biomass of the RCG plants at both plots. On plot WH5 the increase in dry biomass yield for plants grown in soils amended with the mixture when compared to the unamended control was 306%. The combination of GWC and DWTR as a soil amendment for plant growth improvements has not been reported in any available literature due relative novelty of the research. Dayton and Basta (2001) tested the potential of 17 different DWTR as a soil amendment for the growth of tomatoes and found that due to the immobilization of P the yield was poor (Dayton and Basta, 2001). Kerr (2019) combined GWC and DWTR as co amendments for a geotechnical purpose (i.e. flood water retention) and hypothesized that the addition of P from the GWC would mitigate the effect of the immobile P in the DWTR (Kerr, 2019). The findings of this study suggest that this has been achieved as the mixture of the amendment produced mean average dry weight biomass of 1.9g whereas the GWC produced 2.4g dry mass. On plot WH3 this is even more noticeable as the mixture produced mean average dry weight biomass of 0.69g whereas the GWC produced 0.7g dry mass

The overall results of the field trials in terms of the relative productivity of the three RCG varieties give a clear indication of the need for appropriate cultivar selection when growing RCG on contaminated soils. On plot WH3 the SWRF5004 variety produced both the tallest plants and the greatest mean average dried biomass. The two more developed varieties SWRF5004 and SWRF5032 were both found to produce significantly more biomass and taller plants on plot WH3 than BS5257. This finding is supported by reported green harvest mean dwt yield of the three varieties presented by the ORNATE project (ORNATE, 2017). On plot WH5 although, there were no statistically significant differences between the varieties SWRF5004 produced the greatest mean average dried biomass (1.58g) and SWRF5032 the lowest (1.49g).

The mean average dry weight of an individual RCG plants produced at WH5 was 1.54g (n=315) which is approximately three times that at WH3. This increase in yield maybe explained by several factors. The WH5 plot is more sheltered than WH3 which is exposed to the upland weather conditions found at Whiteheaps. The WH5 plot was found to have less Pb (9112 mg/kg) when compared WH3 (13870 mg/kg), which is considered the most phytotoxic elements connected with the plot given its toxicity and concentrations. The amendment rate used at WH5 was greater than that for WH3 to the extent that for WH3 10.5 AR t/ha of GWC were used where as for WH5 20.5 AR t/ha of GWC were used leading to almost double the amount of nutrients being added to the WH5 mine soils. Further details of amendment rates can be found in tables Table 43 and Table 44.

All three metrics (i.e. tiller number, maximum height and dry biomass) used to measure the impact of the amendments on the three different cultivars of RCG produced broadly similar results, suggesting that any of the three could be used with confidence in further studies.

8.5.11 Effect of amendment and variety of RCG on PTE concentrations

The following is the results from the ICP-MS commercial analysis of the RCG grown at Whiteheaps which were bulked into separate samples determined by both amendment and variety. As the groups were independent from each other and had a normal distribution the two tailed t-test was applied to the data to assess whether the means of different groups differed significantly.

8.5.12 Arsenic

Concentrations of As (mg/kg) in dried and bulked RCG biomass grown on plots WH3 and WH5 are shown in Figure 73 and were found to have a low standard deviation with results ranging from 1.7 – 7.2 mg/kg and 1.4 – 3.5 mg/kg respectively. The mean As concentration of both plots do not differ significantly ($n=12$) ($p< 0.05$). At plot WH3 none of the amendments resulted in a significant change in the concentrations of As found in the biomass. The unamended soils at WH3 produced biomass with the lowest mean concentration of As (2.4 mg/kg) and the soils amended with DWTR produced the highest mean concentration (4.1 mg/kg). No significant difference was found between the As concentrations in the three different RCG varieties. Similarly, the concentrations of As found at plot WH5 did not differ significantly between the differently amended soils or the unamended soil. The biomass grown in soils amended with DWTR provided the highest concentrations of As (2.7 mg/kg) and the GWC amended soils the lowest (1.7 mg/kg). No significant difference was found between the As concentrations in the three different RCG varieties.

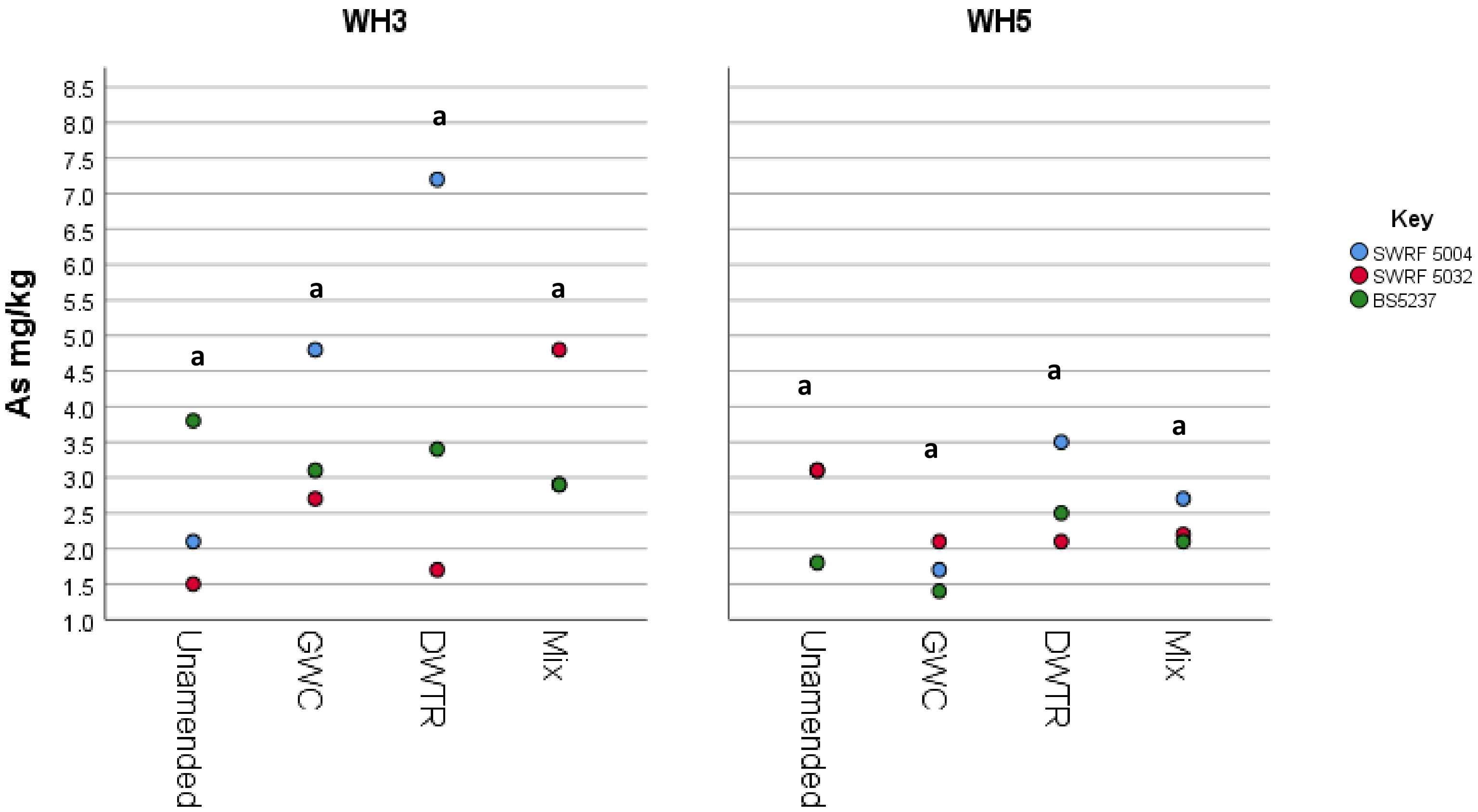

Figure 73 Grouped scatter plots showing concentrations of As (mg/kg) found in dried RCG biomass grown in unamended mine soils and soils amended with green waste compost (GWC), drinking water treatment residue and a 50/50 mixture (mixture) of the two amendments by volume at plots WH3 and WH5. At plot WH3 the amendment was added at the following rate GWC 14.2 % AR, DWTR 18.4% AR and mixture 16.1% AR. At plot WH5 the amendment rate for all amendments was 25 % AR. Amendment factors marked with the same letter have mean concentrations that do not differ significantly according to the two tailed t-test ($p < 0.05$). Each point represents the analysis of bulked biomass from up to 27 plants.

Concentrations of As were also found to be greater in the biomass produced from soils amended with DWTR when compared to biomass produced in soils amended with GWC and the unamended WH5 soil in the analysis of the biomass produced during the pot trials shown in section 1.1.3 As discussed previously in the amendment DWTR has lower As concentrations than the amendment GWC and so amendment concentrations alone do not explain this result. Li *et al* (2021) found that amending soil with GWC considerably reduced the plant available (pakchoi cabbage) concentrations of As (Li *et al.*, 2021). There have been several studies that have evaluated the impact that DWTR has on the mobility of As and it is clear further research is required to understand this behaviour fully (Nagar *et al.*, 2014, 2015; Yuan *et al.*, 2016). In general terms the presence of ferric oxides in DWTS would be expected to sorb the As and so possibly to reduce uptake. Arsenic bioavailability is affected by changes in soil pH (Bradham *et al.*, 2011) and the lowering of soil pH following amendment with DWTR (pH 5.2) and increasing of soil pH following amendment with GWC (pH 8.2) may be responsible for the similar results at both WH3 and WH5.

8.5.13 Cadmium

Concentrations of Cd (mg/kg) in dried and bulked RCG biomass grown on plots WH3 and WH5 are shown in and were found to have a low standard deviation with results ranging from 5.99 – 13.13 mg/kg and 3.35- 6.9 mg/kg respectively. The mean Cd concentration at plot WH3 (8.31 mg/kg) are significantly greater than that of WH5 (5.13 mg/kg) (n=12) ($p < 0.05$).

At plot WH3 none of the amendments resulted in a significant change in the concentrations of Cd found in the biomass. The soils amended with DWTR at WH3 produced biomass with the lowest mean concentration of Cd (6.76 mg/kg) and the soils amended with the MIX produced the highest mean concentration (9.83 mg/kg). No significant difference was found between the Cd concentrations in the three different RCG varieties. The RCG variety BS5237 at WH3 produced biomass with significantly less mean Cd concentrations than that of SWRF5004 and SWRF5032 (n=4) ($p < 0.05$).

At plot WH5 the mean concentrations of Cd found within the biomass produced in GWC (6.07 mg/kg) amended soils was found to be significantly greater (n=3) ($p < 0.05$) than the biomass produced in the two other types of amended soil and the unamended soil. The biomass grown in soils amended with DWTR had the lowest concentrations of Cd on plot WH5 at 4.16 mg/kg. Although no significant difference was found between the Cd concentrations in the three different RCG varieties the variety BS5237 did produce biomass with the lowest mean concentrations (4.56 mg/kg) which was found to be significant at $p < 0.08$ (n=4) when compared with the concentrations found in the SWRF5004 variety (5.69 mg/kg).

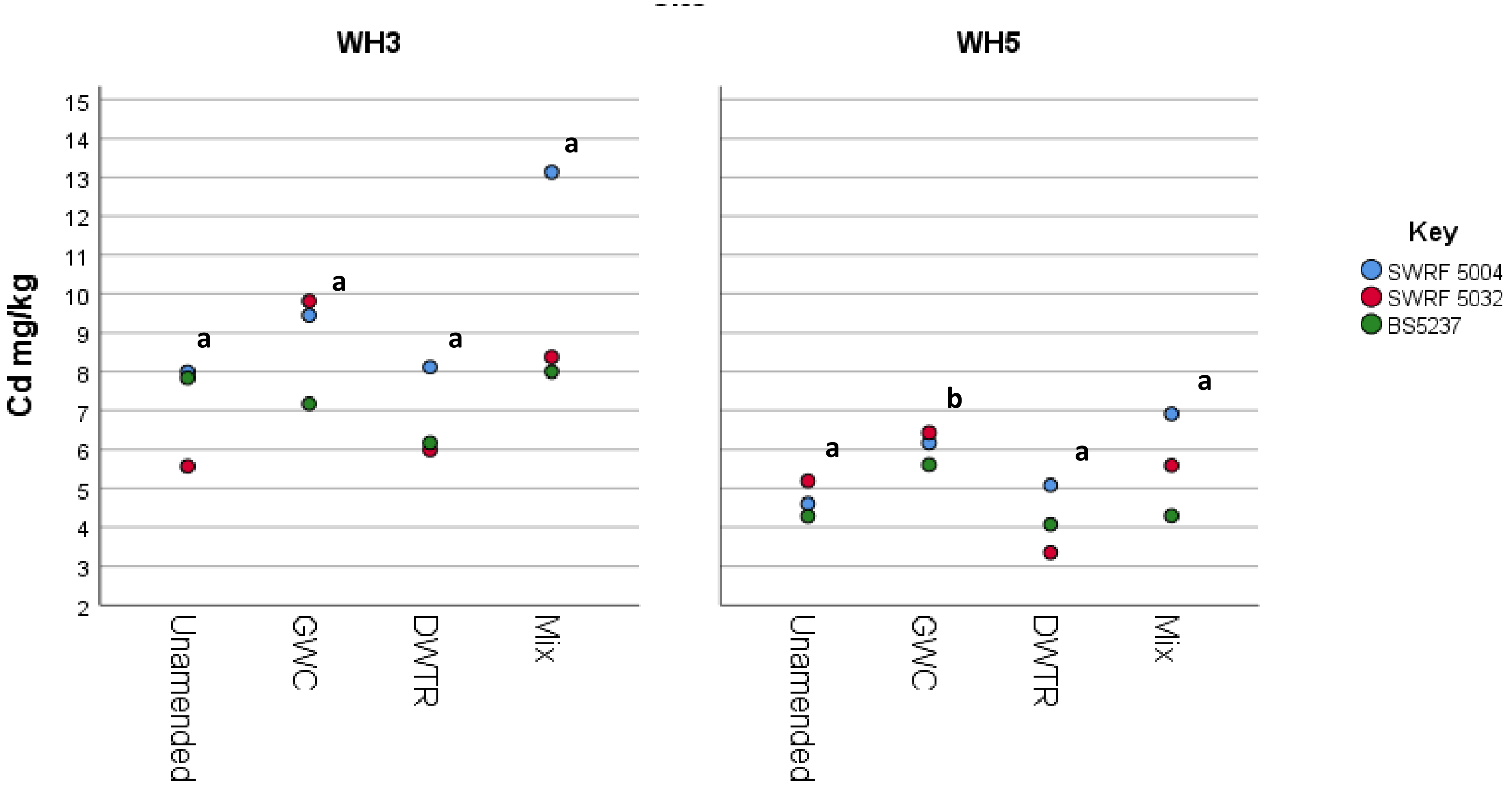

Figure 74 Grouped scatter plots showing concentrations of Cd (mg/kg) found in dried RCG biomass grown in unamended mine soils and soils amended with green waste compost (GWC) , drinking water treatment residue and a 50/50 mixture (mixture) of the two amendments by volume at plots WH3 and WH5. At plot WH3 the amendment was added at the following rate GWC 14.2 % AR, DWTR 18.4% AR and mixture 16.1% AR. At plot WH5 the amendment rate for all amendments was 25 % AR. Amendment factors marked with the same letter have mean concentrations that do not differ significantly according to the two tailed t-test ($p < 0.05$)

8.5.14 Chromium

Concentrations of Cr (mg/kg) in dried and bulked RCG biomass grown on plots WH3 and WH5 are shown in

Figure 75 were found to have range and standard deviation of 11.1 – 42.8 ±9.15 mg/kg and 9.2 – 62.4 ±15.6 mg/kg respectively. The mean Cr concentrations in the biomass produced at plot WH3 was 21.1 mg/kg and at WH5 was 19.8 mg/kg. The mean concentrations of Cr between the two plots were not found to be significantly different (n=12) ($p < 0.05$). At plot WH3 the biomass produced in soils amended with GWC and the MIX had significantly greater concentrations of Cr than the biomass produced in the unamended and DWTR amended soils. Here the results for Cr concentrations for biomass produced in DWTR amended soils had a far greater standard deviation ±16.9 mg/kg than the other soils which were in the range of ±2-7mg/kg. The RCG variety BS5237 was found to have significantly lower Cr concentrations when compared to that of SWRF5004 (n=4) ($p<0.05$).

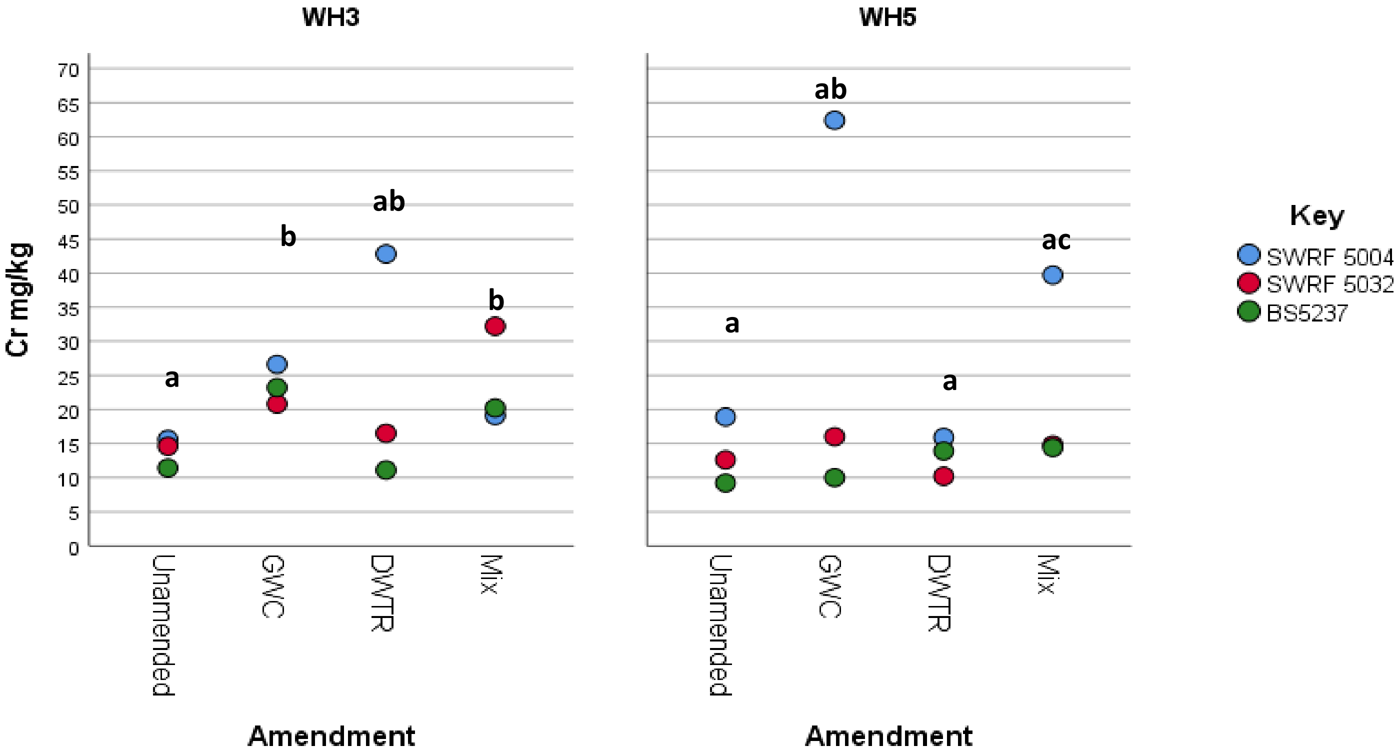

Figure 75 Grouped scatter plots showing concentrations of Cr (mg/kg) found in dried RCG biomass grown in unamended mine soils and soils amended with green waste compost (GWC) , drinking water treatment residue and a 50/50 mixture (mixture) of the two amendments by volume at plots WH3 and WH5. At plot WH3 the amendment was added at the following rate GWC 14.2 % AR, DWTR 18.4% AR and mixture 16.1% AR. At plot WH5 the amendment rate for all amendments was 25 % AR. Amendment factors marked with the same letter have mean concentrations that do not differ significantly according to the two tailed t-test ($p < 0.05$).

At plot WH5 there were no significant differences found between the biomass concentrations of Cr produced in the amended and unamended soils. Both the GWC (29.4 mg/kg) and MIX (22.9 mg/kg) amended soils produced biomass with mean average concentrations of Cr that were greater than those of the biomass produced in the DWTR amended soil (13.3 mg/kg) and the unamended (13.5 mg/kg) and were mutually different. However, the range within the results prevented them from being significant. The RCG variety SWRF5004 (34.2 mg/kg) contained the greatest mean concentrations of Cr when compared to both SWRF5032 (13.4 mg/kg) and BS5237 (11.8 mg/kg). This result was found to be significant (n=4) ($p<0.05$) and was also found in the biomass produced at plot WH3. The variety BS5237 was also found to have the lowest concentrations of Cr as it did on WH3. The above results suggest that the addition of GWC as an amendment on its own and as part of the MIX is increasing the bioavailability of Cr. This finding cannot be attributed to the concentrations of Cr in either the amendment or the soil. The GWC was found to have 25.8 mg/kg Cr and the DWTR was found to have 25.7 mg/kg Cr. Likewise the soils from the two plots were found to not differ a great deal in their Cr content, WH3 containing 18.6 mg/kg Cr and WH5 23.1 mg/kg Cr. The results from the BCR sequential extraction procedure suggested that no significant changes took place concerning the availability of Cr upon the addition of either amendment to the WH5 soil. The Cr in amendments and mine soils and amended mine soils were all found to be mostly in the residual fraction Figure 48. A lack of published research on the subject of Cr speciation following soil amendment with organic waste means that drawing further conclusions is difficult at this stage and suggests that further work needs to be completed.

8.5.15 Copper

Concentrations of Cu (mg/kg) in dried and bulked RCG biomass grown on plot WH3 shown in Figure 76 were found to have a low standard deviation except for those grown in soils amended with DWTR for which the concentrations ranged from 69.27 mg/kg to 221.12 mg/kg. The range of results for DWTR includes both the highest and lowest found Cu concentrations and could be the result of heterogeneity in Cu concentrations across the field trial plot. However, the consistency in the results for the other amendment options does not support this. All three types of amended soils produced biomass that had greater mean concentrations of Cu than the unamended soils with GWC providing the least of the three. No significant difference ($p<0.05$) was found between the varieties of RCG and their Cu concentrations although SWRF5004 was found to have the greatest average contentions (132 mg/kg Cu) and SWRF5032 the lowest (84.8 mg/kg Cu). Concentrations of Cu (mg/kg) in dried and bulked RCG biomass grown on plot WH5, Figure 76, were also found to have high levels of variation between the different amendments. Biomass produced in soils amended with both GWC and the mixture were found to have lower concentrations of Cu with less variation between results when compared to that produced in soils amended with DWTR and the unamended control soil. The greatest concentration of Cu was found in biomass grown in DWTR amended soils at 141.48mg/kg. The greatest mean average of all three bulked samples was found in biomass produced in unamended soils at 97.44mg/kg. The mean concentrations of Cu between the two plots were not found to be significantly different ($n=12$) ($p< 0.05$).

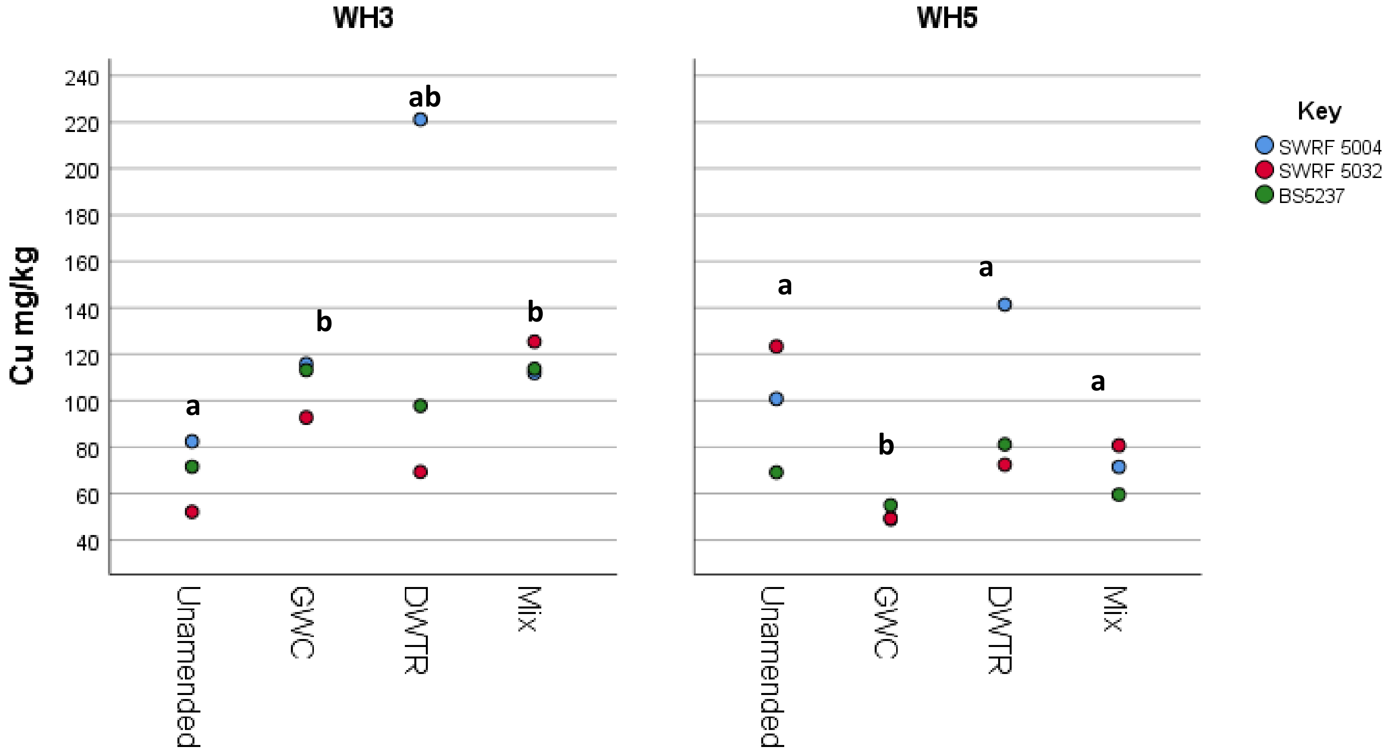

Figure 76 Grouped scatter plots showing concentrations of Cu (mg/kg) found in dried RCG biomass grown in unamended mine soils and soils amended with green waste compost (GWC) , drinking water treatment residue and a 50/50 mixture (mixture) of the two amendments by volume at plots WH3 and WH5. At plot WH3 the amendment was added at the following rate GWC 14.2 % AR, DWTR 18.4% AR and mixture 16.1% AR. At plot WH5 the amendment rate for all amendments was 25 % AR. Amendment factors marked with the same letter have mean concentrations that do not differ significantly according to the two tailed t-test ($p < 0.05$).

Combining data for both sites, biomass produced in soils amended with GWC consistently provided the lowest concentrations of Cu with a mean average of 51.1 mg/kg. The GWC produced biomass that had statistically significantly less mean average Cu in than all of the other amendment options ($p<0.05$) ($n=3$). No significant difference ($p<0.05$) was found between the varieties of RCG and their Cu concentrations although SWRF5004 was found to have the greatest average contentions (90.7 mg/kg Cu) and SWRF5032 the lowest (66.1 mg/kg Cu). The mean concentrations of Cu within the three RCG varieties were not found to be significantly different on either plot and no observable pattern was recorded ($n=12$) ($p< 0.05$).

The results for the biomass concentrations produced across the two plots do not mirror each other. For example, at plot WH3 the biomass with greater concentrations of Cu were grown in the GWC and MIX amendments and biomass grown at plot WH5 in those amendments contained the least concentrations, when compared with the DWTR amended and unamended soils.

The Cu concentrations within the amendments (GWC 72.1 mg/kg and DWTR 27.8 mg/kg) or their possible heterogeneity were unlikely to have effected these results considering the greater concentrations found within the mine soils themselves (WH3 545 mg/kg and WH5 890 mg/kg). The difference between the concentrations in the two mine soils is not reflected in the RCG biomass produced suggesting that RCG has a threshold for Cu accumulation. This observation is supported by the similar biomass Cu concentrations found in the pot trials (see section 6.4.9) which ranged from 11.1 mg/kg to 106.8 mg/kg.

Copper is an essential element to the growth of plants and plays a crucial role in several enzyme processes and in the formation of chlorophyll. However, the quantities that of Cu that plants typically need is typically around 4.5 mg/kg dry biomass (Nagajyoti *et al*., 2010). Indeed the literature suggests that this average is typical for RCG even when it is grown on contaminated soils (Strašil, 2012; Lord, 2015a; Antonkiewicz *et al.*, 2016). Antonijević *et al*., found concentrations of Cu in similarly high concentrations to this experiment (89-2400mg/kg dry weight) in native plants growing on a copper smelter plot and suggested that at these quantities of Cu were phytotoxic (Antonijević *et al.*, 2012). Whilst the results of the BCR procedure (1.11.3) show that the amendments used in this experiment are able to reduce the availability of the metal it is likely that the RCG plants reached a Cu threshold within the remaining plant available Cu. For example, although the addition of 30% (amendment as received weight equivalent/soil dry weight) GWC reduced the exchangeable (plant available) fraction of Cu by approx. 30%, 15% of 900 mg/kg Cu remained in a plant available form.

8.5.16 Nickel

Concentrations of Ni (mg/kg) in dried and bulked RCG biomass grown on plots WH3 and WH5 are shown in Figure 77 and were found to have ranges of 8.4 – 20.0 mg/kg and 9.0 - 42.6 4 mg/kg respectively. The mean Ni concentrations in the biomass produced at plot WH3 was 12.3 ±3.4 mg/kg and at WH5 was 15.7 ±9.5 mg/kg. The mean concentrations of Ni between the two plots were not found to be significantly different (n=12) ($p< 0.05$).

At plot WH3 the biomass produced in the unamended soils had significantly less concentrations of Ni when compared to the biomass produced in the amended soils. No significant difference was found between the concentrations in the biomass produced in amended soils. Here the results for Ni concentrations for biomass produced in DWTR amended soils again had the greatest standard deviation ±5.4 mg/kg than the other soils which were in the range of ±0.6-3.2mg/kg. No significant difference was found between the varieties of RCG although (n=4) ($p<0.05$), BS5237 was found to have the lowest mean concentrations 11.8± 2.5 mg/kg and SWRF5004 the greatest 13.3 ±4.6 mg/kg. As with Cr there were greater concentrations of Ni found in the amendments (GWC 23.8 mg/kg and DWTR 164 mg/kg) than in the mine soils (WH3 BLD and WH5 27.6 mg/kg). The result that there are greater concentrations in the biomass produced in the amended soils when compared to the unamended at WH3 and that the greatest concentrations are found in the soils amended with DWTR clearly confirms the impact the amendments have had on the biomass. Although a complementary result was not found in the pot trial biomass analysis for WH3 (biomass grown in unamended WH3 soils provided the greatest concentrations of Ni), in the WH5 pot trial soils the unamended soil did produce biomass with the lowest concentrations (see section 6.4.10). Analysis of the fractionation patterns for Ni in the amendments found that the Ni in the DWTR was more liable than that in the GWC 7.4.47.5.4 (see sections 7.4.4 and 7.5.4).

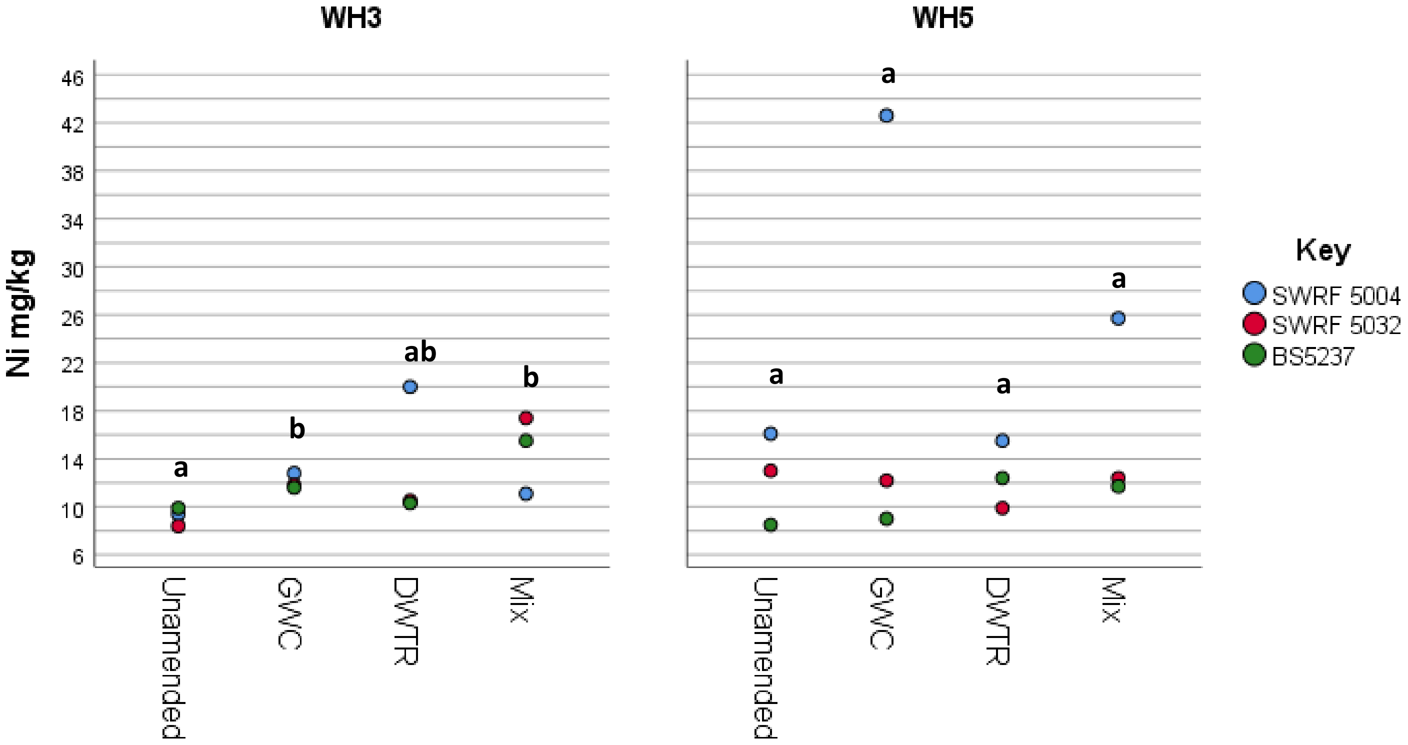

Figure 77 Grouped scatter plots showing concentrations of Ni (mg/kg) found in dried RCG biomass grown in unamended mine soils and soils amended with green waste compost (GWC) , drinking water treatment residue and a 50/50 mixture (MIX) of the two amendments by volume at plots WH3 and WH5. At plot WH3 the amendment was added at the following rate GWC 14.2 % AR, DWTR 18.4% AR and mixture 16.1% AR. At plot WH5 the amendment rate for all amendments was 25 % AR. Amendment factors marked with the same letter have mean concentrations that do not differ significantly according to the two tailed t-test ($p < 0.05$).

At plot WH5 there were no significant differences found between the biomass concentrations of Ni produced in the amended and unamended soils (n=3) ($p<0.05$). Although the GWC and MIX amended soils both resulted in a single outlier results that showed considerably more Ni biomass concentrations (see *Figure 77*). The outlier for the Ni concentrations found in biomass produced in GWC soils (21.2 ±18.5 mg/kg) results in the mean for this factor being far greater than for the biomass produced in the other amended (DWTR 12.6 ±2.8 mg/kg) and MIX (16.6 ±7.8 mg/kg) and the unamended soils (12.5 ±3.8 mg/kg). The RCG variety SWRF5004 produced biomass with significantly greater Ni concentrations (n=4) ($p<0.05$) than the other two varieties. The results for WH5 suggest that the variety of RCG had a greater impact than the soil amendment for Ni biomass concentrations. Further experimentation is required to see if this finding could assist in developing RCG varieties that interact with soil Ni differently. Nickel is recognized as essential for plant growth and development but requirement is the lowest of all essential elements at typically < 0.5 mg per kg of dry weight, although concentrations of 10m/kg Ni in healthy plants have been reported (Chen *et al.*, 2009a). Excessive Ni concentrations can alter plant metabolism and lead to, chlorosis, necrosis of the leaf, stunted growth, inhibition of germination and inhibit dry matter production (Shabnam and Seema, 2011; Gonnelli and Renella, 2013). As Ni is highly mobile within the plant system it can easily be translocated from the rhizosphere to the above ground portions of the plant (Kováčik *et al.*, 2009).

8.5.17 Lead

8.5.17.1 WH3

The figures for biomass concentrations of Pb were separated due to the significantly greater mean concentrations (p<0.05 n=12) found in all biomass grown at plot WH3 (4450 mg/kg) when compared with that grown at plot WH5 (578 mg/kg). Concentrations of Pb (mg/kg) in dried and bulked RCG biomass grown on plot WH3 are shown in Figure 78 and were found to have a low range except for those grown in soils amended with DWTR for which the concentrations ranged from 3107 mg/kg to 7155 mg/kg. Both the GWC (4370 ±494mg/kg Pb) and mixture (5037 ±186mg/kg Pb) amended soils produced biomass that had significantly greater (p<0.05 n=3) mean concentrations of Pb than the unamended soils (3199 ±477mg/kg Pb). The MIX of amendments produced biomass that contained mean concentrations of Pb significantly greater than all other amended soils. The range of results for the biomass produced in the DWTR soils prevents any significant result and any conclusion being drawn from the impact of this amendment here.

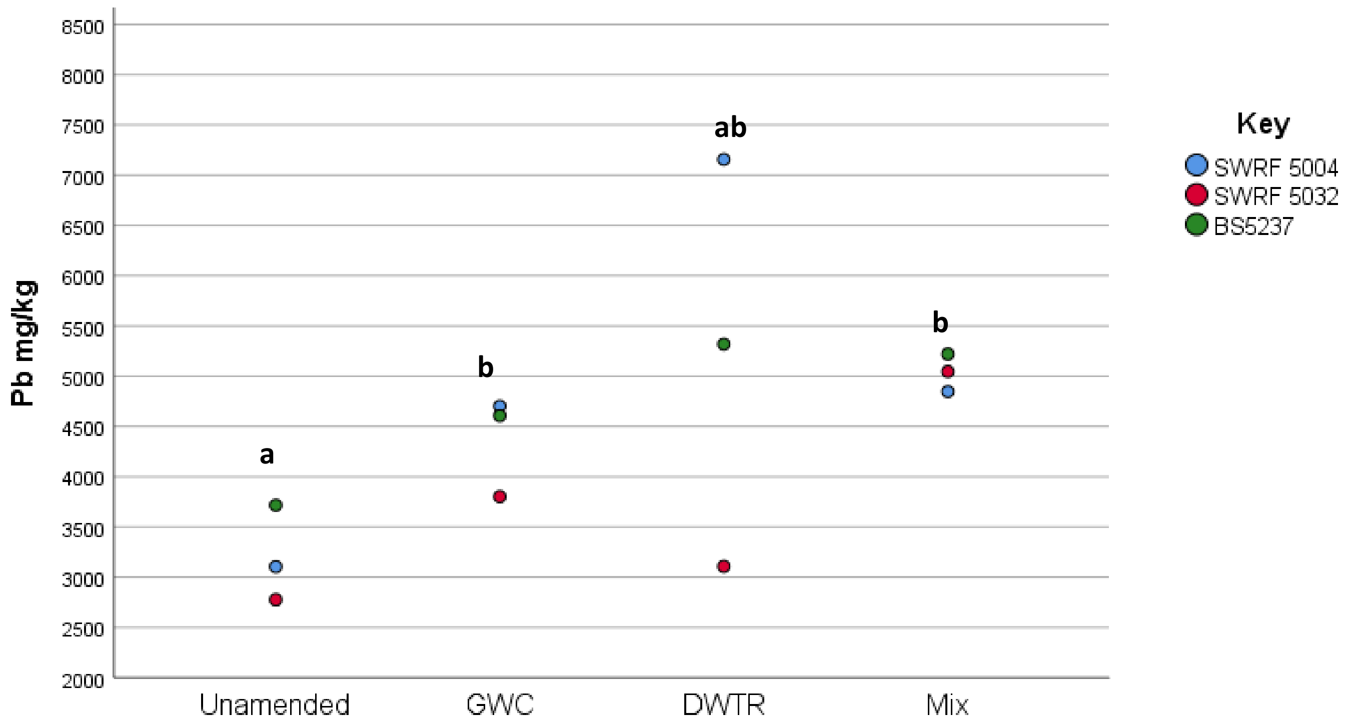

Figure 78 Grouped scatter plots showing concentrations of Pb (mg/kg) found in dried RCG biomass grown in unamended mine soils and soils amended with green waste compost (GWC) (amendment rate 14.2 % AR) , drinking water treatment residue (amendment rate 18.4% AR) and a 50/50 mixture (MIX) of the two amendments by volume (amendment rate 16.1% AR) at plot WH3. Amendment factors marked with the same letter have mean concentrations that do not differ significantly according to the two tailed t-test (p < 0.05).

No significant difference ($p<0.05$ $n=4$) was found between the varieties of RCG and their Pb concentrations although SWRF5004 was found to have the greatest mean average contentions (4952 mg/kg Cu) and SWRF5032 the lowest (3682 mg/kg Cu). It is clear that amending the mine soil with GWC and the MIX has promoted RCG Pb uptake at plot WH3. The concentrations found in all the biomass from plot WH3 are far in excess of those found in WH5 and those reported in relevant published literature on RCG (see section 3.4.1). Concentrations of Pb in biomass have been found in other plant species particularly *Brassica sp.* (Tangahu *et al.*, 2011), but this is rare, and unseen in grass species. It would possible to explain this result as an effect of cross contamination with a soil sample from the plot or the effect of an inadequate washing procedure during biomass analysis but neither explanation can answer for the similar biomass concentrations between WH3 and WH5 found for all other analytes apart from Pb. The analyses for all analytes mentioned in this chapter took place on the same samples. The Pb concentrations of the amendments and the mine soils do not explain this result either. The GWC was found to contain 159 mg/kg and the DWTR 50.5 mg/kg, neither being enough to influence this result significantly. The unamended mine soils WH3 and WH5 were found to contain 13,900 mg/kg Pb and 9110 mg/kg Pb respectively. Although there was more Pb found in the mine soils from WH3 the difference is not great enough to explain the difference in the Pb concentration for the biomass produced at either plot, which are approximately by a factor of 10. The biomass produced at plot WH3 was far less than that of WH5 and this is potentially due to a lack of nutrients as a result of the differences in amendment mass between the plots (see Table 43). However, the phytotoxicity of Pb may have stunted the plant growth at

WH3 and caused an issue with the physiochemical processes of the plants resulting in Pb saturation of the biomass.

The difference in concentrations between the two plots cannot be explained by a difference in the ratio of biomass to Pb containing dust left over on the plant material. A Pb 'Offtake value' can be found by multiplying the total dry biomass obtained from each plot and their average Pb concentrations, shown in Table 49. If these values were very close it would suggest that the greater concentrations of Pb in the WH3 plants were due to the smaller dry biomass. This however is not the case as the offtake value for WH3 (716,000) is greater by a factor of 2 than the value for WH5 (282,000). Whilst the greater biomass produced in plot WH5 may have resulted in lower concentrations of Pb it is clearly not the only reason for the differences between the two sites

Table 49 Offtake values for plots WH3 and WH5 found by multiplying average Pb concentrations with the total biomass obtained from each plot.

Factor	Total dry biomass obtained in plot (g)	Average Pb concentration (mg/kg) (n=12)	Offtake value (#)
WH3	161	4450±1230	716,000
WH5	488	578± 143	282,000

If the key factor in the greater concentrations of Pb were the greater amounts of mine soil dust on the analysed biomass, then a calculation of the percent dust required to give the biomass concentrations found could provide an indication as to whether this is the case. The unamended mine soils at plot WH3 were found to contain 13900 mg/kg Pb and the average Pb concentrations in the biomass analysis were 4450 mg/kg. The Pb concentrations found in the biomass are 32% of the soil concentrations. It is very unlikely that the mass of the analysed biomass would be 32% mine soils/dusts following the washing procedure and if it were, this would be noticeable by the laboratory practitioner.

8.5.17.2 WH5

The concentrations of Pb (mg/kg) in dried and bulked RCG biomass grown on plot WH5 are found in Figure 79 and were found to have high levels of variation between the different amendments. Similar to the results for Cu at plot WH5 the biomass produced in soils amended with both GWC (459 ±67.2mg/kg Pb) and the MIX (542 ±64.3mg/kg Pb) was found to have lower mean concentrations of Pb with less variation between results when compared to that produced in soils amended with DWTR (636 ±187mg/kg Pb) and the unamended soil (671 ±165mg/kg Pb). The greatest concentration of Pb was found in biomass grown in the DWTR amended soils at 851.83 mg/kg. Biomass produced in soils amended with GWC consistently provided the lowest mean average concentrations of Pb which were found to be significantly less than that of the DWTR and the MIX ($p < 0.05$ n=3) but not the unamended soils.

Despite an observable pattern of the SWRF5004 variety biomass having greater concentrations of Pb than the other RCG varieties (see fig. 20) the result was not found to be significant. The variety SWRF5004 was found to be greater than BS5237 at a p value < 0.08 n=4.

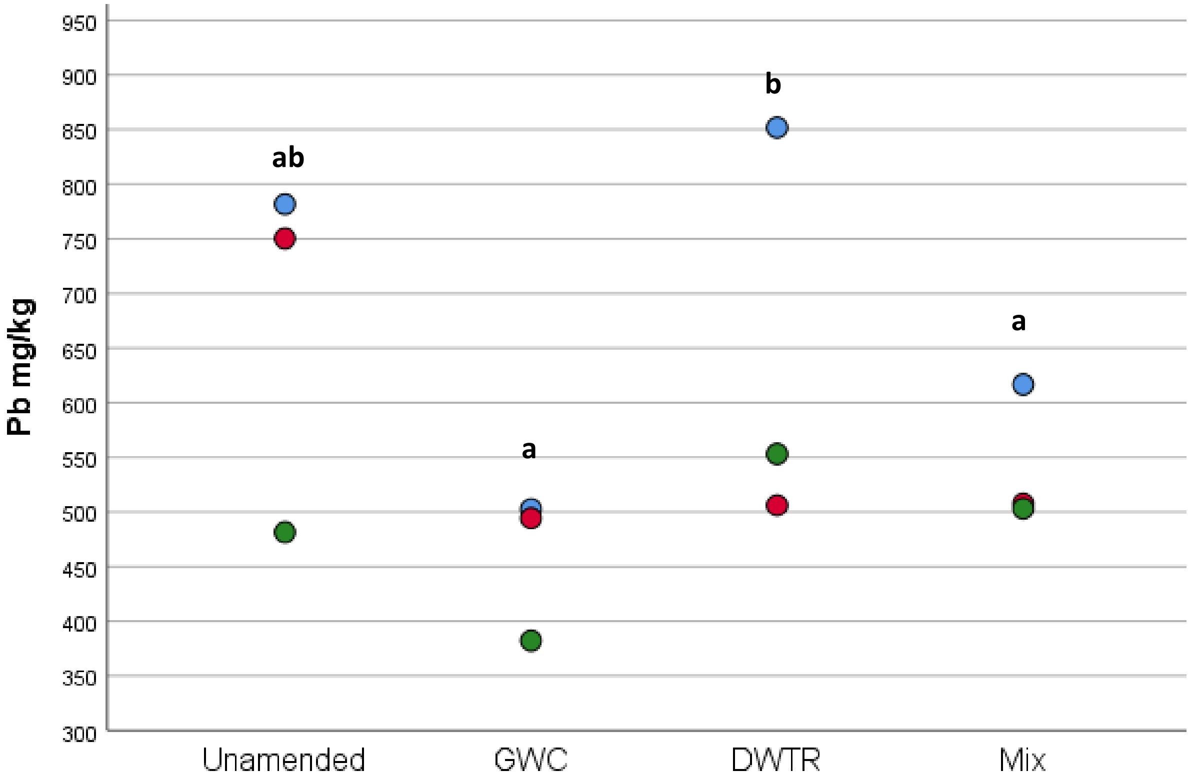

Figure 79 Grouped scatter plot showing concentrations of Pb (mg/kg) found in dried RCG biomass grown in unamended mine soils and soils amended green waste compost (GWC) (amendment rate 25 % AR) , drinking water treatment residue (DWTR) (amendment rate 30% AR) and a 50/50 mixture (mixture) of the two amendments by mass (amendment rate 30% AR) at plot WH5. Amendment factors marked with the same letter have mean concentrations that do not differ significantly according to the two tailed t-test ($p < 0.05$).

The result that the biomass produced in the GWC and MIX amended soils had lower concentrations of Pb when compared to the unamended soil is one that can be supported by both the other experiments presented in this thesis and the relevant literature. The analysis of the biomass grown during the pot trial (see section 6.4.11) showed a significant reduction in Pb concentrations for plants grown in soils amended with GWC. The analysis of GWC amended WH5 soils at 30% (amendment as received weight equivalent/soil dry weight) following extraction using the BCR procedure showed that Pb decreased in availability in the amended soil. As both organic matter and an increase of pH are well understood factors in decreasing the solubility of Pb (Alvarenga *et al.*, 2008; Li *et al.*, 2021) it can be concluded here that the addition of GWC has resulted in less Pb in the biomass. This could also be the case for the MIX result which produced biomass with slightly more Pb in as 50% of the MIX is GWC (as received). Although the addition of DWTR has provided similar results to the GWC in the pot trial results described in section 6.4.11 and in the relevant literature (Rodríguez *et al.*, 2009), a similar result is not seen here. That is the biomass produced in soils amended with DWTR had similar concentrations to that in the unamended soil. This maybe as a result of the difference between the physical structure of GWC and DWTR resulting in DWTR application to the mine soils not being as homogeneous as the GWC. This difference is discussed in greater detail in section 5.4.

8.5.18 Zinc

Concentrations of Zn (mg/kg) in dried and bulked RCG biomass grown on plots WH3 and WH5 are shown in Figure 80 and were found to have range of 1820 – 2430 mg/kg and 1750 - 2350 mg/kg respectively. The mean Zn concentrations in the biomass produced at plot WH3 was 2070 ±270 mg/kg and at WH5 was 1920 ±170 mg/kg. The mean concentrations of Zn between the two plot were not found to be significantly different (n=12) ($p < 0.05$). At plot WH3 the mean concentrations of Zn in the biomass produced in the unamended soils (1970 ±323 mg/kg) and the soils amended with DWTR (1930 ±120 mg/kg) had significantly less concentrations of Zn when compared to the biomass produced in the soils amended with GWC 1820 ±92.7 mg/kg) and the MIX 1942 ±72.5 mg/kg) (n=3) ($p < 0.05$). No significant difference was found between the varieties of RCG although (n=4) ($p<0.05$), BS5237 was found to have the greatest mean concentrations 2070± 260 mg/kg and SWRF5004 the lowest 2020 ±283 mg/kg. At plot WH5 there were no significant differences found between the biomass concentrations of Zn produced in the amended and unamended soils (n=3) ($p<0.05$). The mean average biomass Zn concentrations were found to all be so similar for the unamended (1970 ±332 mg/kg) and the GWC (1820 ±92.7 mg/kg), DWTR (1930 ±120 mg/kg) and the MIX (1940 ±72.5 mg/kg) amended soils that drawing any worthwhile inference from their differences would be difficult. No significant difference was found between the Zn concentrations found in the three varieties of RCG either (n=4 $p<0.05$).

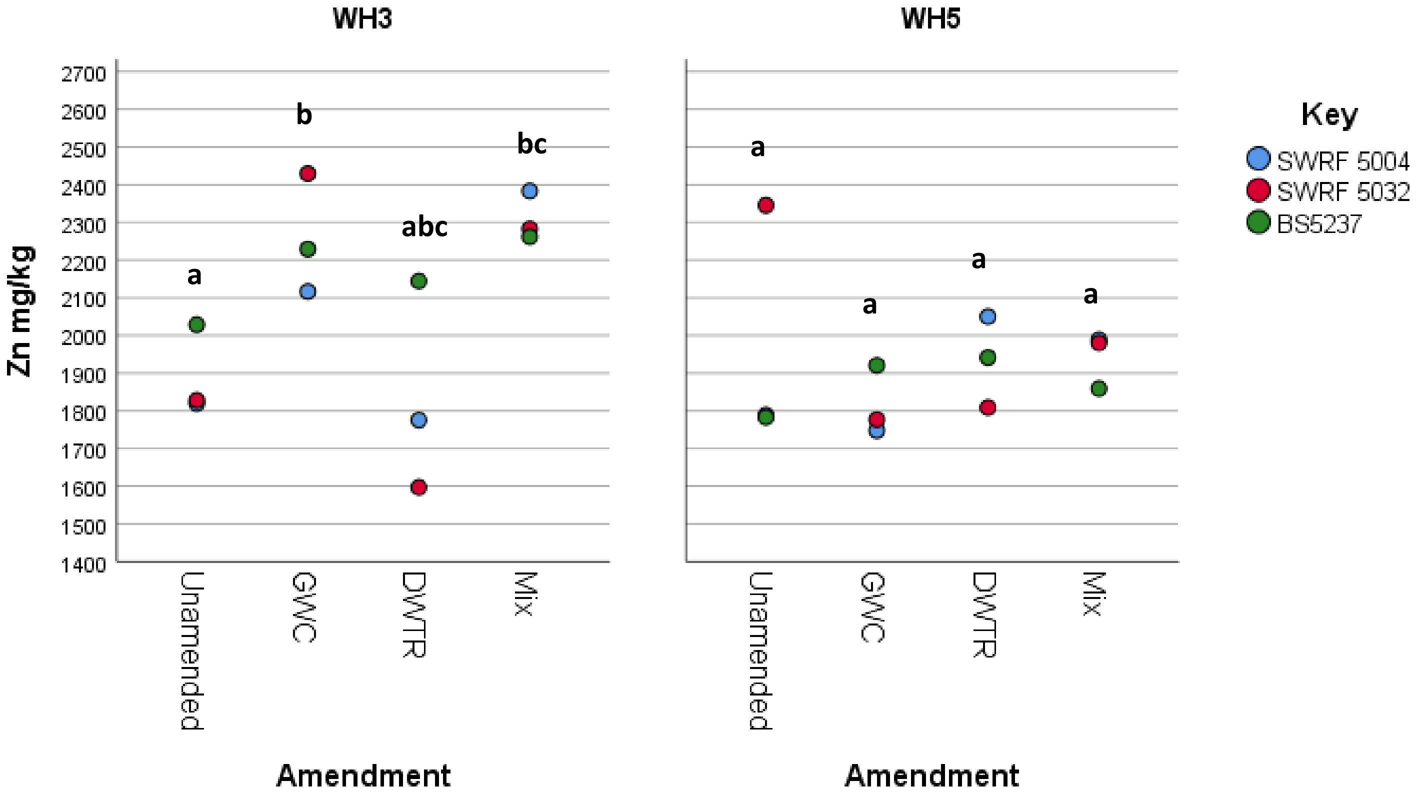

Figure 80 Grouped scatter plots showing concentrations of Zn (mg/kg) found in dried RCG biomass grown in unamended mine soils and soils amended with green waste compost (GWC) , drinking water treatment residue and a 50/50 mixture (MIX) of the two amendments by volume at sites WH3 and WH5. At site WH3 the amendment was added at the following rate GWC 14.2 % AR, DWTR 18.4% AR and mixture 16.1% AR. At site WH5 the amendment rate for all amendments was 25 % AR. Amendment factors marked with the same letter have mean average concentrations that do not differ significantly according to the two tailed t-test ($p < 0.05$).

8.6 Biological Accumulation Coefficient (BAC)

The BAC was used to evaluate the impact that the amendments had on the mobility of PTE. A description of the BAC and the equation used in its application to the results are found in section 6.5 of the current study. Calculations for the concentrations of PTE have been made for the amended soils using known concentrations in the mine soils and amendments and the 'as received' (AR) rate of amendment. Concentrations of PTE in biomass are calculated as the mean average (n=3) concentration found in bulk samples of RCG grown in each amendment on each plot. The results are shown in Table 50. The BAC results for As show that the addition of all amendments on both field trial plots had little effect on the plant availability of the analyte. The amendments had similar effects on the BAC of Cd and Cr which was to increase their availability to the RCG on both plots. This effect was most noteworthy on plot WH3 were the addition of amendments resulting in plants with the greatest BAC values (Cd) and the greatest increase in BAC from an unamended soil value below 1 (Cr). This effect was not seen in the pot trial test where the amended soils produced plants which gave far lower BAC values than the unamended (see section 6.5). This result does however support the findings of the BCR extraction which showed that none of the amendment options had a considerable impact on reducing the bioavailability of Cd and Cr and in fact may have increased it slightly (see section 7.4). The increase in BAC for Cd and Cr when compared to the unamended soil is seen the least in the WH5 soils amended with DWTR.

Table 50 The biological accumulation coefficient (BAC) for reed canary grass plants following a 1.5 year field trial at two plots (WH3 and WH5) using green waste compost (GWC), drinking water treatment residue (DWTR) and a mixture of the two (MIX) to amend soils..

Element	**As**	**Cr**	**Cd**	**Cu**	**Ni**	**Pb**	**Zn**
WH3							
Unamended	0.06	0.75	1.41	0.13	0.92	0.23	1.02
GWC 14.2 % AR	0.10	1.20	1.99	0.22	1.01	0.37	1.39
DWTR 18.4% AR	0.12	1.18	1.53	0.29	0.35	0.46	1.14
MIX 16.1 % AR	0.10	1.21	2.23	0.25	0.62	0.43	1.42
WH5							
Unamended	0.04	0.59	0.74	0.11	0.45	0.074	0.38
GWC 30% AR	0.03	1.24	1.25	0.07	0.80	0.067	0.46
DWTR 30% AR	0.05	0.56	0.82	0.15	0.20	0.09	0.48
MIX 30% AR	0.05	0.97	1.12	0.10	0.38	0.08	0.49

<1 plant suitable for phytostabilisation
>1 plant suitable for phytoextraction (Brooks, 1998)

For Cu there is a slight decrease in the BAC of the analyte in the soils amended with GWC on plot WH5. On plot WH3 the soils amended with GWC have a lower BAC when compared to that of the other two amendment options but not the unamended soils. The impact of GWC on reducing the mobility of Cu was shown in both the pot trials and in the extract analysis following the BCR procedure (see section 1.6 and 1.12). However, the unamended soils produced biomass with the lowest BAC for Cu on plot WH3 suggesting that the reported effect of the amendments found in the pot trials and BCR extraction results has not been completely replicated in the field trial.

There is a clear impact on the BAC of Ni for the plants grown in soils amended with DWTR when compared to the unamended soils as at both plots the BAC is reduced by above 60%. The effect is also seen in both the BAC for the pot trial and the analysis of the BCR extracts. Conversely the plants grown in the soils amended with GWC show an increase in BAC when compared to the unamended soils for both plots WH3 and WH5 an effect that was not seen in the previous experiments.

The pattern of the results for the BAC in regards to Pb are very similar to that of As and Cu. The unamended soils for WH3 gives BAC values that are slightly lower than the amended soils suggesting that the amendment has increased the bioavailability of Pb. The other amendment options here give BAC values that are above that of the GWC. For plot WH5 the BAC value for the GWC amended soils is slightly lower than that of the unamended soils and the other amendment options are slightly above the unamended soils. There is also a large difference in the BAC values for Pb between the two plots, WH3 and WH5. Values for WH3 are at least greater by a factor of 10, this is a reflection of the far greater biomass concentrations. It is interesting to note that when applying BAC to these results that the plants with high Pb concentrations grown in plot WH3 were still not classed as suitable for phytoextraction, this is due to the high Pb soil concentrations.

The BCR sequential extraction analysis results in section 1.10.5 shows that both GWC and DWTR had a considerable effect in reducing the concentrations of Pb in the most plant available fraction. The pot trial results also showed a clear reduction in Pb biomass concentrations in amended soils compared with unamended soils from both plots. As discussed, the lack of effect on the BAC seen in the DWTR and to a lesser extent the MIX maybe as a result of the failure to homogenise the sticky DWTR with the mine soil to the same extent as for the granular GWC.

The BAC results for Zn show the clearest contrast between the two plots. On WH3 the BAC values are all above 1 and are the highest for both the MIX and GWC amended soils. On plot WH5 the BAC values are all below 1 and there is not a great deal of difference between the unamended and amended soils. The BCR extracts analysis suggested that the amendment of the mine soils with GWC slightly increased the concentrations of Zn in the plant available fractions although it was not clear if this effect was due to leaching of the analyte during the pot trial process. There are considerable differences between the Zn concentrations in the mine soils at plots WH3 (1850 mg/kg) and WH5 (5150 mg/kg) and this is reflected in the BAC. As discussed previously the increase in Zn mine soil concentrations at plot WH5 does not result in an increase in Zn in the biomass.

8.7 X-ray computed tomography

In order to understand better the high concentrations of Pb found within the RCG biomass from plot WH3, grass samples were collected from both WH3 and WH5 plots for X-ray computed tomography (XCT) analysis. The technique, which allows for the penetration of dense materials in order to image their 3D structures, has been recently applied for biomass analysis (Boigné *et al.*, 2021; Sun *et al.*, 2021). As the species SWRF5004 grown in DWTR at plot WH3 had the greatest concentrations of Pb (7160 mg/kg), it was selected for analysis. The respective sample from WH5 was also selected in order to provide a comparison. Figure 81 shows XCT images of unwashed but dried grass samples from both WH3 and WH5 at a resolution of 60µm. The calibration standards 100 and 1000 mg/kg Pb provided a detectable difference in density and the XCT images were produced successfully. No detectable difference was found between the 10 and 100 mg/kg Pb standards. The production of X-ray attenuation images is linked to sample density. The attenuation of the sample can be extrapolated by understanding the attenuation of parts of the scan with a known density. The polypropylene of the centrifuge tube that the grass is placed in has a density of 0.92 g/cm^3 (Gahleitner and Paulik, 2014). The density of the air filled parts of the scanned image 0.001 g/cm3. As the plastic and air parts of the scan shown in fig 22 have attenuation of 19.6 /m and 0.2 /m respectively, it can be extrapolated that parts of the images with an attenuation rate of over 20 /m have a density of 10 g/cm^3. Lead has a density of 11.29 g/cm^3 and its most likely compounds in regards to this mine soil have densities of approximately 6 g/cm^3 (galena 6.23 g/cm^3, anglesite (PbSO4) 6.3 g/cm^3 or cerussite (PbCO3) 6.58 g/cm^3).

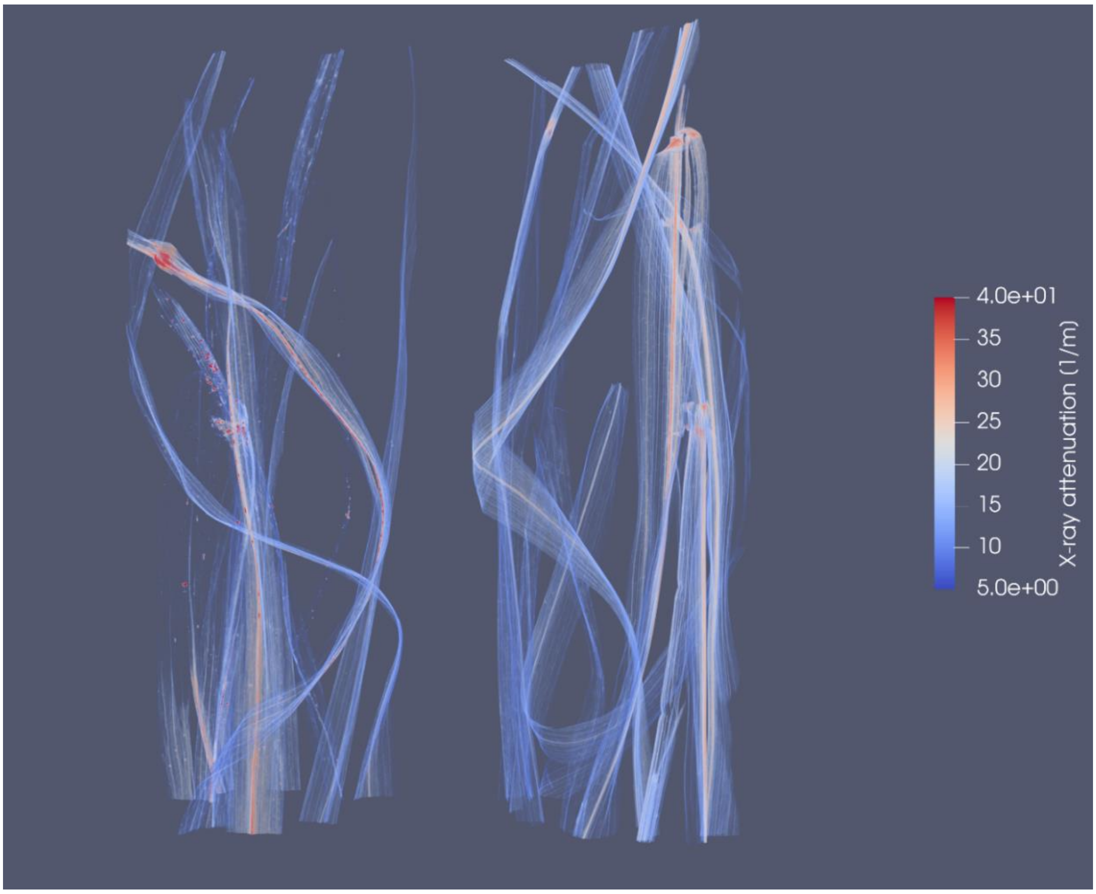

Figure 81 XCT images of RCG (variety SWRF 5004) from plot WH3 and WH5 grown in DWTR amended soils at a resolution of 60µm

As the plants that have been scanned have been found to contain 800 mg/kg (WH5) and 7160 mg/kg (WH3) it is plausible to suggest that the parts of the image in fig 22 with an attenuation rate of over 20 /m represent Pb or Pb compounds. The scan displayed in Figure 82 shows that there are greater areas of density within the grass sample from plot WH3 when compared to the grass sample from plot WH5.

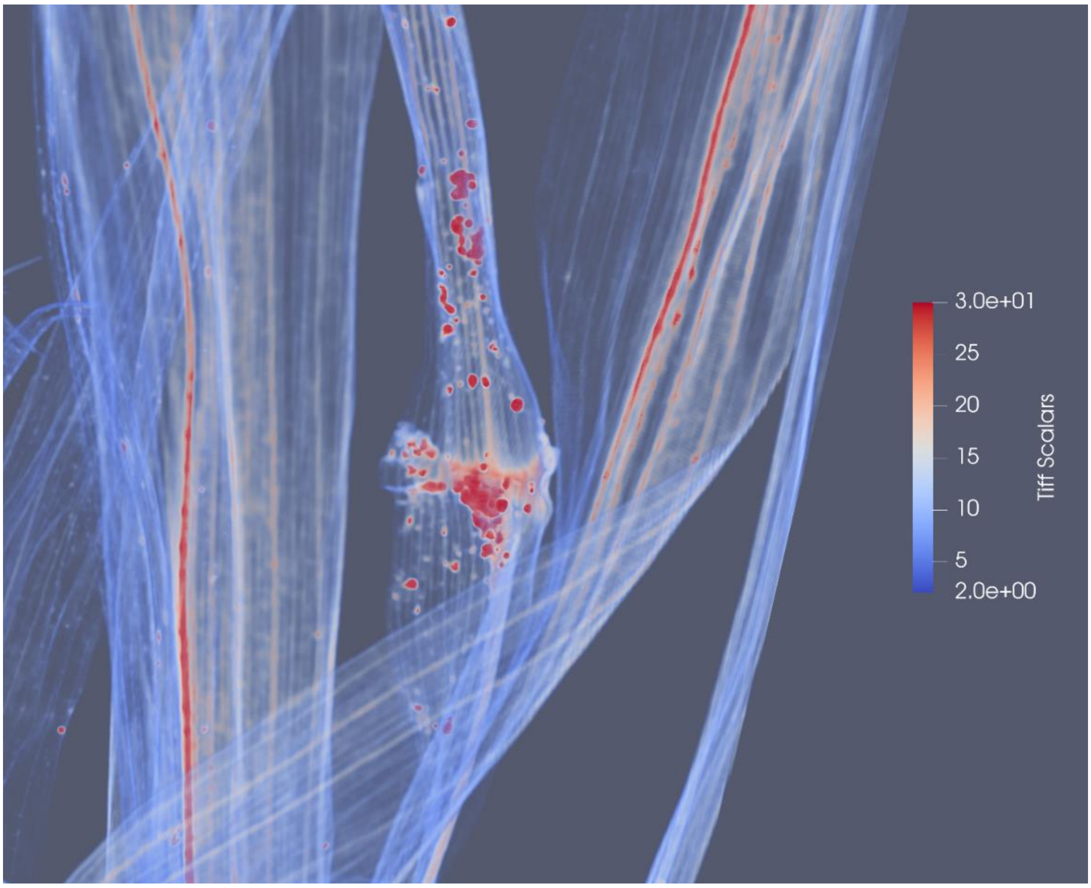

Figure 82 XCT image of RCG (variety SWRF 5004) from plot WH3 grown in DWTR amended soils at a resolution of 60µm

The image shown in Figure 82is ‘zoomed in’ on an area of high density within the WH3 grass sample. The scan shows that there are two forms in which the areas of high density take in this sample. There are areas of high density within the stem of the grass and also pockets of high density particularly around the ligule (the thin outgrowth at the junction of a grass leaf) of the plant. This suggests that some of the Pb found in the analysis of the biomass may have been trapped dust from the mine soils which were found to contain 13870 mg/kg Pb. The WH5 grass sample shown in Figure 81 was found to have fewer focused high density areas that potentially indicate Pb contaminated dusts. The grass at plot WH3 was found to be far shorter than that of WH5 so was potentially more likely to receive dust through the effect of rain splashing into the soils. The soils at plot WH3 were also found to be dryer and finer and so more susceptible to mobilisation due to rain splash and wind.

Figure 83 is an image produced through an XCT scan of two further samples of RCG from plots WH3 and WH5 that were washed thoroughly in deionised water prior to analysis. The images in Figure 83 were taken at a much higher resolution of 22µm.

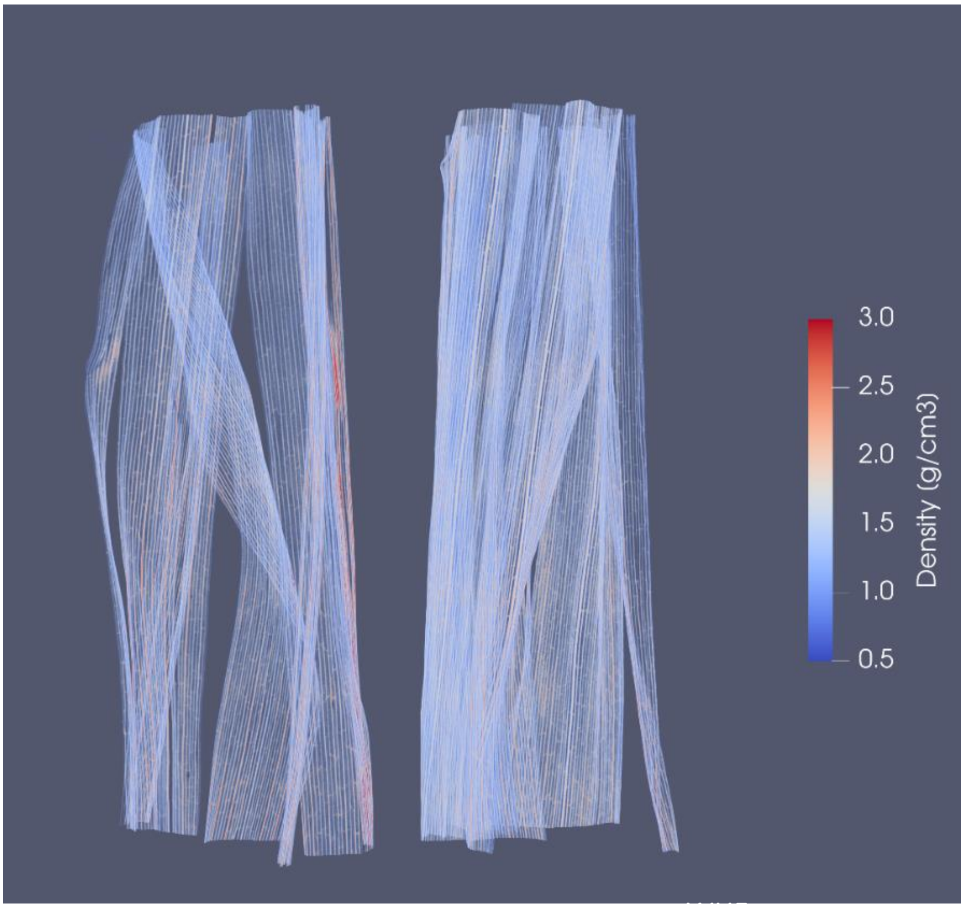

Figure 83 XCT images of washed RCG (variety SWRF 5004) from plot WH3 and WH5 grown in DWTR amended soils at a resolution of 22µm

It is clear from Figure 83 that neither of the scans picked up similar areas of high density as were found in the unwashed samples. This suggests that the majority of the Pb found in the analysis of the biomass bulk samples from was in fact dust on the plant. However, there are uniformly dense areas situated vertically in the grass plants and overall, the WH3 sample has denser regions in some leaves. Unlike the previous scan shown in Figure 81 these regions have a density of around 4 g/cm^3 which is closer to biogenic/amorphous silica (2.22 g/cm^3) than any the PTE discussed by this study (Wang *et al.*, 2012a). Silica is known to occur in Phalaris (ref) and results in its higher SiO2 content compared to other grasses (Tao *et al.*, 2012) or biomass ashes (Lord, 2015a). The samples analysed in Figure 83 were thoroughly washed as individual grass blades rather than as a bulk biomass sample and the differences in washing procedure makes a further conclusion difficult. Further work would need to be carried out in order to confirm whether the concentrations of PTE found during this study represent plant absorbed or mine dust.

8.8 Chapter conclusions

The aim of this work was to evaluate the potential of reed canary grass grown with organic waste soil amendments as a phytostabilising remediation option for historic mine soils. The combination of the high plant survival rates, successfully implemented metrics for monitoring growth rate and significant results in both the dry weight yields and analysis of the biomass for PTE show that the field trial discussed in this chapter has provided robust evidence that addresses the aim. The GWC at both amendment rates clearly had a positive effect on the growth of RCG. The GWC used alone and as a mixture with DWTR provided the highest mean average heights and dry yields throughout the trial at both plots. The DWTR had significant effects on the height of the plants at plot WH3 and the dry biomass produced at WH5 when compared to the unamended control soils. The preferential growing conditions for RCG in GWC when compared to DWTR were found to be potentially linked to the lower nutrient content and immobilisation of essential nutrients especially P in the DWTR. The difference in bulk densities of the plots and moisture content of the amendments did lead to variations in the amended soil nutrient concentrations. Further work that assesses the effects of GWC and DWTR, or any organic waste amendment, should take these parameters into account when looking to make robust comparisons. Of the three RCG varieties the SWRF 5004 outperformed the SWRF 5032 and BS 5237 on both plots. This provides a clear indication of the development potential for variety selection of RCG when grown on contaminated land.

The physical characteristics of the mine soils potentially played more of a significant role in the inhibition of growth of the RCG plants than the presence of PTE. Plot WH3 produced plants that had an interquartile range approximately 21-23 cm whereas plot WH5 produced plants with an interquartile range of approximately 40-45cm. Plot WH3 also had a lower plant survival rate than plot WH5. Whilst the two plots shared similar PTE concentrations the moisture content and bulk density of the soils was quite different. Plot WH3 was also situated at a greater elevation to WH5 and was far more exposed to the wind and rain, to the extent that some of the plants were lost due to sheet flooding. Plot WH5 was positioned close to a mine adit that fed water to the plot throughout the year. Despite the addition of the amendments having significant effects on reducing the bioavailability of PTE in the mine soils when extracted using the BCR procedure (as discussed in the previous chapter), little evidence of these effects were recorded in the analysis of the biomass produced by the field trial. In several cases the application of the organic waste amendments increased the concentrations of PTE in the biomass. This is best shown in the results of the bioaccumulation coefficient which tended to be greater for biomass grown in amended soils when compared to that grown in unamended soils. The results of the XCT analysis showed that, despite washing the samples, at least a considerable portion of the PTE (especially Pb) found in the analysis were dust on the plant rather than absorbed into the biomass. Considering that the plants grown in the amended soils had more biomass, it possible to conclude that a plant with greater biomass would have a greater surface area for contaminated dusts to settle. This potentially explains the counter intuitive results found in the biomass analysis. Further work should be carried out to investigate this, as if RCG plants are not translocating metals into their biomass (even at the very high soil concentrations found in this study) then the case for the plant being selected for phytostabilisation approach in the remediation of contaminated land becomes stronger.

9 Thesis Conclusion

The objectives of this study are outlined in section 1.2. These objectives were met through the course of this thesis allowing for several conclusions to be drawn.

Chapter 2 was designed to represent a general literature review of all the topics discussed in this study and to discover what gaps there were in the published literature and therefore what contributions this study could make. Several gaps in the literature were identified. For instance, there is little published literature on the use of DWTR as a soil amendment for historic mine sites, despite its considerable potential especially as it can be (as in the case of this work) a locally ‘produced’ option due to the effects of mine sites on aquifers and the upland location of many historic mines.

It is clear that the research topic of phytoremediation suffers from a lack of large scale field trials and a focus on what is practically achievable when using plants for remediation. This is highlighted in the review and discussions of the work by Reeves and Bolan (Bolan *et al.*, 2014; Reeves *et al.*, 2018). Claims of hyperaccumulation tend to be made in laboratory pot trials where conditions are controllable. Much more work should be conducted on the standardisation of the phytoremediation approach in order for it to be a ‘ready to use’ option for industry and regulators.

Chapter 3 of this study set out to perform a comprehensive review of the published data on concentrations of PTE found in RCG when grown on impacted soils and compare that with date on two other lignocellulosic biofuel crop species. The initial finding of the review was that there is a research gap concerning lignocellulosic biofuel crop species grown on soils with high levels of PTE. Very few papers were found for SRC Willow and Miscanthus and no papers for RCG that used soils with similar concentrations to the ones in this study. For example, the majority of soil concentrations shown in the relevant literature were not greatly above the average for UK soils. This provided a clear justification for this study and also highlighted a limitation to the literature review. The review did, however, find several potential relationships between RCG biomass adsorption and soil concentrations of PTE, clear differences in PTE adsorption between the three species and between cultivars of the same species, thus highlighting the importance of cultivar selection and reporting. It is clear from the review of literature that RCG is likely to play an important role in the future of bioenergy crops and thus there need for further research on its growth in PTE impacted soils and accession development (Lord, 2015; Oleszek *et al*., 2014).

The aim of Chapter 5 was to gain a full understanding of the materials used throughout the study. One of the key conclusions of the chapter, following a desk study of the site and the analysis of soils and sediments, was that it was highly likely that the mine site was impacting local waterways. This justified the remediation focus and experimentation with the use RCG and the organic waste soil amendments. The low nutrient content and high levels of PTE found in the mine soils showed the need for soil amendment and justified experiments with the organic waste soil amendments. The analysis of the mine soils provided another justification for the study as their concentrations of PTE were found to be greater (in some cases by a factor of 100) than those found in the published literature on RCG and its use in remediation i.e. there is no published work on RCG grown in such high concentrations. This study therefore, contributes towards addressing that gap in the literature.

The two organic waste soil amendments used throughout this study were found to have differing qualities. The amendments have different pH, concentrations of PTE, conductivity and total/available nutrients. These found differences made their comparisons throughout the results chapters valuable as interest in the use of both soil amendments is growing. Chapter 5 provided a foundation for which further conclusions on the nature of the mine soils and soil amendments could be drawn throughout the following chapters of the study.

Chapter 6 considered the effect that two organic waste soil amendments had on the growth and PTE concentrations of reed canary grass biomass produced over a ten-week pot trial. The initial finding of the experiment was that the different characteristics of the organic waste soil amendments meant that it was difficult to amend the soils equally. Due to different bulk densities and moisture contents for example the addition of the same mass of amendments potentially led to different nutrient addition/PTE dilution. This is a limitation of comparing organic waste soil amendments which are often different in their composition. Further work should be undertaken to standardise a process that allows for adequate comparison of these amendments. The various tests applied as part of the pot trials were successful in showing the effect of the soil amendments and many conclusions were drawn, see section 6.6. The results provided a clear justification for upscaling the experiment to a field trial. A significant finding of the biomass analysis of the plant material grown as part of the pot trials was that the greatest concentrations of most of the PTE tested (Cr, Cu, Pb and Zn) were generally found in the biomass produced in the unamended soils. This provided an indication that the organic waste soil amendments may have reduced the mobility of some PTE in the amended soils resulting in the lower biomass concentrations. However, it may have also been the results of a breakdown in normal physiological processes due to phyto toxic PTE or that the PTE were absorbed in greater quantities at the beginning of growth and thus the concentrations were diluted in the plants that achieved greater biomass production as they did in the amended soils. The pH of the mine soils was also altered considerably through the application of the amendments (reduced by the DWTR and increased by the GWC) and this was thought to be likely a factor in any changes in the PTE bioavailability. These findings provided a clear justification for further tests on the bioavailability of the PTE.

Chapter 7 of this study involved the application of the BCR sequential procedure to the pot trial soils. The results obtained through the analysis of the samples taken through the sequential extraction procedure had consistently low variability between triplicates, an uncommon occurrence given the complexity of the procedure and the origin of the samples i.e soils from three different pots that had been individually amended and taken through the pot trial process. The quality of the results was thought to be likely an indication of the benefits of successful homogenisation of the different material used prior to the pot trials i.e. the mine soils and amendments. The miniaturisation of the BCR method was found to be successful, as shown by the good recovery results of the CRM in section 7.3.3 . This is an important finding of this study as it allows for this procedure to the undertaken without a laboratory having to invest in less common centrifuge equipment i.e. a centrifuge that uses the larger 100ml centrifuge tubes as required by the standard method.

Changes in the fractionation patterns for almost all of the PTE analysed were found to occur in the pot trial soils following amendment and plant growth, the most significant were that of Cu and Pb. The Cu in the unamended mine soil was found mainly in the exchangeable and oxidisable fractions, which is typical when applying the BCR procedure as Cu has strong affinity for organic matter. Significant increases in the oxidisable fraction and decreases in the exchangeable fraction of the DWTR amended soil when compared to the unamended soil led to the conclusion that the high OM content of the amendment led to a reduction in the mobility of Cu, this effect has been seen in many similar studies as highlighted in sections 2.19.2 and 7.4.3. For the soils amended with GWC it was the reducible fraction that increased when the exchangeable fraction decreases when compared to the unamended soils. The effect of the addition both amendments was to considerably lower the RAC value for Cu suggesting scope for further research into their effects on Cu contaminated soils.

The high concentrations of Pb found in the exchangeable fraction of the WH5 mine soil made Pb the most mobile of all the PTE studied in this work. This is important as Pb is also one of the key PTE concerned with this study due to its toxicity and high concentration in the mine soils. The fractionation pattern found suggests that the Pb is most likely carbonate bound and therefore cerussite. In the mine soils amended with DWTR the Pb was predominantly in the reducible fraction which indicates that Pb was occurring in a form bound to Fe/Mn oxides which are common in DWTR due to the water treatment process. This movement in Pb from the exchangeable to the reducible fractions constituted a significant change in mobility and suggests that DWTR could be used to remediate Pb contaminated soils. The addition of GWC also resulted in significant increases in the reducible fraction and decrease in the exchangeable. This change was attributed to increases in OM content (humic materials) and pH deceasing the solubility of Pb. There is a possibility the high P content of the GWC also played a role in decreasing the mobility of Pb through the formation of an insoluble pyromorphite-like mineral as discussed in sections 2.4 and 5.4.1. Further research on the effects of P when used in mine soil remediation should be conducted to confirm this finding. Both amendments had the effect of considerably lowering the risk assessment code (RAC) value for Pb from a very high risk to a medium risk. However, the type of RAC applied in this study only took into account the reduction in concentrations from the exchangeable fraction. If the reducible fraction were also included, as is done in several similar studies, the effects of the amendments on reducing the mobility of Pb would be less pronounced.

The aim of Chapter 8 in essence was to realise the feasibility of the application of the findings of the previous chapters at field scale. As stated regularly throughout this study and others studies including key literature reviews (referenced in the relevant sections), field trails concerning organic waste soil amendments and also biofuels grown on contaminated land are relatively rare due to several factors predominantly related to their risk of failure, cost and the time it takes to establish them. Field trials are, however, essential if laboratory based results are to be realised in a 'real' scenario. To meet this aim Chapter 8 involved an evaluation of the potential of reed canary grass grown with organic waste soil amendments to act as a phytostabilising remediation option for historic mine soils. The design of the field was an attempt to match that of the pot trials as realistically as possible. As with the pot trials a key difficulty with accurately comparing the soil amendments was the many differences in the characteristics. A detailed description of this is given in section 8.3 highlighting how comparing amendments performance whether they are added to soil based on mass, nutrient concentration or volume can lead to very different results. This is an often understated issue in published literature on the topic resulting in difficulty for comparing one study to another and one amendment to another. Standardisation of one metric for which soil amendment was applied in similar studies to this one would alleviate this issue e.g. by dry mass or by as received volume. For practical reasons, most amendments will be measured by wet mass or volume, with dry matter content back-calculated retrospectively. In regards to the results found in this chapter the combination of the high plant survival rates, successfully implemented metrics for monitoring growth rate and significant results in both the dry weight yields and analysis of the biomass for PTE show that the field trial has provided robust evidence that addresses the aim.

The GWC at both amendment rates clearly had a positive effect on the growth of RCG, as it did in the pot trials. The GWC used alone and as a mixture with DWTR provided the highest mean average heights and dry yields throughout the trial at both plots. The DWTR had significant effects on the height of the plants at plot WH3 and the dry biomass produced at WH5 when compared to the unamended control soils. The preferential growing conditions for RCG in GWC when compared to DWTR were found to be potentially linked to the lower nutrient content and immobilisation of essential nutrients, especially P, in the DWTR. The difference in bulk densities of the plots and moisture content of the amendments did lead to variations in the amended soil nutrient concentrations.

Of the three RCG varieties the SWRF 5004 outperformed the SWRF 5032 and BS 5237 on both plots. This provides a clear indication of the scope for the development potential for variety selection of RCG when grown on contaminated land. As discussed in section 3.2.4 RCG is a very underdeveloped biofuel crop (despite outcompeting willow and miscanthus in previous studies on non-agricultural sites (Lord, 2015a)) and has not been developed at all for phytostabilisation of contaminated land. The species is clearly resistant to high concentrations of PTE and the other biogeochemical challenges presented on historic metal mine sites. Further development of the species is a key recommendation of this study.

The differences in growth rates between the two plots at the field trial suggested the physical characteristics of the mine soils potentially played more of a significant role in the inhibition of growth of the RCG plants than the presence of PTE. Plot WH3 produced plants that had an interquartile range approximately 21-23 cm whereas plot WH5 produced plants with an interquartile range of approximately 40-45cm. Plot WH3 also had a lower plant survival rate than plot WH5. Whilst the two plots shared similar PTE concentrations the moisture content and bulk density of the soils was quite different. Plot WH3 was also situated at a greater elevation to WH5 and was far more exposed to the wind and rain, to the extent that some of the plants were lost due to sheet flooding. Plot WH5 was positioned close to a mine adit that fed water to the plot throughout the year. This illustrates the difficulties of achieving true replicate field trials on mine sites, since the fundamental principle of homogeneity cannot be assumed.

The results of the biomass analyses did not follow the results found in previous chapters i.e. those in sections 6.4 and 7.4. In several cases the application of the organic waste amendments increased the concentrations of PTE in the biomass rather than decreased it. This might be expected from the results of the BCR procedure and the pot trial biomass analysis. This is best shown in the results of the bioaccumulation coefficient which tended to be greater for biomass grown in amended soils when compared to that grown in unamended soils. The results of the XCT analysis showed that, despite washing the samples, at least a considerable portion of the PTE (especially Pb) found in the analysis were present as dust on the plant rather than absorbed into the biomass, even after standard washing. Considering that the plants grown in the amended soils had more biomass, it is possible to conclude that a plant with greater biomass would have a greater surface area for contaminated dusts to settle. This potentially explains the counter intuitive results found in the biomass analysis. Heterogeneity of the mine soil may also explain this although there are several other factors which could also have influenced the results. Discrepancies such as this highlight the need for site-specific pot trials to be verified with field trials and vice versa if any firm conclusions are to be drawn from work in this field. Although there have been several laboratory incubation and glasshouse experiments that have examined the effects of amendments and RCG for the immobilization of PTE, a limited number of studies have examined their value under field conditions. Further work should be carried out to investigate this, as if RCG plants are not translocating metals into their biomass (even at the very high soil concentrations found in this study) then the case for the plant being selected for phytostabilisation approach in the remediation of contaminated land becomes much stronger. Regardless of the translocation of PTE is it clear that RCG, a marginal wetland species, can still tolerate the very challenging mine soil conditions presented at Whiteheaps. The areas used for the trials had remained unvegetated for over 30 years and were left with plants of above 1-meter-tall (especially on GWC amended soils – WH5). The two organic waste soil amendments had statistically significant effects on the dry mass production of RCG. This suggests that their use could replace the costs prohibitive alternatives whilst supporting a range of eco system services.

9.1 Future work and policy recommendations

- Further research on cultivar selection and genomic development of RCG.

- An increase in the establishment of field trials to support and verify published claims from laboratory or ‘pot’ trials in literature in regards to the interaction of both plants and soil amendments with PTE.

- Research into the geotechnical benefits of establishing RCG for phytostabilisation– i.e. quantifying the effect to which the plants prevent erosion of sediments in to water ways.

- Further work on the miniaturisation of the BCR sequential extraction procedure.

- Further research on the effects of organic waste soil amendments on the bioavailability of PTE and essential plant nutrients.

- Further work with policy makers and industry in crossing the gap between small scale research and the large scale implementation of growing second generation biofuel crops on land contaminated with PTE.

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10 Appendix

10.1 Results of soils analysis by Nothumbrian Water Ltd. at Whiteheaps historic mine site 2017

Table 51 showing PTE concentrations found by Northumbrian Water Ltd at White heaps July 2017

Sample site name	Cd mg/kg	Cu mg/kg	Pb mg/kg	Ni mg/kg	Zn mg/kg	Grid Ref
WHITE HEAPS 1	0.29	110	4400	67	120	NY 94963 46614
WHITE HEAPS 2	0.58	250	16000	30	270	NY 94944 46614
WHITE HEAPS 3	2	320	5600	14	970	NY 94938 46621
WHITE HEAPS BANK RESERVOIR	4.9	660	14000	52	1800	NY 94584 46575
WHITE HEAPS SLOPE DOWN MILL	2.5	570	19000	86	1100	NY 94861 46632
WHITE HEAPS WASHING FLOOR	2.6	580	5900	91	1400	NY 94844 46537
WHITE HEAPS MILL	3.9	300	8300	110	1400	NY 94753 46602

10.2 Photos of initial sample sites at Whiteheaps

Figure 84 Sample site WH1 NY945465

Figure 85 Sample site WH 2 NY945465

Figure 86 Sample site WH3 NY946465

Figure 87 Sample site WH4 NY947465

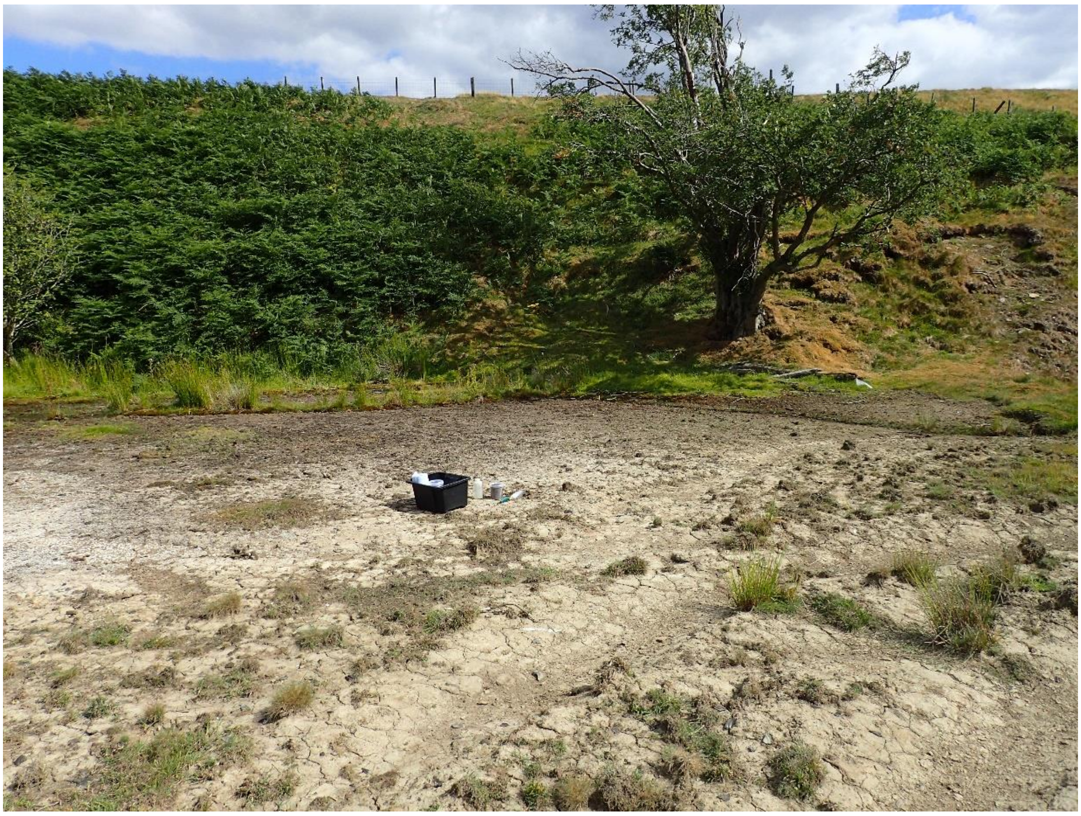

Figure 88 Sample site WH5 NY948465

Figure 89 Sample site WH6 NY949466

10.3 ANOVA results for sequential extraction procedure – Drinking water treatment residue

Table 52One-way ANOVA with a Bonferroni post hoc test at 0.05 significance level for PTE concentration (mg/kg; n = 3) in step 1 - 4 of the BCR sequential extraction scheme for pot trial soils amended with drinking water treatment residue at 30% (amendment as received weight equivalent/soil dry weight) compared to an unamended control and calculated concentrations. An l or m indicates that the result is significantly less or more than then respective results found in the amended soil

Element	Step	Unamended Soil	Calculated
Cd	Exchangeable	1.000	0.000m
	Reducible	0.000m	0.000m
	Oxidisable	0.000m	0.000l
	Residual	1.000	1.000
Cr	Exchangeable	1.000	1.000
	Reducible	1.000	1.000
	Oxidisable	1.000	1.000
	Residual	0.043m	1.000
Cu	Exchangeable	0.000l	0.000l
	Reducible	0.000l	0.183
	Oxidisable	0.653	0.000m
	Residual	1.000	1.000
Ni	Exchangeable	1.000	0.289
	Reducible	1.000	0.000l
	Oxidisable	1.000	1.000
	Residual	1.000	1.000
Pb	Exchangeable	0.000l	0.000l
	Reducible	0.000m	0.000l
	Oxidisable	0.160	0.009m
	Residual	1.000	1.000
Zn	Exchangeable	0.000l	0.000l
	Reducible	1.000	1.000
	Oxidisable	1.000	0.859
	Residual	1.000	1.000

10.4 ANOVA results for sequential extraction procedure – Green waste compost

Table 53One way ANOVA with a Bonferroni post hoc test at 0.05 significance level for PTE concentration (mg/kg ; n = 3) in step 1 - 4 of the BCR sequential extraction scheme for pot trial soils amended with green waste compos at 30% (amendment as received weight equivalent/soil dry weight) compared to an unamended control and calculated concentrations. An l or m indicates that the result is significantly less or more than then respective results found in the amended soil

.

Element	Step	Unamended Soil	Calculated
Cd	Exchangeable	0.000m	0.000m
	Reducible	0.000m	0.000m
	Oxidisable	1.000	1.000
	Residual	1.000	1.000
Cr	Exchangeable	1.000	1.000
	Reducible	1.000	1.000
	Oxidisable	1.000	1.000
	Residual	0.000m	0.000m
Cu	Exchangeable	0.000l	0.000l
	Reducible	0.000m	0.000m
	Oxidisable	0.000m	0.000m
	Residual	1.000	1.000
Ni	Exchangeable	1.000	0.169
	Reducible	1.000	1.000
	Oxidisable	1.000	1.000
	Residual	1.000	1.000
Pb	Exchangeable	0.000l	0.000l
	Reducible	0.000m	0.000m
	Oxidisable	1.000	1.000
	Residual	1.000	1.000
Zn	Exchangeable	0.000m	0.000m
	Reducible	0.000m	0.000m
	Oxidisable	1.000	1.000
	Residual	1.000	1.000

10.5 Botanical survey of Whiteheaps Mine by Dr Janet Simkin

Botanical survey of Whiteheaps Mine

by Dr Janet Simkin MCIEEM, for Northumbria Water

May 2019

1. Background

Whiteheaps Mine, near Ramshaw in County Durham, was established by the London Lead Company and worked for lead and silver from about 1715. The veins in this area are not rich in galena, not worth mining for the lead but with a high silver content that helped keep the mine profitable. This ceased in 1883 but in the 1920s and again from the 1950s until final closure in 1983 it was worked for fluorspar as part of the Whiteheaps-Sikehead group of mines. Since then it has been flattened and little remains of the mine structures. The main spoil heap has been landscaped and partially covered in places soil, presumably with a view to establishing grassland.

Calaminarian habitats are those associated with areas of heavy metal mines where the rock or ground is contaminated with phytotoxic levels of lead, zinc, cadmium, copper or another metal. The plants, lichens and fungi able to grow in these places are known as metallophytes. Some are species only found on contaminated substrates, and these are of particular conservation interest. In the North Pennines the most characteristic of these are the plants spring sandwort (*Minuartia verna*), alpine penny-cress (*Noccaea caerulescens*), and the local form of mountain pansy (*Viola lutea*). Others are ecotypes of species that can also be found elsewhere, such as thrift (*Armeria maritima*) and pyrenean scurvy-grass (*Cochlearia pyrenaica*). Mechanisms of heavy metal tolerance or avoidance vary, as does the degree of tolerance of the different metals, making these calaminarian plant communities very variable. Bryophytes and lichens are often important on highly contaminated ground.

2. Objectives

Northumbrian Water are involved with a Strathclyde University research project involving phytoremediation on an old abandoned metals mine site. A field trial is to be set up at Whiteheaps Mine, consisting of two 9x9m experimental plots into which reed canary-grass (*Phalaris arundinacea*) is to be planted.

The plots are shown as WH3 and WH5 on the map provided, and prior to the survey they had been marked out on the ground by wooden pegs. Each is to be fenced to exclude rabbits, so the effective area is 12 x 12m.

The main objective of this survey was to assess those two plots and the surrounding area for botanical interest, on this site likely to be calaminarian grassland species, in case there was anything of conservation interest that might be impacted by the experiment. The rest of the mine site was also assessed briefly to set those findings in context.

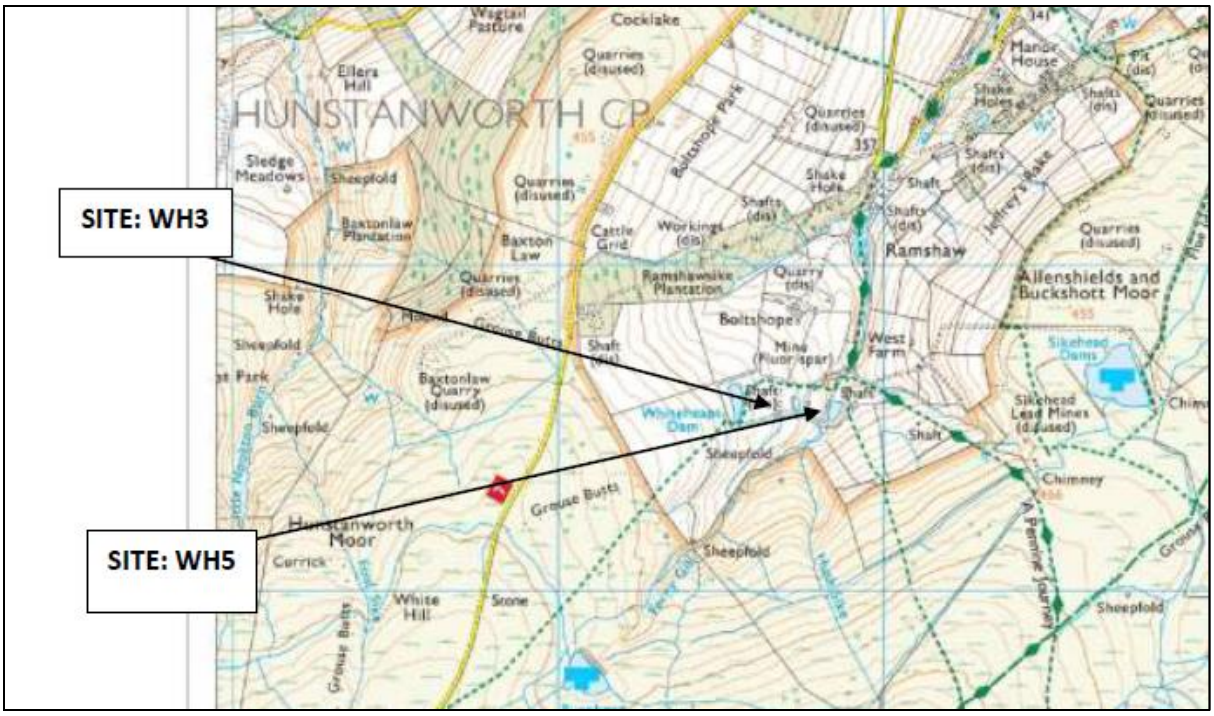

Figure 1. Location of the mine and experimental plots.

3. Results

The site was visited twice, on 20th and 23rd May 2019.

The landscaped area was found to have no calaminarian grassland or other vegetation characteristic of metal-contaminated ground, apart from occasional small patches of pale thread-moss (*Bryum pallens*, Figure 6) and a dog lichen (*Peltigera neckeri*, Figure 7). These are both lead and zinc tolerant and are often found on damp ground on mines in the North Pennines. Here they may be remnants of a more widespread calaminarian grassland community that has otherwise been lost, but soil testing has suggested that metal levels are high, in places at least, so the loss may be due to the nature of the fluorite mine spoil and the landscaping since the mine closed.

The absence of metallophytes is typical of sites that have been mined or reprocessed for fluorite. Sometimes they survive in sheltered nooks and crannies that have escaped the reprocessing and landscaping, but here the landscaping has been thorough and nothing was found. The most interesting area was found to be the remains of an old wall crossing the spoil heap where there was a greater variety of plants, lichens and bryophytes than elsewhere, but nothing of conservation importance.

Plot WH5

Within the plots no botanical interest was found. However, there is a wet flush between the plot and the small pool behind it (Figures 3-5) which has sedges (*Carex* sp), not identifiable at this time of year, hare-tail cotton-grass (*Eriophorum vaginatum*), and toad rush (*Juncus bufonius*). Bryophytes include *Bryum pallens*, *B. pseudotriquetrum*, *Philonotis fontanum* and a variety of other mosses and liverworts. A drier hummock has *Peltigera neckeri* in the grass. This flush appears to be fed by water from an old washing floor and so is likely to have high metal levels, and yet there are no metallophyte plants, not even pyrenean scurvy-grass (*Cochleria pyrenaica*) which favours such sites. Trampling of this wet ground should be avoided if possible, but the conservation interest does not warrant any special measures or moving the experimental plot.

Drier ground nearby has grassland with sheeps fescue (*Festuca ovina*), common bent (Agrostis capillaris), glaucous sedge (*Carex flacca*), field woodrush (*Luzula campestris*), heath bedstraw (*Galium saxile*), wood sorrel (Oxalis acetosella), wood sage (*Teucrium scorodonia*), and bird's-foot trefoil (*Lotus corniculatus*).

Plot WH3

This is on higher ground close to the old engine house. It appears to have been disturbed and compacted by bikes or vehicles, and more recently by rabbits. It may be seasonally wet but at the time of this visit the fine mineral substrate was very dry, with small stones and slag fragments sorted to the surface.

Vegetation within the plot is very sparse and of no interest, presumably because of the disturbance and compaction. These conditions are unlikely to be suitable for reed canary grass. The only plants are tussocks of common bent (*Agrostis capillaris*), Yorkshire fog (*Holcus lanatus*), sheeps fescue (*Festuca ovina),* tussock grass (*Deschampsia cespitosa*) and mat grass (*Nardus stricta*), all heavily grazed by rabbits, with the moss *Weissia controversa* between. The edge of the bare ground also has mouse-ear (Cerastium fontanum), self-heal (*Prunella vulgaris*), and the mosses *Bryum pallens* and *Brachythecium albicans*. Dry banks nearby are grassed over with more plants, including lesser burdock (Arctium minus), nettles (*Urtica dioica*) and, rather surprisingly, daffodils!

Figure 2. Plot WH3 on high ground. The grassland is very sparse and the spoil at this time was compacted and very dry.

Figure 3. Plot WH5 on the valley floor, viewed from the hillside above. The wet flush can be seen beyond the trial plot, with the small pool in the remains of the washing floor to the left.

Figure 4. The pool in the remains of the washing floor, close to plot WH5.

Figure 5. Wet ground between plot WH5 and the pool. Small clumps of *Bryum pallens* show red, and the *Peltigera neckeri* is on the grassy patch in the foreground.

Figure 6. Pale thread-moss *Bryum pallens*.

Figure 7. The dog lichen *Peltigera neckeri*.

4. Plant and lichens species recorded

Plants

Agrostis capillaris

Agrostis stolonifera

Anthoxanthum odoratum

Arctium minus

Bellis perennis

Cardamine pratensis

Carex flacca

Carex sp.

Cerastium fontanum

Cirsium arvense

Cirsium palustre

Cirsium vulgare

Crataegus monogyna

Deschampsia cespitosa

Epilobium brunnescens

Eriophorum vaginatum

Festuca ovina

Galium saxatile

Holcus lanatus

Juncus bufonius

Juncus effusus

Lotus corniculatus

Luzula campestris

Nardus stricta

Oxalis acetosella

Prunella vulgaris

Ranunculus repens

Rumex acetosa

Sagina procumbens

Succisa pratensis

Taraxacum vulgare

Teucrium scorodonia

Ulex europeus

Urtica dioica

Veronica persica

Bryophytes

Brachythecium albicans

Bryum pallens

Bryum pseudotriquetrum

Pellia epiphylla

Philonotis fontanum

Pseudoscleropodium purum

Rhytidiadelphus Squarrosus

Weissia controversa

Lichens

Cladonia furcata

Lecanora polytropa

Micarea lignaria

Peltigera hymenina

Peltigera neckeri

Peltigera rufescens

Peltigera membranacea

Porpidia macrocarpa

Porpidia tuberculosa

Rhizocarpon reductum

Trapelia coarctata

Trapelia placodioides