Department of Civil and Environmental Engineering
University of Strathclyde

Biochar for assisted phytomanagement of contaminated soils – short term effects on contaminant availability and plant growth

A Thesis presented for the Degree of Doctor of Philosophy
by
Aoife Brennan

April 2014

Abstract

There has been much interest in using biochar as a low cost sorbent amendment to reduce the risk posed by contaminated sites but an understanding of biochar interactions with plants in a contaminated soil context is still in its early stages. This thesis is based on the overall hypothesis that biochar amendment would improve soil health and plant growth in addition to reducing the availability of organic and inorganic contaminants.

Biochars from three different feedstocks (maize stover, olive tree pruning and pine woodchip) and coal-derived activated carbon were used in experimental studies designed to test the thesis hypothesis. Soils contaminated with copper and arsenic (Chapters 2 and 4), mercury (Chapter 3), and polycyclic aromatic hydrocarbons (Chapters 4 and 5) were used in experiments designed to investigate the different effects biochar amendment had on specific contaminant behaviour and mobility in soils.

Innovative passive sampling techniques were used to monitor changes in freely dissolved concentrations of the contaminants studied. Rhizon samplers extracted porewater from soil for selected inorganic contaminant analysis while polyoxymethylene (POM) samplers were used in laboratory equilibrium tests to determine freely dissolved concentrations of organic contaminants in soil.

Biochars consistently reduced plant uptake (in plant species maize and Italian ryegrass) for both organic and inorganic contaminants. Biochars had a generally beneficial effect on plant growth. Freely dissolved concentrations were reduced for inorganic contaminants copper and arsenic (with the exception of arsenic with olive tree pruning in Chapter 2). Limited to no effects on porewater concentrations were

observed for mercury (Chapter 3) and organic contaminants. Activated carbon was more effective at removing organic contaminants from porewater than biochar.

By defining the conditions in which sorbent amended soils successfully reduced contaminant bioavailability and improved plant growth, this thesis demonstrates how biochar may prove a valuable tool in the phytomanagement of contaminated soils.

Table of Contents

Declaration

This thesis is the result of the original research conducted by Aoife Brennan. Where others have contributed or other sources are quoted, full acknowledgement has been made. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed

Date

Publications and conference presentations arising from thesis

The following papers have been published or are under review:

Brennan, A., Moreno Jiménez, E., Puschenreiter, M., Albuquerque, J., Switzer, C. 2014. Effect of biochar amendment on root structure and contaminant availability of maize plants in a copper and arsenic impacted soil. Plant and Soil, 1-10. doi: 10.1007/s11104-014-2074-0

Brennan, A. Moreno Jiménez, E., Albuquerque, J., Knapp, C.W., Switzer, C. 2014. Effects of biochar and activated carbon amendment on maize growth and the uptake and measured availability of polycyclic aromatic hydrocarbons (PAHs) and potentially toxic elements (PTEs). Under review in Environmental Pollution, submitted March 2014.

The following paper will be submitted to an appropriate journal:

Brennan, A., Knapp, C.W., Switzer, C. 2014. Assessing the effects of carbonaceous sorbent amendment on *Lolium multiflorum* growth and the uptake and measured availability of polycyclic aromatic hydrocarbons (PAHs)

Conference contributions:

Oral:

Brennan, A., Knapp, C.W., Switzer, C. 2014. Effect of carbonaceous sorbent amendment on observed plant growth and predicted bioavailability in a multi-pollutant contaminated soil, SETAC Europe Annual Meeting 2014

Brennan, A., Switzer, C. Biochar and the remediation of soil micropollutants *Geophysical Research Abstracts* Vol. 14, EGU2012-781, 2012 EGU General Assembly 2012 http://meetingorganizer.copernicus.org/EGU2012/EGU2012-781.pdf

Poster:

Brennan, A., Moreno Jiménez, E., Switzer, C. 2013 Effect of biochar amendment on mercury soil pore water concentrations in soil contaminated by mercury mining activities, International Conference on Mercury as a Global Pollutant 2013 (poster)

Brennan, A., Moreno Jiménez, E., Switzer, C. 2013 Effect of carbonaceous sorbent amendment on observed plant growth and predicted bioavailability in a multi-pollutant contaminated soil, SETAC Europe Annual Meeting 2013 (poster)

Other publications by author:

Brennan, A., Moreno Jimenez, E., 2014. Advances in field soil sampling: obtaining relevant *in situ* data for the environmental management of contaminated soils, Contaminated Soils: A Guide to Sampling and Analysis. Future Science Ltd, pp. 18-30.

Acknowledgements

Firstly, thanks to my supervisors Drs Christine Switzer and Charles Knapp, for the opportunity to embark on a course of research at Strathclyde and who have supported me during my time here. Thanks to the Faculty of Engineering, for providing my doctoral scholarship.

Very special thanks must go to Dr Eduardo Moreno Jiménez, who has been a constant source of inspiration and encouragement and was always available whenever crisis struck.

Thanks to all my colleagues at Strathclyde for their friendship and support over the last few years. Special mentions go to: Mara, for making my final year a much more pleasant experience thanks to her efficiency and general helpfulness; Andy Robson, for his help with the contaminated soil episode; Andy Pape, for being a source of knowledge and sensible thinking; Lisa, for being willing to help me understand analytical instruments in those early months.

I was fortunate to have the opportunity to develop collaborations with research groups overseas over the course of my PhD research. A European Cooperation in Science and Technology (COST) grant for Action TD1107, *Biochar as an Option for Sustainable Resource Management*, enabled me to work with Dr Moreno at the Universidad Autónoma de Madrid for three months in the winter of 2012. I am grateful to Mariana, Rebeca, Teresa and Carlos for being such good company during the long hours in the lab and for being patient with my rusty Spanish.

A second research stay abroad, funded by the Strathclyde/EPSRC International Exchange Program, was spent at the University of Maryland Baltimore County in the group of Prof Upal Ghosh in March 2013. I am especially grateful to Dr José Gomez-

Eyles. Thanks to him, I learned a great deal about optimising GC-MS methods which I was able to apply to my own research. Prof Ghosh and Dr Gomez-Eyles both went out of their way to make me feel welcome in their department and ensure my short stay went as smoothly as possible. Thanks to Huan, Carmen, Natasha and James, who also helped make my stay a pleasant experience.

Thanks to the EU COST Action TD1107 for funding my place at a European Biochar Research Network Summer School in September 2013. It was a unique opportunity to meet my peers in the biochar research community and attend workshops given by experts in the field in a relaxed and informal atmosphere.

Last, but by no means least, thanks to Mum and Dad, and to Andy Hodge.

List of Figures and Tables

Chapter 2

Chapter 3

Chapter 4

1

There has been much interest in using biochar as a sorbent amendment to reduce the risk posed by contaminated sites, at a lower cost than traditional ex-situ treatments, but an understanding of biochar interactions with plants in a contaminated soil context is still largely unknown. This thesis presents experimental work that was carried out in order to further understand biochar-plant-contaminated soil interactions in the short term. In this chapter, an overview of contamination issues and the challenges of remediation and contaminant risk assessment is given (Section 1.2). Biochar research and alternative soil amendments are briefly reviewed (Section 1.3). A brief discussion follows for each of the key elements that influenced the research presented in this thesis: sorption; bioavailability and the methods used for its determination; and phytostabilisation (Section 1.4). Finally, each of the experimental chapters is introduced (Section 1.5).

1.1 Context

Contamination of soils, sediments and waters due to anthropogenic activities in industry, agriculture and urban development is a globally significant problem and is often costly and time consuming to remediate. This reality seriously challenges the global community's declared ambition, as set out in Millennium Development Goal target 7, to ensure sustainable development, i.e. "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland, 1987). To give just one example of the scale of the problem, a recent assessment of contamination across Europe as part of the EIONET-SOIL (European Environment Information and Observation Network for soil) study

estimated a total cost of 6.5 billion euros for remediation efforts on sites across the EU (Panagos et al., 2013). The same study estimated that 2.5 million sites were "potentially contaminated" and 342,000 sites were "contaminated". Traditional soil remediation strategies tend to be aggressive techniques involving *ex-situ* chemical or heat treatments, focused on removing the total concentration of contaminants at a value which exceeds recommended national guidelines. For where this approach may be economically unfeasible, but contaminant concentrations may still pose risk, less invasive *in situ* techniques which focus on reducing the biologically available fraction of a given contaminant are often more appropriate.

National regulatory agencies such as the Environment Agency in the UK or international agencies such as the World Health Organization (WHO) are predominantly concerned with risk to humans. However, European level biodiversity plans such as Natura 2000, international environmental protection agreements such as Convention on Biological Diversity and conservation bodies such as Natural England and Scottish Natural Heritage have endowed a certain official level of concern over risk to other organisms, giving a real world relevance to research assessing bioavailability of contaminants in soil environments.

Many contaminated sites are a result of our historic legacy and rather than pushing development in pristine sites, it is our responsibility develop these brownfield sites or restore them to green spaces. Development on brownfield sites can take many forms, from car parks to housing. This thesis is, however, set against the backdrop of an alternative proposal, one which explores plant establishment. From the possibility of using brownfield sites for bioenergy production to restoration of these sites to green space for community regeneration, phytomanagement of brownfield sites is increasingly being researched (Pediaditi et al., 2010; Smith et al., 2013;

Nsanganwimana et al., 2014). Balancing the necessity for food security with the need for bioenergy for meeting CO2 reduction targets and reducing fossil fuel dependence makes re-utilisation of brownfield sites for this purpose an attractive option. Restoring plant cover on brownfield sites where bioenergy crops are not an option (whether through economic or practical feasibility issues) could potentially contribute to reductions of atmospheric CO2 (Manning and Renforth, 2012).

The development of a risk based approach to contaminated land assessment has shifted focus from assessing total concentrations to level of risk posed by exposure to these concentrations. The source-pathway-receptor model drives this risk-based approach. Nonetheless, national soil guideline values for acceptable levels of total contaminants are in place and for some countries vary according to land use, i.e. pristine and agricultural areas, residential areas, industrial areas (Carlon, 2007). In the UK, values are also dependant on contaminant physico-chemical properties, soil organic matter content and soil type. Most importantly, classification in the UK is highly dependent on the source-pathway-receptor model whereby receptor exposure (e.g. children below 3 years old) drives remediation targets (Environment Agency, 2004). Consequently, there is no globally applicable, universal value for each contaminant at which any given site can be classed as "contaminated" and a certain level of background knowledge and interpretation is required where contamination is concerned.

With regards to the soils used in this thesis, total contaminant concentrations exceeded all consulted guidelines for residential, allotment and natural areas (Carlon, 2007), but not necessarily those for industrial areas. No guidelines exist for soil porewater values and values derived for drinking waters (ground and surface waters) are not necessarily the most appropriate yardstick to measure against. Nonetheless, they provide an indication of desired aqueous concentrations. To give some examples relevant to this

thesis, maximum contaminant levels (MCLs) for drinking water set down by the US Environmental Protection Agency (USEPA) are 1.3 mg L^{-1} for copper, 10 μg L^{-1} for arsenic, 2 μg L^{-1} for inorganic mercury, 0.2 μg L^{-1} for benzo(a)pyrene (USEPA, 2009).

1.2 Why biochar?

Biochar is defined as charcoal produced by biomass (preferably waste) under low oxygen conditions. The use of waste materials or by-products is preferable due to pressures on virgin biomass resources and can range from non-treated wood wastes from forestry activities to sewage sludge or poultry litter. Biochar can be produced at small or large scale, under fast or slow pyrolysis conditions and at temperatures ranging from 300°C to 800°C (Joseph and Taylor, 2014). This wide variety of feedstocks and production conditions means that biochars can differ greatly in their properties (Zhao et al., 2013; Joseph and Taylor, 2014), leading to variety of effects on soil, as observed in the literature. However, the potential for biochar to sorb both inorganic and organic contaminants warrants further investigation for its use as a soil amendment, due to its similarities to black carbon and its potential as a cheaper alternative to activated carbon (Section 1.3).

Interest in biochar as a soil amendment had its roots in the increasing awareness of the anthropogenic origins of Terra Preta soil in the Brazilian Amazon (highly fertile, high organic matter soils)(Glaser and Birk, 2012). With the concern over climate change and increased CO_2 levels in the atmosphere at a peak (IPCC, 2007), the concept of "new Terra Preta" has gained ground in the international scientific community. In this scenario, biochar amended soils would improve soil fertility and provide a carbon sink, with greatest effects in soils naturally low in organic matter. Biochar research in an agricultural context is driven by an attempt to understand the effects of biochar amendment on soil and soil biota (Sohi et al., 2010; Lehmann et al., 2011). From the

climate change perspective, biochar's potential for long term CO_2 and N_2O abatement is being investigated (Lehmann, 2007; Gaunt and Lehmann, 2008; Lehmann and Joseph, 2009; Downie et al., 2011). This dual role of crop productivity improvements and climate change mitigation is also applicable to biochar use in brownfield/contaminated soils, and provides incentive for use of biochar under such circumstances.

Within the context of soil remediation, the use of soil amendments to enhance plant establishment and reduce contaminant risk represents an important avenue for research. The state of the science for biochar use in contaminated land is much less developed than compost or fly ash (a quick keyword search in www.sciencedirect.com of "biochar" "contaminated" "land" vs. "compost" "contaminated" "land" and "fly ash" "contaminated" "land" yielded hits of 268, 3288 and 3149 research/review articles respectively). Green waste compost and fly ash have been heavily investigated (Farrell and Jones, 2009; Lopareva-Pohu et al., 2011; Park et al., 2011; Pourrut et al., 2011; Clemente et al., 2012). Both of these amendments have clear potential for improving soil quality and have demonstrated efficacy in long term field trials (Ram et al., 2006; Lopareva-Pohu et al., 2011; Clemente et al., 2012). One clear advantage posed by compost use is its wide availability as a result of municipal compost production schemes set up to meet waste reduction targets (Farrell and Jones, 2009). In addition, clear guidelines are in place for compost standards (BSI PAS 100). Due to its widespread availability, compost costs are negligible. Fly ash is a by-product of coal combustion and produced in large quantities on a global scale; it is therefore inexpensive and widely available, given that estimated use of fly ash residues worldwide is 25% (Ram and Masto, 2014). However, these amendments have thus far shown best results for inorganic contaminants.

Despite comprehensive reviews of the potential role of biochar in the remediation of contaminated soils (Beesley et al., 2011; Gomez-Eyles et al., 2013), many questions remain over its application, particularly with regards to biochar-plant-contaminant interactions. As such, it was decided to make experiments designed to explore the possibility of biochar application to contaminated soils the main focus of this thesis. The following section highlights the three main themes that underpin the experiments in this thesis.

1.3 Sorption, bioavailability, phytostabilisation

As a natural sorbent, the remediation of contaminants may represent an important biochar application (Beesley et al., 2011; Gomez-Eyles et al., 2013; Ahmad et al., 2014). Once the role of organic matter in controlling contaminant availability was understood to a greater extent, particularly for organic contaminants, it became clear that sorption of environmental contaminants to carbonaceous sorbents is an important sink for environmental contaminants (Luthy et al., 1997; Ghosh et al., 2003; Cornelissen et al., 2005; Zhu and Pignatello, 2005; Cornelissen et al., 2006). More recently, focus has shifted to how anthropogenically-produced activated carbons and biochars can mimic the behaviour of black carbon and thus reduce the detrimental impact of both organic and inorganic environmental contaminants on biota. This is achieved by reducing contaminant concentrations in the freely dissolved phase, predominantly in sediments (Beckingham and Ghosh; Zimmerman et al., 2004; Cornelissen et al., 2008; Ghosh et al., 2011), but also increasingly in soils as an amendment (Cao et al., 2009; Beesley et al., 2010; Karami et al., 2011; Denyes et al., 2012; Hale et al., 2012).

Research has moved towards assessment of bioavailability following the recognition that not all forms of a contaminant were available for organism uptake or degradation and that remediation targets that require removal of total concentrations are

excessively conservative in terms of actual risk posed, as well as being time-consuming and costly. This move follows a greater understanding of the role of organic matter in controlling contaminant availability as discussed in the previous paragraph (Pignatello and Xing, 1995; Luthy et al., 1997; Alexander, 2000; Sauvé et al., 2000). Put simply, biological availability, or bioavailability, refers to the fraction of a contaminant that is available for uptake by a given organism (Semple et al., 2004). Freely dissolved concentrations are increasingly used as an analogue for bioavailability of contaminants in soils. Passive samplers such as rhizon samplers for inorganic contaminants (Beesley and Dickinson, 2011; Moreno-Jiménez et al., 2011a) and polyoxymethylene samplers for organic contaminants (Jonker and Koelmans, 2001; Cornelissen et al., 2009; Gomez-Eyles et al., 2011) are frequently employed for measuring these freely dissolved concentrations. Both techniques are relatively cost effective and adaptable, suitable for both field and laboratory applications. Other bioavailability assessment techniques such as cyclodextrin extractions (Reid et al., 2000; Hickman et al., 2008; Beesley et al., 2010) and passive sampling techniques such as diffusive gradients in thin films (DGT) for inorganic contaminants (Degryse et al., 2009) and solid phase micro-extraction (SPME) or silicon for organic contaminants (Mayer et al., 2011; Cui et al., 2013) are also widely used in research, but were not used during the course of this PhD due to considerations over cost and ease of deployment for monitoring purposes and are therefore not discussed further.

Phytoremediation is an attractive option for restoring degrading soils, particularly in the form of phytostabilisation, which aims to restore soil structure and reduce soil erosion (Schnoor et al., 1995). Natural attenuation through the re-establishment of plant growth and encouraging natural succession is increasingly being employed as a low impact *in situ* strategy (Kidd et al., 2009; Onwubuya et al., 2009; Moreno-Jiménez et al., 2011b). As discussed in section 1.1, using brownfield sites to grow biomass crops

for bioenergy involves simultaneous restoration of a degraded site, production of an energy source and development of an income stream and therefore holds definite appeal in the modern economy, in addition to redirecting the growth of these crops from agricultural land (Conesa et al., 2012; Witters et al., 2012a; Witters et al., 2012b; Van Slycken et al., 2013). The use of soil amendments such as compost, waste residues and activated carbons and biochars has been shown in a number of studies to assist plant establishment and occasionally reduce contaminant availability and uptake, but this is contaminant-dependent (Hilber et al., 2009; Beesley and Dickinson, 2011; Karami et al., 2011; Clemente et al., 2012; Jakob et al., 2012). What's more, only a few studies look at the effects of plants and carbonaceous sorbent amendments in multi-contaminated soils (Jakob et al., 2012; Khan et al., 2013, Waqas et al.,2014).

1.4 Thesis outline

The current research aims to address specific knowledge gaps in biochar interactions with contaminated soils and plants, using field contaminated soils where possible.

This chapter has described the context for the research undertaken in the thesis. Chapters 2-5 each take the form of an independent paper which has been prepared for journal publication and as such, each chapter can stand on its own as a piece of research. Chapter 2 investigates specific root traits and assesses the effect of biochar amendment on contaminant availability. Chapter 3 is a preliminary study on the effects of biochar amendment on mercury mobility and seed germination success. Chapter 4 presents data from controlled growth chamber plant experiments that were set up to grow maize in soil contaminated with polycyclic aromatic hydrocarbons (PAHs) and metals. Chapter 5 presents data from outdoor weather-exposed pot experiments using PAH contaminated soil to grow Italian ryegrass. Through investigation of the effects of biochar amendment under different conditions and for different types of

contamination, these chapters were designed to answer the question of whether or not biochar improves plant growth in early development and reduces contaminant availability. A final chapter summarises the findings and conclusions and highlights opportunities for further research.

1.5 References

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S., 2014. Biochar as a sorbent for contaminant management in soil and water: A review. Chemosphere 99, 19-33.

Alexander, M., 2000. Aging, Bioavailability, and Overestimation of Risk from Environmental Pollutants. Environmental Science & Technology 34, 4259-4265.

Beckingham, B., Ghosh, U., Polyoxymethylene passive samplers to monitor changes in bioavailability and flux of PCBs after activated carbon amendment to sediment in the field. Chemosphere.

Beesley, L., Dickinson, N., 2011. Carbon and trace element fluxes in the pore water of an urban soil following greenwaste compost, woody and biochar amendments, inoculated with the earthworm Lumbricus terrestris. Soil Biology and Biochemistry 43, 188-196.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution 158, 2282-2287.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B., Sizmur, T., 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environmental Pollution 159, 3269-3282.

Brundtland, G. (1987). Our Common Future - Report of the World Commission on Environment and Development. Technical report. World Commission on Environment

and Development. Published as Annex to General Assembly Document A/42/427, Development and International Co-operation: Environment.

http://www.un-documents.net/wced-ocf.htm (cited 5th June 2014).

Cao, X., Ma, L., Gao, B., Harris, W., 2009. Dairy-Manure Derived Biochar Effectively Sorbs Lead and Atrazine. Environmental Science & Technology 43, 3285-3291.

Carlon, C.E., 2007. Derivation methods of soil screening values in Europe. A review and evaluation of national procedures towards harmonization. European Commission, Joint Research Centre, Ispra, p. 306.

Clemente, R., Walker, D.J., Pardo, T., Martínez-Fernández, D., Bernal, M.P., 2012. The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions. Journal of Hazardous Materials 223–224, 63-71.

Conesa, H.M., Evangelou, M.W.H., Robinson, B.H., Schulin, R., 2012. A Critical View of Current State of Phytotechnologies to Remediate Soils: Still a Promising Tool? The Scientific World Journal 2012, 10.

Cornelissen, G., Breedveld, G.D., Kalaitzidis, S., Christanis, K., Kibsgaard, A., Oen, A.M.P., 2006. Strong Sorption of Native PAHs to Pyrogenic and Unburned Carbonaceous Geosorbents in Sediments. Environmental Science & Technology 40, 1197-1203.

Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., van Noort, P.C.M., 2005. Extensive Sorption of Organic Compounds to Black Carbon, Coal, and Kerogen in Sediments and Soils: Mechanisms and Consequences for Distribution, Bioaccumulation, and Biodegradation. Environmental Science & Technology 39, 6881-6895.

Cornelissen, G., Okkenhaug, G., Breedveld, G.D., Sørlie, J.-E., 2009. Transport of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in a landfill: A novel

equilibrium passive sampler to determine free and total dissolved concentrations in leachate water. Journal of Hydrology 369, 253-259.

Cornelissen, G., Pettersen, A., Broman, D., Mayer, P., Breedveld, G.D., 2008. Field testing of equilibrium passive samplers to determine freely dissolved native polycyclic aromatic hydrocarbon concentrations. Environmental Toxicology and Chemistry 27, 499-508.

Cui, X., Mayer, P., Gan, J., 2013. Methods to assess bioavailability of hydrophobic organic contaminants: Principles, operations, and limitations. Environmental Pollution 172, 223-234.

Degryse, F., Smolders, E., Zhang, H., Davison, W., 2009. Predicting availability of mineral elements to plants with the DGT technique: a review of experimental data and interpretation by modelling. Environmental Chemistry 6, 198-218.

Denyes, M.J., Langlois, V.S., Rutter, A., Zeeb, B.A., 2012. The use of biochar to reduce soil PCB bioavailability to Cucurbita pepo and Eisenia fetida. Science of The Total Environment 437, 76-82.

Downie, A., Munroe, P., Cowie, A., Van Zwieten, L., Lau, D.M.S., 2011. Biochar as a Geoengineering Climate Solution: Hazard Identification and Risk Management. Critical Reviews in Environmental Science and Technology 42, 225-250.

Environment Agency, 2004. Model Procedures for the Management of Land Contamination- CLR11- full report. p. 203.

Farrell, M., Jones, D.L., 2009. Critical evaluation of municipal solid waste composting and potential compost markets. Bioresource Technology 100, 4301-4310.

Gaunt, J.L., Lehmann, J., 2008. Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production. Environmental Science & Technology 42, 4152-4158.

Ghosh, U., Luthy, R.G., Cornelissen, G., Werner, D., Menzie, C.A., 2011. In-situ Sorbent Amendments: A New Direction in Contaminated Sediment Management. Environmental Science & Technology 45, 1163-1168.

Ghosh, U., Zimmerman, J.R., Luthy, R.G., 2003. PCB and PAH Speciation among Particle Types in Contaminated Harbor Sediments and Effects on PAH Bioavailability. Environmental Science & Technology 37, 2209-2217.

Glaser, B., Birk, J.J., 2012. State of the scientific knowledge on properties and genesis of Anthropogenic Dark Earths in Central Amazonia (terra preta de Índio). Geochimica et Cosmochimica Acta 82, 39-51.

Gomez-Eyles, J.L., Beesley, L., Moreno-Jimenez, E., Ghosh, U., Sizmur, T., 2013. The potential of biochar amendments to remediate contaminated soils. in: Ladygina, N., Rinea, F. (Eds.). Biochar and Soil Biota. CRC Press, p. 278.

Gomez-Eyles, J.L., Jonker, M.T.O., Hodson, M.E., Collins, C.D., 2011. Passive Samplers Provide a Better Prediction of PAH Bioaccumulation in Earthworms and Plant Roots than Exhaustive, Mild Solvent, and Cyclodextrin Extractions. Environmental Science & Technology 46, 962-969.

Hale, S.E., Elmquist, M., Brändli, R., Hartnik, T., Jakob, L., Henriksen, T., Werner, D., Cornelissen, G., 2012. Activated carbon amendment to sequester PAHs in contaminated soil: A lysimeter field trial. Chemosphere 87, 177-184.

Hickman, Z.A., Swindell, A.L., Allan, I.J., Rhodes, A.H., Hare, R., Semple, K.T., Reid, B.J., 2008. Assessing biodegradation potential of PAHs in complex multi-contaminant matrices. Environmental Pollution 156, 1041-1045.

Hilber, I., Wyss, G.S., Mäder, P., Bucheli, T.D., Meier, I., Vogt, L., Schulin, R., 2009. Influence of activated charcoal amendment to contaminated soil on dieldrin and nutrient uptake by cucumbers. Environmental Pollution 157, 2224-2230.

IPCC, 2007. Climate Change 2007: The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. in: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Cambridge University Press.

Jakob, L., Hartnik, T., Henriksen, T., Elmquist, M., Brändli, R.C., Hale, S.E., Cornelissen, G., 2012. PAH-sequestration capacity of granular and powder activated carbon amendments in soil, and their effects on earthworms and plants. Chemosphere 88, 699-705.

Jonker, M.T.O., Koelmans, A.A., 2001. Polyoxymethylene Solid Phase Extraction as a Partitioning Method for Hydrophobic Organic Chemicals in Sediment and Soot. Environmental Science & Technology 35, 3742-3748.

Joseph, S., Taylor, P., 2014. 16 - The production and application of biochar in soils. in: Waldron, K. (Ed.). Advances in Biorefineries. Woodhead Publishing, pp. 525-555.

Karami, N., Clemente, R., Moreno-Jiménez, E., Lepp, N.W., Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. Journal of Hazardous Materials 191, 41-48.

Khan, S., Wang, N., Reid, B.J., Freddo, A., Cai, C., 2013. Reduced bioaccumulation of PAHs by Lactuca satuva L. grown in contaminated soil amended with sewage sludge and sewage sludge derived biochar. Environmental Pollution 175, 64-68.

Kidd, P., Barceló, J., Bernal, M.P., Navari-Izzo, F., Poschenrieder, C., Shilev, S., Clemente, R., Monterroso, C., 2009. Trace element behaviour at the root–soil interface: Implications in phytoremediation. Environmental and Experimental Botany 67, 243-259.

Lehmann, J., 2007. A handful of carbon. Nature 447, 143-144.

Lehmann, J., Joseph, S., 2009. Biochar for environmental management: science and technology. Earthscan.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biology and Biochemistry 43, 1812-1836.

Lopareva-Pohu, A., Pourrut, B., Waterlot, C., Garçon, G., Bidar, G., Pruvot, C., Shirali, P., Douay, F., 2011. Assessment of fly ash-aided phytostabilisation of highly contaminated soils after an 8-year field trial: Part 1. Influence on soil parameters and metal extractability. Science of The Total Environment 409, 647-654.

Luthy, R.G., Aiken, G.R., Brusseau, M.L., Cunningham, S.D., Gschwend, P.M., Pignatello, J.J., Reinhard, M., Traina, S.J., Weber, W.J., Westall, J.C., 1997. Sequestration of Hydrophobic Organic Contaminants by Geosorbents. Environmental Science & Technology 31, 3341-3347.

Manning, D.A.C., Renforth, P., 2012. Passive Sequestration of Atmospheric CO2 through Coupled Plant-Mineral Reactions in Urban soils. Environmental Science & Technology 47, 135-141.

Mayer, P., Olsen, J.L., Gouliarmou, V., Hasinger, M., Kendler, R., Loibner, A.P., 2011. A Contaminant Trap as a Tool for Isolating and Measuring the Desorption Resistant Fraction of Soil Pollutants. Environmental Science & Technology 45, 2932-2937.

Moreno-Jiménez, E., Beesley, L., Lepp, N.W., Dickinson, N.M., Hartley, W., Clemente, R., 2011a. Field sampling of soil pore water to evaluate trace element mobility and associated environmental risk. Environmental Pollution 159, 3078-3085.

Moreno-Jiménez, E., Vázquez, S., Carpena-Ruiz, R.O., Esteban, E., Peñalosa, J.M., 2011b. Using Mediterranean shrubs for the phytoremediation of a soil impacted by pyritic wastes in Southern Spain: A field experiment. Journal of Environmental Management 92, 1584-1590.

Nsanganwimana, F., Pourrut, B., Mench, M., Douay, F., 2014. Suitability of Miscanthus species for managing inorganic and organic contaminated land and restoring ecosystem services. A review. Journal of Environmental Management 143, 123-134.

Onwubuya, K., Cundy, A., Puschenreiter, M., Kumpiene, J., Bone, B., Greaves, J., Teasdale, P., Mench, M., Tlustos, P., Mikhalovsky, S., Waite, S., Friesl-Hanl, W., Marschner, B., Müller, I., 2009. Developing decision support tools for the selection of "gentle" remediation approaches. Science of The Total Environment 407, 6132-6142.

Panagos, P., Van Liedekerke, M., Yigini, Y., Montanarella, L., 2013. Contaminated Sites in Europe: Review of the Current Situation Based on Data Collected through a European Network. Journal of Environmental and Public Health 2013.

Park, J.H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N., Chung, J.-W., 2011. Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. Journal of Hazardous Materials 185, 549-574.

Pediaditi, K., Doick, K.J., Moffat, A.J., 2010. Monitoring and evaluation practice for brownfield, regeneration to greenspace initiatives: A meta-evaluation of assessment and monitoring tools. Landscape and Urban Planning 97, 22-36.

Pignatello, J.J., Xing, B., 1995. Mechanisms of Slow Sorption of Organic Chemicals to Natural Particles. Environmental Science & Technology 30, 1-11.

Pourrut, B., Lopareva-Pohu, A., Pruvot, C., Garçon, G., Verdin, A., Waterlot, C., Bidar, G., Shirali, P., Douay, F., 2011. Assessment of fly ash-aided phytostabilisation of highly contaminated soils after an 8-year field trial: Part 2. Influence on plants. Science of The Total Environment 409, 4504-4510.

Ram, L.C., Masto, R.E., 2014. Fly ash for soil amelioration: A review on the influence of ash blending with inorganic and organic amendments. Earth-Science Reviews 128, 52-74.

Ram, L.C., Srivastava, N.K., Tripathi, R.C., Jha, S.K., Sinha, A.K., Singh, G., Manoharan, V., 2006. Management of mine spoil for crop productivity with lignite fly ash and biological amendments. Journal of Environmental Management 79, 173-187.

Reid, B.J., Stokes, J.D., Jones, K.C., Semple, K.T., 2000. Nonexhaustive Cyclodextrin-Based Extraction Technique for the Evaluation of PAH Bioavailability. Environmental Science & Technology 34, 3174-3179.

Sauvé, S., Hendershot, W., Allen, H.E., 2000. Solid-Solution Partitioning of Metals in Contaminated Soils: Dependence on pH, Total Metal Burden, and Organic Matter. Environmental Science & Technology 34, 1125-1131.

Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Wolfe, N.L., Carreira, L.H., 1995. Phytoremediation of Organic and Nutrient Contaminants. Environmental Science & Technology 29, 318A-323A.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., Harms, H., 2004. Peer Reviewed: Defining Bioavailability and Bioaccessibility of Contaminated Soil and Sediment is Complicated. Environmental Science & Technology 38, 228A-231A.

Smith, S.L., Thelen, K.D., MacDonald, S.J., 2013. Yield and quality analyses of bioenergy crops grown on a regulatory brownfield. Biomass and Bioenergy 49, 123-130.

Sohi, S., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use and function in soil. Advances in Agronomy 105, 47-82.

USEPA, 2009. List of Contaminants and their Maximum Contaminant Levels (MCLs). http://water.epa.gov/drink/contaminants/. (cited 5th June 2014)

Van Slycken, S., Witters, N., Meers, E., Peene, A., Michels, E., Adriaensen, K., Ruttens, A., Vangronsveld, J., Du Laing, G., Wierinck, I., Van Dael, M., Van Passel, S., Tack, F.M.G., 2013. Safe use of metal-contaminated agricultural land by cultivation of energy maize (Zea mays). Environmental Pollution 178, 375-380.

Waqas, M., Khan, S., Qing, H., Reid, B.J., Chao, C., 2014. The effects of sewage sludge and sewage sludge biochar on PAHs and potentially toxic element bioaccumulation in Cucumis sativa L. Chemosphere 105, 53-61.

Witters, N., Mendelsohn, R., Van Passel, S., Van Slycken, S., Weyens, N., Schreurs, E., Meers, E., Tack, F., Vanheusden, B., Vangronsveld, J., 2012a. Phytoremediation, a sustainable remediation technology? II: Economic assessment of CO2 abatement through the use of phytoremediation crops for renewable energy production. Biomass and Bioenergy 39, 470-477.

Witters, N., Mendelsohn, R.O., Van Slycken, S., Weyens, N., Schreurs, E., Meers, E., Tack, F., Carleer, R., Vangronsveld, J., 2012b. Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement. Biomass and Bioenergy 39, 454-469.

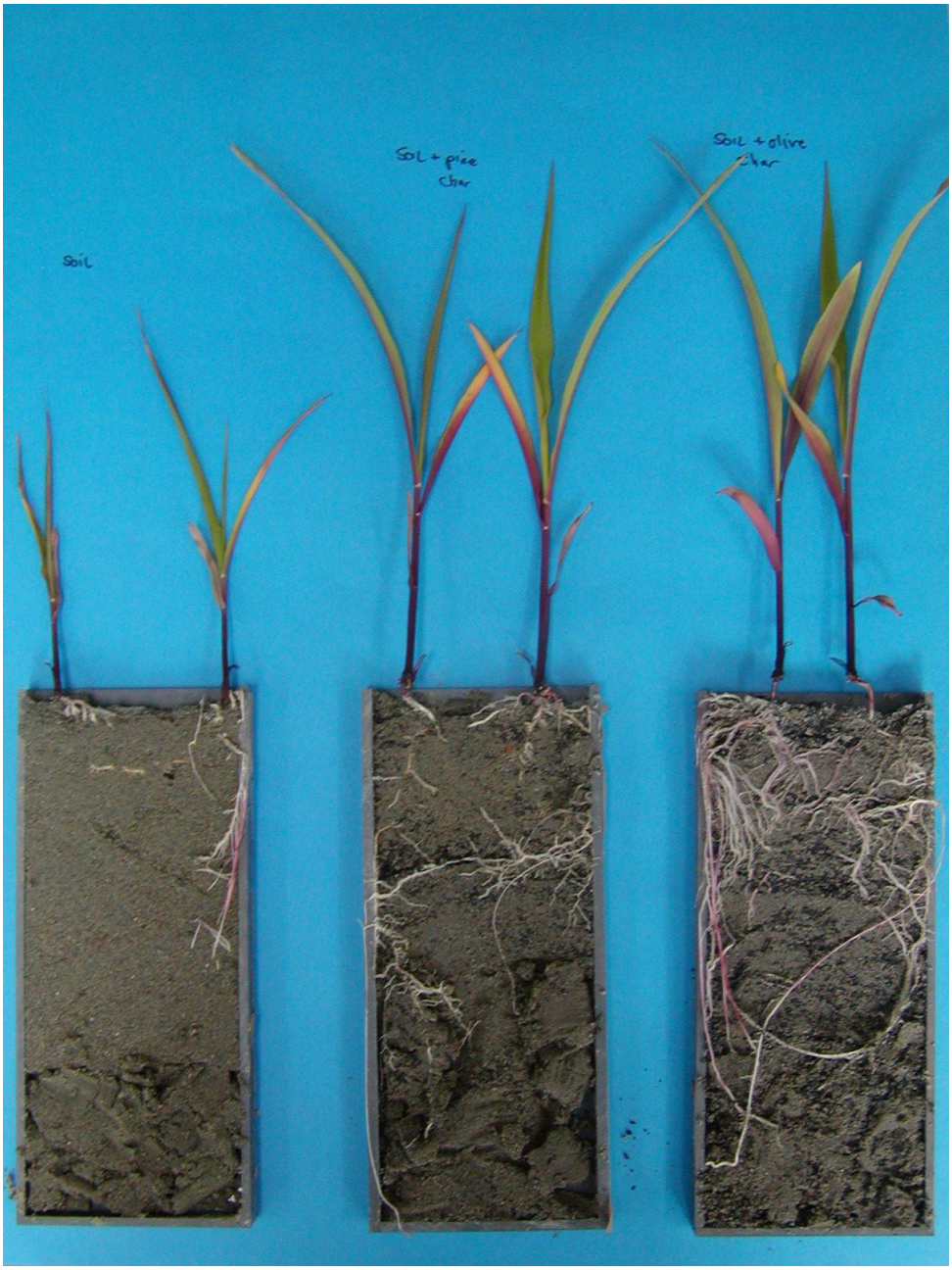
Zhao, L., Cao, X., Mašek, O., Zimmerman, A., 2013. Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. Journal of Hazardous Materials 256–257, 1-9.

Zhu, D., Pignatello, J.J., 2005. Characterization of Aromatic Compound Sorptive Interactions with Black Carbon (Charcoal) Assisted by Graphite as a Model. Environmental Science & Technology 39, 2033-2041.

Zimmerman, J.R., Ghosh, U., Millward, R.N., Bridges, T.S., Luthy, R.G., 2004. Addition of Carbon Sorbents to Reduce PCB and PAH Bioavailability in Marine Sediments: Physicochemical Tests. Environmental Science & Technology 38, 5458-5464.

2

Effects of biochar amendment on root traits and contaminant availability of maize plants in a copper and arsenic impacted soil



2.1 Introduction

Restoring degraded soils using low impact, cost-effective remediation techniques has been increasingly investigated over the last few decades, given the extremely high number of contaminated sites generated in the wake of anthropogenic activities and the expense involved in remediating these sites (Onwubuya et al. 2009). Phytoremediation in the form of phytostabilisation is one low impact remediation option which aims to stabilise soil structure and reduce negative contaminant effects simultaneously (Kidd et al. 2009).

The use of degraded sites for biomass crop generation is a proposed solution for deriving commercial benefit from a phytoremediation approach (Atkinson et al. 2008). Maize (*Zea mays*) is one potential crop choice due to its quick growth cycle and high biomass production. It has been previously used to investigate contaminant impact on plant health and growth (Lin et al. 2008) in addition to in studies assessing its potential as a biomass crop grown in contaminated soil (Witters et al. 2012a; Witters et al. 2012b).

Successful phytoremediation relies on good plant development in the form of healthy root structure and high root and shoot biomass in conjunction with minimal root to shoot translocation of contaminants to minimise transfer across the food chain (Karami et al. 2011; Wenzel 2009). However, plant establishment on a contaminated site can be problematic and the use of amendments, particularly organic materials, can enhance biomass yield and improve plant health (Clemente et al. 2012). The use of biochar as an amendment for re-establishing plant growth in contaminated environments (Beesley et al. 2011; Karami et al. 2011) is one potentially cost effective approach, particularly if waste-derived biochars are used, but field derived data are scarce mainly due to concerns over increased mobility of some contaminants, particularly arsenic (Beesley

et al. 2013; Beesley et al. 2010). The literature available for biochar amendment of uncontaminated/agricultural sites have highlighted the positive effects of biochar amendment on root growth (Lehmann et al. 2011; Prendergast-Miller et al. 2011; Prendergast-Miller et al. 2013) but this has not yet been fully studied in the context of contaminated soils. A wider knowledge of the effects of biochar amendment on root growth in contaminated systems is essential in addition to a better understanding of contaminant behaviour patterns before confident field scale application.

Based on the hypothesis that biochar amendment alters root growth and available contaminant pools in polluted soils, the objectives of this work were to: investigate root morphology and architecture in a contaminated soil amended with biochar using a rhizobox approach and; to assess the effect of biochar amendment on available/mobile contaminant pools in the soils and on measured plant uptake.

2.2 Materials and methods

2.2.1 Experiment set up

Soil was collected from the topsoil (0-15 cm) of the vicinity of the tailings dump of the disused copper mine El Fernandito in Garganta de los Montes (40°55'3.14"N; 3°40'23.36"W), near Madrid, Spain, sieved to 2mm, and air dried. The soil had a sandy loam texture (54% sand, 39% silt and 7% clay), pH of 6.8, low organic matter content (1.08%) and high total As and Cu concentrations (74 and 404 mg kg^{-1} d.w., respectively). The soil also contained enhanced levels of Zn and Mn (260 and 606 mg kg^{-1} d.w., respectively). Soil analysis details are provided in Section 2.2.3.

To put the metal values into context, the Spanish guidelines are determined by each regional authority and as a rule of thumb a soil is contaminated with metals if it exceeds the mean plus twice the standard deviation of soil background values. As no

background values are available for where the soil was sampled, Italian guidelines state screening values as follows: As 20 mg kg^{-1} d.w., Cu 150 mg kg^{-1} d.w., Zn 150 mg kg^{-1} d.w. (Carlon 2007)

Two biochars, derived from the slow pyrolysis of pine woodchip (PB) and olive tree pruning (OB), were used to amend the contaminated soil and were lightly crushed and sieved to 0.5 to 2mm. Biochars were produced in a pilot plant at 450 °C with a residence time in the reactor of approximately 15 minutes. Biochar samples were produced by the University of León (Natural Resources Institute, Spain) in the framework of the project "Proyecto Biocar: Estudio del Biocarbón como Sumidero de Carbono" (IPT-440000-2010-8). The biochars differed greatly in their properties as shown in Table 2.1. Section 2.2.3 describes the methodology used for deriving the values shown in Table 2.1.

Table 2.1 *Main characteristics (on a dry weight basis) of the two biochars (PB: pine woodchip biochar and OB: olive tree pruning biochar). *Data provided by J.A. Albuquerque.*

Parameters	**PB**	**OB**
Bulk density (g cm^{-3})	0.63	0.36
pH[a]	7.52	9.34
Electrical conductivity[a] (µS cm^{-1})	256	2430
Organic matter (g kg^{-1})	981.9	900.3
C (g kg^{-1})	837.1	755.2
N (g kg^{-1})	3.6	11.0
P (mg kg^{-1})	148	1464
K (mg kg^{-1})	1708	9159
Mn (mg kg^{-1})	153	50
Zn (mg kg^{-1})	42	24
Cu (mg kg^{-1})	134	114
As (mg kg^{-1})	1.7	6.1
Specific surface area (m^2 g^{-1})	288	265
Germination index (lettuce, %)	92	100
Germination index (cress, %)	117	84
Cation exchange capacity (cmol kg^{-1})	12.6	36.6
Water-soluble fractions		
Water-soluble organic C (WSC, mg kg^{-1})	920	1527
Water-soluble inorganic C (mg kg^{-1})	122	1020
Water-soluble N (WSN, mg kg^{-1})	10	19
WSC/WSN	90	82
Water-soluble P (mg kg^{-1})	6	17
Water-soluble K (mg kg^{-1})	256	2546

[a]*water extract 1:10 (w/v).*

Maize seeds were washed and pre-germinated before planting to ensure only viable seeds were used. They were washed by sonicating in 10% sodium hypochlorite for 30 minutes and then in deionised water for 30 minutes. They were then placed on tissue paper moistened with deionised water and several drops of calcium sulphate (1.5 mM) and incubated at 28°C for 72 hours for germination. (Clark et al. 1999)

The pre-germinated maize seeds were grown in rhizoboxes (25 cm x 10 cm x 1 cm) for 21 days in a controlled growth chamber (temperature day 25°C (night 20°C); relative humidity day 40% (night 60%); hours of light day 13 hours (night 11 hours); light intensity 520µmol m^{-2} s^{-1}). Rhizoboxes were filled according to three treatment scenarios, each with five replicates and two germinated seeds: control with no amendment (300g soil), PB amended soil (300g soil + 9g biochar), OB amended soil (300g soil + 9g biochar). The biochar amended soil were thoroughly hand mixed before adding to rhizoboxes. A fine cloth was inserted into the bottom of the rhizobox to allow watering by capillary action, maintaining constant moisture content within the boxes. The rhizoboxes were covered with foil to exclude light and angled at 65° to encourage the roots to grow on the rhizobox/soil interface (Marschner and Römheld 1983). (Fig 2.1)

Fig 2.1 *Rhizobox setup in growth chamber*

A column experiment adapted from NEN 7343:1995 (Dutch Environment Agency) set up to run in parallel to the rhizoboxes explored the differences in leaching patterns between washed and unwashed olive tree pruning biochar. Washing consisted of sonication in 100% ethanol for two hours before rinsing three times with deionised water and air drying. 700 g of soil only or 700 g of a soil and 3% char mix was packed into Perspex columns (cylinders 40 cm long x 5 cm diameter) (Fig 2.2). Each treatment was performed in duplicate columns. A peristaltic pump was set to run deionised water from the base of each column upwards, collecting eight fractions on an accumulated volume basis. In total, 13500 ml of leachate was collected over 14 days and this volume simulated about 9 years of rainfall at the site from which the soil was removed.

Fig 2.2 *Column experiment setup*

2.2.2 Harvesting

After 21 days, the maize plants were harvested. Shoots were cut 1 cm above the soil surface, rinsed with deionised water, dried with tissue paper. Roots were sonicated twice in deionised water, rinsed and dried with tissue paper. All plant material was weighed for total fresh weight. Of the two plants per treatment replicate, one plant was used for enzymatic analyses (nitrate reductase in the shoots and acid phosphatase in the roots), and the second plant was used to determine concentrations of potentially toxic elements in the shoots after being dried at 60°C for 72 hours. The fresh roots of

the second plant were stained with 5% Giemza Blue solution and kept at 4°C in a ziplock bag in deionised water until root characteristics analysis was carried out using WinRhizo® software.

Leaf area was determined by scanning (HP Photosmart C4280) and processing the images in GIMP 2 software. Root length, root surface area, diameter and diameter classes were determined by WinRhizo software following root preparation as described in the previous paragraph. Roots were scanned after being placed carefully on a transparent tray in 2-3mm of water (Himmelbauer et al. 2004).

2.2.3 Soil and biochar analysis

Soil particle size distribution was determined using standard method ISO 11277:2009 (ISO 2009). pH and electrical conductivity (EC) of the soil and soil and biochar samples were determined in the water extract 1:5 (w/v) and 1:10 (w/v) respectively after stirring the mixture mechanically for 2 hours. Organic matter content (OM) was determined by loss on ignition at 550°C for soil and following the Test Methods for the Examination of Composting and Compost (TMECC) method for biochar (TMECC 2002).

Biochar C and N contents were determined using an elemental analyser (LECO CHN-600). The water-soluble organic C (WSC), water-soluble inorganic C (WSIC) and water-soluble N (WSN) were determined using an automatic analyser for liquid samples (TOC-V CSN+TNM-1 Analyser, Shimadzu). Total P, K, Ca, Mg, Fe, Mn, Zn and Cu were determined after dry ash sample digestion using method 04.12-C (TMECC 2002). Total and water soluble (1:10 w/v) components were analysed as follows: P was determined colorimetrically (Murphy and Riley 1962); K by atomic emission spectroscopy; Ca, Mg, Fe, Mn, Zn and Cu by atomic absorption spectrophotometry and; As by atomic fluorescence spectroscopy (Millennium Excalibur, PS Analytical).

The biochar bulk density was estimated by weighing 10 mL of milled sample. The CO_2 adsorption method (273 K) using a Micromeritics ASAP 2020 instrument was performed to determine the surface area of the biochar samples. All biochar samples were degassed under vacuum at 200°C for 8 hours prior to analysis. Cation exchange capacity (CEC) was measured by a modified ammonium-acetate compulsory displacement method (Gaskin et al. 2008). The germination index (GI) for the biochars was determined using cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) (Zucconi et al. 1981).

2.2.4 Plant enzyme analysis and soil and plant tissue analysis of potentially toxic elements (PTEs)

Samples of 0.25 g (+/- 0.005 g) fresh shoot material and 0.25 g (+/- 0.005 g) fresh root material were extracted for nitrate reductase activity (Ruiz et al. 1999) and acid phosphatase activity respectively (Barrett-Lennard and Greenway 1982).

Total and extractable As concentrations in the treatments were determined by atomic fluorescence spectroscopy (Millennium Excalibur, PS Analytical) and Cu, Zn and Mn was determined by atomic absorption spectroscopy (AA800, Perkin Elmer) following autoclaving (Lozano-Rodriguez et al. 1995) and ammonium sulphate extraction (Vázquez et al. 2008) respectively. For total soil concentrations, 0.5 g (+/- 0.005 g) of soil was transferred into 50 ml autoclave bottles to which 6 ml of MilliQ water, 6 ml of 65% HNO_3 and 4 ml of 33% H_2O_2 were added. The autoclave was set at a pressure of 1.5 kg cm^{-2} (125°C) for 30 minutes, samples were left to cool, then filtered and made up to 50 ml. Total plant concentrations (shoot tissue) were determined by weighing 0.1 g (+/- 0.001 g) dried shoot tissue into 20 ml autoclave bottles to which 2 ml of MilliQ water, 1.5 ml of 65% HNO_3 and 1 ml of 33% H_2O_2 were added and made up to 5 ml once autoclaved, cooled and filtered. Extractable PTEs in the soils were determined by extracting 1.5 g (+/- 0.005 g) of soil with 15 ml of $(NH_4)_2SO_4$ 0.1M in 50 ml tubes and

shaking for four hours at 180 rpm. The samples were then filtered and 0.1 ml of HNO_3 was added by volumetric pipette.

2.2.5 Column leachate analysis

Column leachate fractions were analysed for pH, EC and dissolved organic carbon (DOC). Nitrate, chloride, phosphate and sulphate were analysed by ion chromatography (Dionex). As and Cu in the leachate were determined as described in the previous section.

2.2.6 Data analysis

Statistical analyses were carried out on statistical software IBM® SPSS® Statistics Version 21. Data were checked to fit the hypothesis of normality and homoscedasticity (population distributions of equal variance). All data were normally distributed and were tested with a one-way ANOVA. Tukey's test was used as post-hoc for mean comparisons of the homoscedastic data. Games-Howell's test was used for the comparisons of non-homoscedastic data.

2.3 Results

2.3.1 Effect of biochar on shoot and root traits

Biochar amendment had a positive effect on most of the measured plant characteristics (Figs. 2.3-2.5). Biochar amendment significantly affected fresh shoot/root ratio (Fig. 2.3), which for both pine woodchip (PB) and olive tree pruning biochar (OB) amendments was greater than the control. In the PB treatment, the higher shoot/root ratio was due to an increase in shoot biomass while root biomass did not significantly differ compared to the control. When amended with OB, both root and shoot biomass increased significantly compared to the control, in addition to the increase in the observed shoot/root ratio.

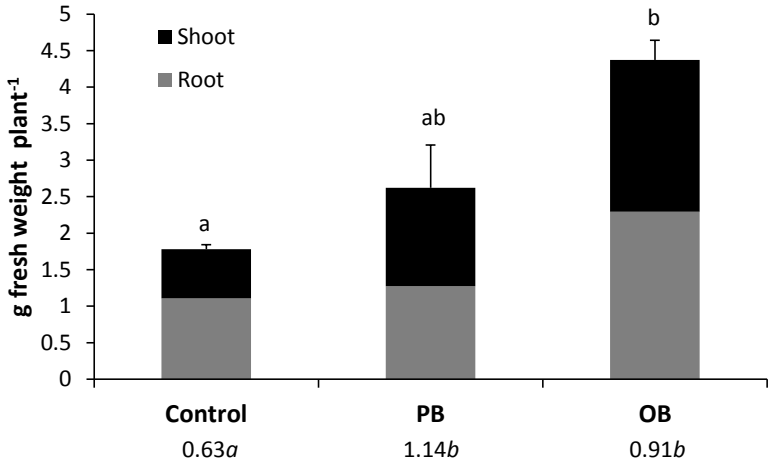


Fig. 2.3 *Plant biomass (g of fresh weight per maize plant) in the mine soil with different biochars (PB: pine woodchip biochar and OB: olive tree pruning biochar). Mean ± SE (n=4-5). The shoot: root ratio was calculated and shown on the bottom of the x axis. Different letters indicate statistical differences between groups at p<0.05.*

Leaf surface area significantly increased ($p<0.05$) in both biochar amended soils compared to the control. The same pattern was observed for root length and root surface area (both at $p<0.01$) (Fig. 2.4). By classifying the different root diameters into percentage composition (Fig. 2.5), root diameters <0.4mm seem predominant in the biochar amended soils (>50%) compared to less than 30% in the control while root diameters of >1mm represent less than 5% in the char amended soils compared to

more than 25% in the control. These results suggest that both PB and OB promoted root growth and production of fine roots. Likewise, both OB and PB amendment led to significantly higher root length density ($p<0.01$) compared to the control. Specific root length was significantly higher in the PB amended soil compared to the control ($p<0.05$), but this was not the case in the OB amended soil. In terms of root morphology, root length: root volume ratios were similar across treatments, while root tissue density was significantly lower in the PB treatment compared to the control at 0.1% versus 0.5% ($p<0.05$). Biochar amendment had a generally beneficial effect on plant resource allocation below ground, but this was not the case for all parameters. OB amended soils had a significantly higher root mass density than both PB amended soils and the control soils ($p<0.01$). Root length ratio was significantly greater in the PB treatment than both the control and the OB treatment ($p<0.05$). Root weight ratio was significantly lower in the PB amended soils compared to the control ($p<0.05$), suggesting PB amendment enabled the plant to allocate more resources to above ground (Table 2.2).

Table 2.2 *Root architecture (1); root morphology (2); plant resource allocation below ground (3). Letters denote statistical significance between treatments, p < 0.05*, <0.01**, <0.001***, n.s. not significant. PB: pine woodchip biochar and OB: olive tree pruning biochar.*

			Control	PB	OB	Sig
1	root length density	root length/ soil volume mg cm^{-3}	0.447 (0.036) a	1.36 (0.162) b	1.289 (0.233) b	**
	specific root length	root length/root biomass cm mg^{-1}	0.106 (0.015) a	0.313 (0.08) b	0.141 (0.025) a	**
2	root length: root volume ratio	root length/root volume cm cm^{-3}	453.3 (35.9)	475.3 (72.2)	510.0 (38.9)	n.s.
	root tissue density	root biomass/root volume mg cm^{-3}	4523.0 (521.2) b	1682.9 (257.1) a	4412.1 (1217.7) b	*
3	root mass density	root biomass/ soil volume mg cm^{-3}	4.424 (0.431) a	5.106 (1.294) a	9.173 (0.499) b	**
	root weight ratio	root biomass/plant biomass mg mg^{-1}	0.619 (0.028) b	0.484 (0.055) a	0.526 (0.018) ab	**
	root length ratio	root length/plant biomass cm mg^{-1}	0.065 (0.009) a	0.143 (0.029) b	0.074 (0.013) a	**

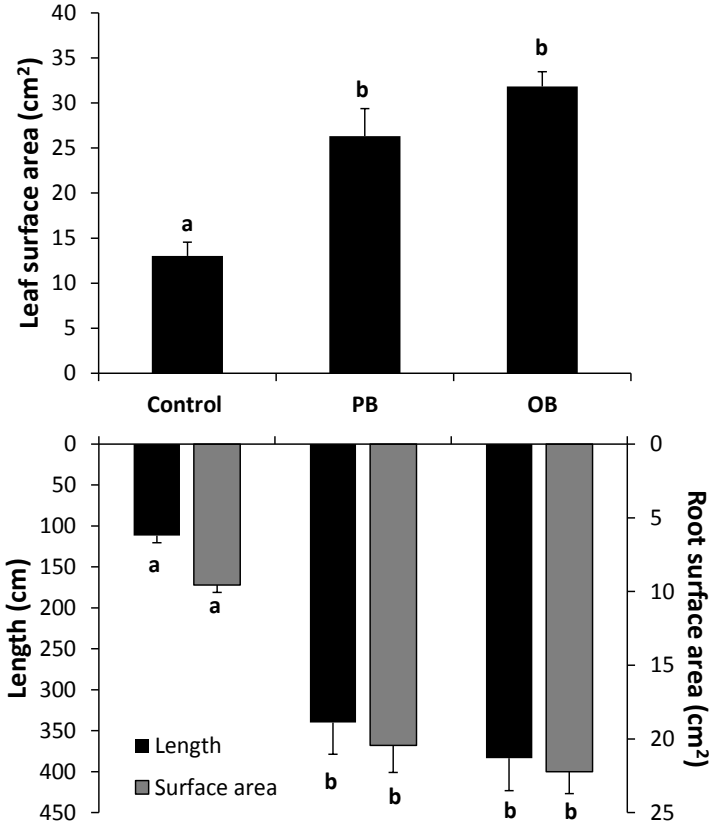


Fig. 2.4 *Plant morphology as affected by biochar application to a mine soil: leaf surface area (top) and root length and root surface area (bottom). Mean ± SE (n=4-5). Different letters mean statistical differences between groups at p<0.05. PB: pine woodchip biochar and OB: olive tree pruning biochar.*

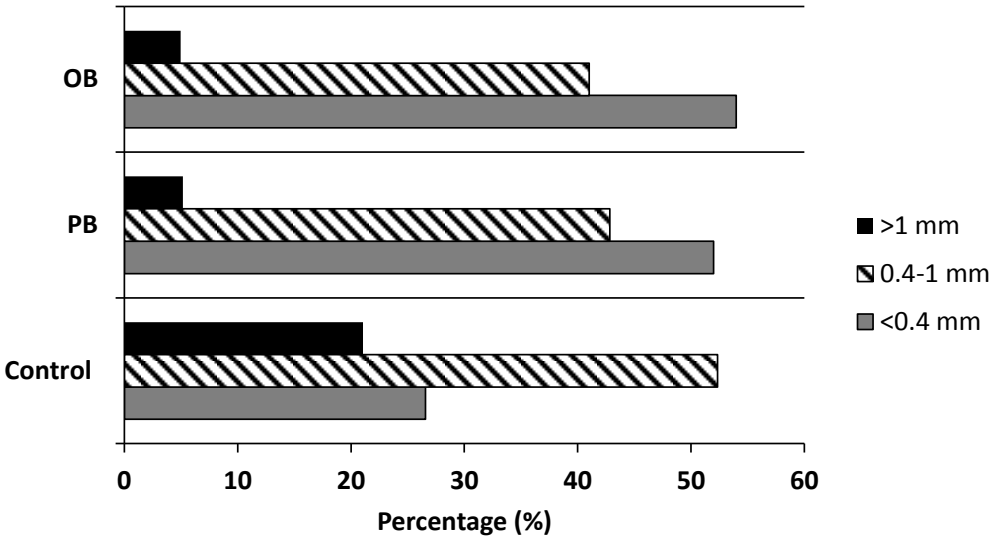


Fig. 2.5 *Root diameter classes as affected by biochar application to a mine soil. Mean ± SE (n=4-5), where absent, error bars fall within symbols. PB: pine woodchip biochar and OB: olive tree pruning biochar.*

2.3.2 Effect of biochar on PTE extractability and plant uptake

The different biochars behaved very differently with regards to PTE extractability in the soils (Fig. 2.6). There was no significant difference in arsenic extractability between the unamended control soil and PB amended soil, while OB amended soils had significantly higher extractable arsenic (at $p<0.05$). In contrast, both biochar amendments significantly decreased copper extractability compared to the control ($p<0.01$). Zinc extractability significantly decreased across treatments, in the order Control>PB>OB ($p<0.05$) while no significant differences were observed for Mn extractability (Fig. 2.7).

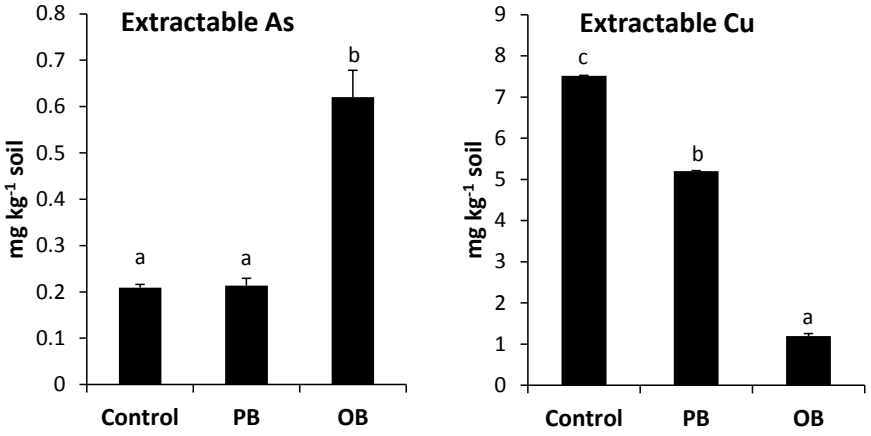


Fig. 2.6 *Ammonium sulphate-extractable As and Cu in a mine soil with different biochar treatment (PB: pine woodchip biochar and OB: olive tree pruning biochar). Mean ± SE (n=5). Different letters signify statistical differences between treatments at p<0.05.*

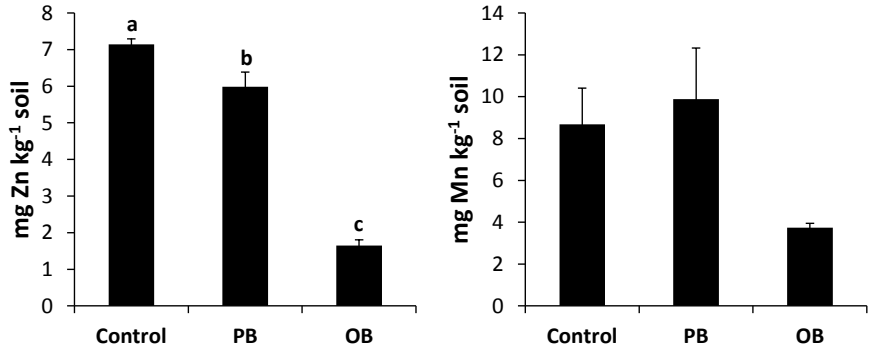


Fig. 2.7 *Ammonium sulfate-extractable Zn and Mn in a mine soil with different biochar treatment (PB: pine woodchip biochar and OB: olive-tree pruning biochar). Mean ± SE (n=5). Different letters signify statistical differences between treatments at p<0.05.*

The patterns observed in PTE shoot uptake were slightly different (Fig. 2.8-2.9). Arsenic uptake in the shoots differed significantly between the two biochar treatments, with PB treatments had significantly less shoot arsenic than OB treatments ($p<0.05$), although neither amendment differed significantly to the control. On the other hand, there was significantly less copper in the plant shoots from both the biochar treatments compared to the control ($p<0.01$). Shoot Zn and Mn concentrations were significantly reduced in the OB treatment compared to the control ($p<0.05$), but not in the PB treatment compared to the control.

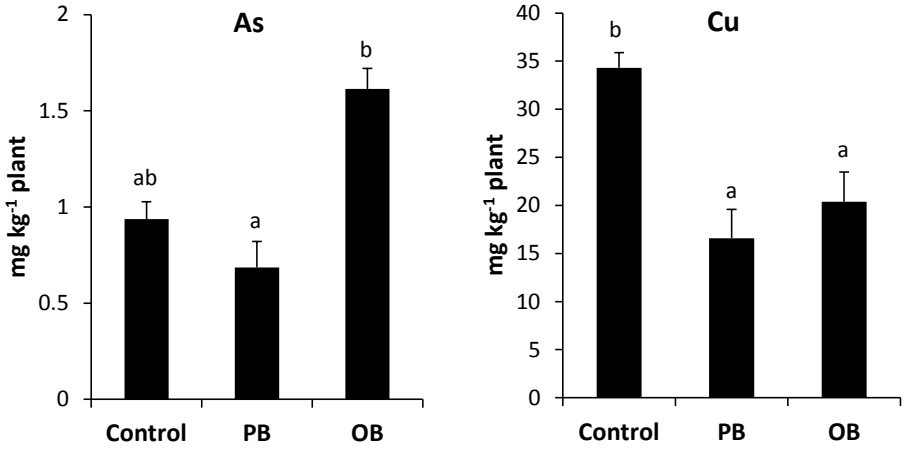


Fig. 2.8 *Arsenic and copper concentration in shoots of maize plants growing on a mine soils with different biochar treatment (PB: pine woodchip biochar and OB: olive tree pruning biochar). Mean ± SE (n=4-5). Different letters mean statistical differences between groups at p<0.05.*

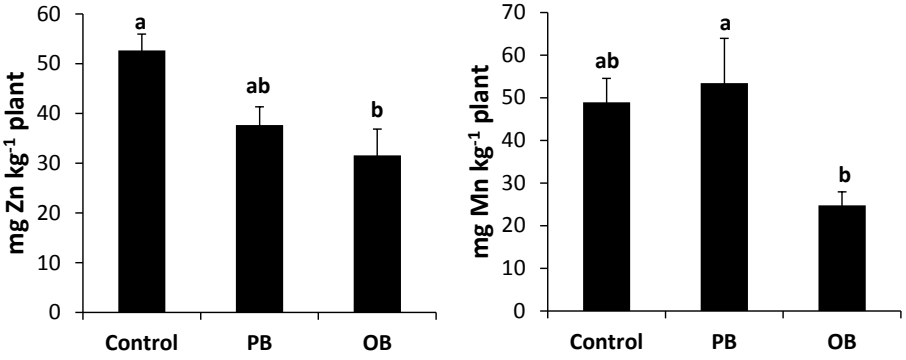


Fig. 2.9 *Zinc and manganese concentration in shoots of maize plants growing on a mine soils with different biochar treatment (PB: pine woodchip biochar and OB: olive-tree pruning biochar). Mean ± SE (n=4-5). Different letters mean statistical differences between groups at p<0.05.*

2.3.3 Plant enzymatic activities as affected by biochar application

A significant downward trend was observed for nitrate reductase activity in plant shoots for OB amended soils compared to the control, while no differences were observed between the control and PB amendment. No significant differences were observed in acid phosphatase activity in the roots across treatments (Fig. 2.10).

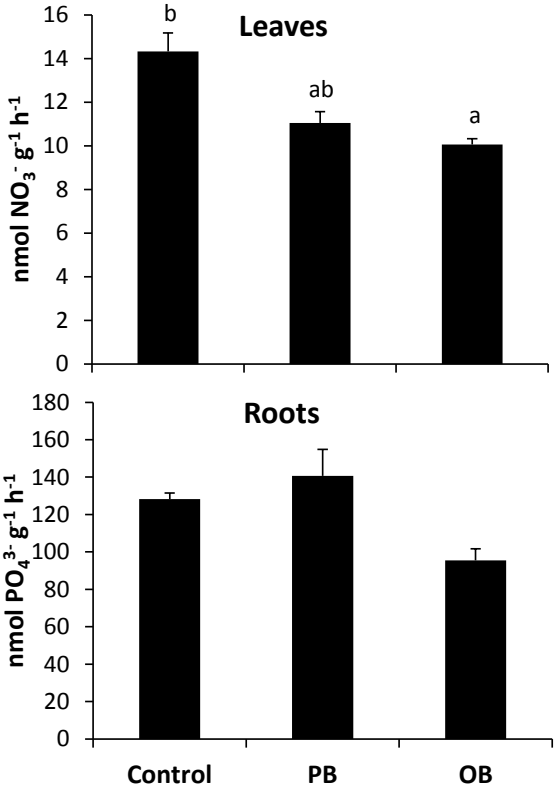


Fig. 2.10 *Nitrate reductase activity in leaves and acid phosphatase activity in roots of maize growing on a mine soils with different biochar treatment (PB: pine woodchip biochar and OB: olive tree pruning biochar). Mean ± SE (n=3-5). Different letters signify statistical differences between groups at p<0.05.*

2.3.4 Arsenic, copper and anion leaching after biochar application

Whereas As leaching rates were constant in the control soils, the olive tree pruning biochar significantly increased arsenic concentrations in the percolate water (Fig. 2.11). Although the washed biochar (WBC) reduced arsenic leaching from the column

compared to the unwashed biochar (BC), the leachable portion of arsenic is very similar between the washed and unwashed chars (Table 2.3).

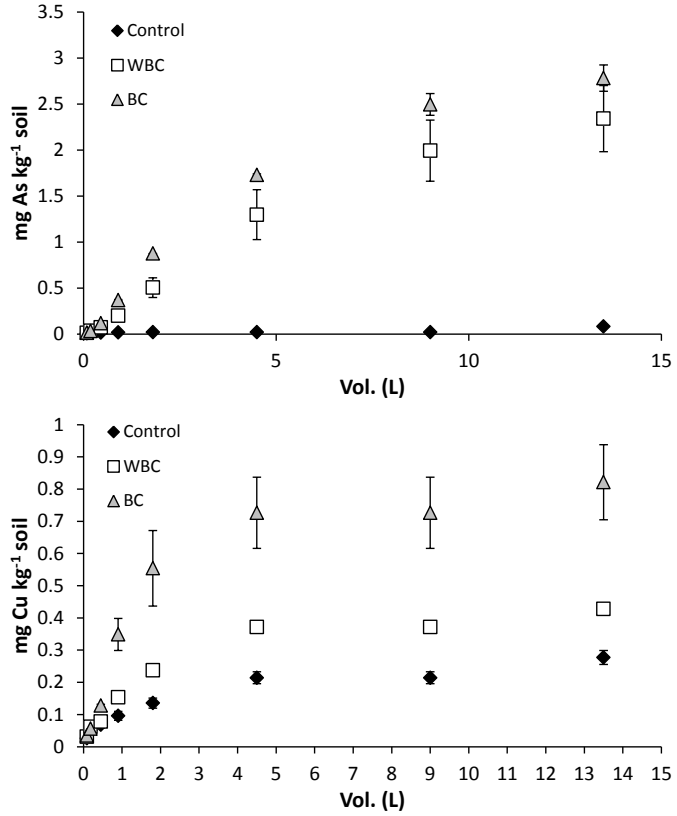


Fig. 2.11 *Leaching of As (top) and Cu (bottom) in mg per kg of soil (on a dry weight basis) as evaluated by a leaching column experiment using washed (WBC) and unwashed (BC) olive tree pruning biochar over 14 days. Mean ± SE (n=2).*

Table 2.3 *Curve parameters after fitting As and Cu leaching data to volume of leachate (after Fig. 7). In the hyperbolic curve, the term a is associated to the maximum cumulative leaching of As/Cu (in mg kg^{-1}) and b is associated to the volume (in L) needed to leach half of the maximum leaching. Statistical significance is indicated by ***p<0.001. WBC: washed olive tree pruning biochar and BC: unwashed olive tree pruning biochar.*

As				
	Linear curve: $y=ax+y_0$			
	a	y_0	Adj. R^2	Sig.
Soil	$4.1 \cdot 10^{-6}$	0.0086	0.66	***
	Hyperbola: $y=ax/(b+x)$			
	a	b	Adj. R^2	Sig.
Soil+WBC	4.7	12.8	0.99	***
Soil+BC	4.5	7.5	0.99	***
Cu				
	Hyperbola: $y=ax/(b+x)$			
	a	B	Adj. R^2	Sig.
Soil	0.29	2.0	0.96	***
Soil+WBC	0.48	1.8	0.98	***
Soil+BC	0.91	1.5	0.97	***

Dissolved organic carbon increased across treatments as highlighted by the cumulative totals: 1.5 mg kg^{-1} (+/- 0.02 SE) in the soil columns, 220.6 mg kg^{-1} in the unwashed biochar columns and 95.7 mg kg^{-1} (+/- 15.9 SE) in the washed biochar columns. Chloride leaching decreased with OB amendment while nitrate leaching significantly increased. Most phosphate fractions were below detection limits while there was no observed effect on sulphate concentrations (Table 2.4).

Table 2.4 *Cumulative total of 8 column leachate fractions (+/- SE n=2) expressed as mg kg^{-1} dry weight soil*

	Control (soil only)	3% washed OB	3% unwashed OB
NO3	113 (70)	386 (115)	1189 (59.6)
PO4	2.73 (2.27)	2.98 (1)	1.5 (0.71)
SO4	31 (6)	20.2 (5.08)	32.32 (4.12)
Cl	532 (347)	74.5 (5.3)	61.4 (12.1)

2.4 Discussion

2.4.1 Effect of biochar amendment on contaminant mobility

Results from the current study suggest that biochar amendment promotes root growth, increases available pools of arsenic for plant uptake while decreasing those of copper and zinc. The variable effect on arsenic availability in the soil according to char type (As in OB > As in PB) may be explained by the OB having a more available arsenic content as a result of its more alkaline pH and relatively high carbonate and soluble P contents compared to PB (Table 2.1). Additional soil and plant interactions over the course of the experiment may also have contributed to the observed data. As others have also suggested, char type needs to be chosen on a site specific basis (Beesley et al. 2011). Increased arsenic availability has also been observed in other studies in soil only and soil and plant systems (Beesley et al. 2010; Karami et al. 2011). The arsenic content in the olive tree pruning biochar itself and the increased pH caused by OB amendment (Table 2.1) may also be contributing to the increased arsenic release observed in the

columns (Fig. 2.11). The leachable pool of copper observed in the unamended soil columns was doubled in the washed biochar and soil columns and tripled in the unwashed biochar and soil columns (Table 2.3). This is likely related to the enhanced concentrations of dissolved organic carbon (DOC) with biochar addition. Comparing the patterns observed for arsenic and copper in the soil control column, Cu is more easily leachable while arsenic appears to be poorly mobile. The data from the biochar amended columns illustrate that OB mobilises As to a greater extent than Cu. One previous study suggested that biochar amendment triggered a higher leaching capacity for As but not for Cu (Beesley and Marmiroli 2011). There are several possible explanations for this, the most likely being due to differences in biochar feedstock properties although differences in experimental design between the present study and the cited study may also be a contributing factor in the patterns observed. Nonetheless, although column studies are useful to get an idea of contaminant leaching capacity, they do not fully represent a field scenario where plant interactions also have an effect on contaminant leaching.

2.4.2 Effect of biochar amendment on nutrient availability

Nitrate reductase is the enzyme responsible for reducing nitrate to nitrite. Lower activity here indicates less availability of nitrate to the plant shoots for conversion, which may have been caused by sorption of nitrate to the biochar particles (Jones et al. 2012). Biochar localises nitrate in the rhizosphere of biochar amended soils, resulting in less nitrate uptake by plants (Prendergast-Miller et al. 2011). Nonetheless, N dynamics are highly complex and a number of factors may be at play for the results presented (Clough et al. 2013).

In the case of the phosphatase enzyme, the reduced activity may be due to the increased uptake of arsenic observed in the plants and therefore reduced phosphate

uptake, considering As is a well reported P analogue (Meharg and MacNair 1992; Moreno Jimenez et al 2008). Overall, the reduced enzymatic activity may be due to nutrient, enzyme or substrate sorption to the biochar (Lehmann et al. 2011). Variability in soil enzyme activity in the presence of char has been reported elsewhere (Bailey et al. 2011; Jones et al. 2012; Lehmann et al. 2011) although no specific data are available for plant enzymes in biochar amended soils.

The availability of other nutrients (e.g. K, Mg, Ca) in each treatment was not determined, thus the possibility that the addition of K or other nutrients due to biochar amendment (see biochar properties in Table 2.1) may have contributed to the improved plant growth cannot be ruled out. Although this aspect may be a potentially confounding factor in the results presented, this potential nutrient addition from biochar amendment would play a more significant role in agricultural soils or in a longer term experiment. Further studies elucidating interactions between nutrients and contaminants in contaminated soils with respect to plant growth are required before making any conclusions on this matter.

2.4.2 Root response to biochar amendment in contaminated systems

Contaminant availability tends to be the principal limiting factor affecting plant growth in contaminated soils. The nutrient limitation commonly found in mine soils is another important factor. However, given the significant reductions in copper availability with biochar amendment (both in terms of extractability and actual uptake) and the corresponding improvements in root development with biochar amendment, our results suggest that excess copper was limiting plant establishment and survival in the presented study. The less consistent behaviour of the other contaminants compared to copper lend credence to this theory.

This study suggests that biochar has no clear detrimental effect on root establishment and, by reducing copper availability significantly in both char treatments, a net positive effect was observed, particularly with regards to root mass density and root length density. Another study found that biochar effects on root traits in agricultural soils were not as indicative of root behaviour as quantifying rhizosheath development and biochar particles in the rhizosphere (Prendergast-Miller et al. 2013). However, in contaminated soils, root traits appear to be useful indicators of root responses to biochar amendment compared to unamended contaminated controls, with significant differences observed across the majority of indicators. There is a scarcity of data investigating specific root responses to biochar amendment in contaminated systems, apart from some qualitative assessment (Beesley et al. 2013) and further studies are needed in order to fully evaluate the effects on a range of plants and in a range of contaminated soils.

2.4.3 Implications for phytomanagement of mine soils

These results suggest that biochar addition to contaminated mine soils may enhance plant cover by improving root development and promoting higher biomass both above and below ground. Not only are these soils affected by contamination, they tend to have poor physical properties and low nutrient and carbon statuses which can make plant establishment difficult. It appears that biochar amendment reduces soil toxicity to plants growing under these difficult conditions, at least in early stages of plant establishment, and may play a role in limiting contaminant dispersion. In terms of improving soil health, other studies have highlighted the beneficial effects of biochar addition to poor soils, for example, improved water holding capacity and cation exchange capacity (Busscher et al. 2010; Carter et al. 2013; Revell et al. 2012; Sukartono et al. 2011). If the trends observed in this study can be further demonstrated

under field conditions, biochar will become a valuable yet affordable tool in the phytomanagement of degraded soils.

2.5 Conclusions

Root establishment in contaminated soils can be enhanced by biochar amendment but choice of biochar is key to maximising soil improvement, controlling contaminant availability to plants and controlling contaminant mobility overall.

2.6 Acknowledgments

Co-authors in the published article of this work are Eduardo Moreno Jiménez, Markus Puschenreiter and José A. Albuquerque. The experimental work was carried out during a STSM awarded to the A.B. by EU COST Action TD1107 *Biochar as an option for sustainable resource management*. Dr Peter Anderson at SETN (Scottish Environmental Technology Network) is thanked for the IC analysis.

2.7 References

Atkinson J, Lane A, Lord R, Street G, Scurlock J (2008) Biomass, Remediation, Re-Generation (BioReGen Life Project): Reusing Brownfield Sites for Renewable Energy Crops. GeoCongress 2008.

Bailey VL, Fansler SJ, Smith JL, Bolton Jr H (2011) Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biology and Biochemistry 43: 296-301. doi: http://dx.doi.org/10.1016/j.soilbio.2010.10.014.

Barrett-Lennard EG, Greenway H (1982) Partial Separation and Characterization of Soluble Phosphatases from Leaves of Wheat Grown Under Phosphorus Deficiency and Water Deficit. Journal of Experimental Botany 33: 694-704. doi: 10.1093/jxb/33.4.694.

Beesley L, Marmiroli M (2011) The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environmental Pollution 159: 474-480. doi: 10.1016/j.envpol.2010.10.016.

Beesley L, Marmiroli M, Pagano L, Pigoni V, Fellet G, Fresno T, Vamerali T, Bandiera M, Marmiroli N (2013) Biochar addition to an arsenic contaminated soil increases arsenic concentrations in the pore water but reduces uptake to tomato plants (Solanum lycopersicum L.). Science of The Total Environment 454–455: 598-603. doi: http://dx.doi.org/10.1016/j.scitotenv.2013.02.047.

Beesley L, Moreno-Jiménez E, Gomez-Eyles JL (2010) Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution 158: 2282-2287. doi: 10.1016/j.envpol.2010.02.003.

Beesley L, Moreno-Jiménez E, Gomez-Eyles JL, Harris E, Robinson B, Sizmur T (2011) A review of biochars' potential role in the remediation, revegetation and restoration of

contaminated soils. Environmental Pollution 159: 3269-3282. doi: 10.1016/j.envpol.2011.07.023.

Busscher WJ, Novak JM, Evans DE, Watts DW, Niandou MAS, Ahmedna M (2010) Influence of Pecan Biochar on Physical Properties of a Norfolk Loamy Sand. Soil Science 175: 10-14.

Carlon CE (2007) Derivation methods of soil screening values in Europe. A review and evaluation of national procedures towards harmonization. European Commission, Joint Research Centre, Ispra.

Carter S, Shackley S, Sohi S, Suy TB, Haefele S (2013) The Impact of Biochar Application on Soil Properties and Plant Growth of Pot Grown Lettuce (Lactuca sativa) and Cabbage (Brassica chinensis). Agronomy 3: 404-418. doi: doi:10.3390/agronomy3020404.

Clark RB, Zeto SK, Ritchey KD, Baligar VC (1999) Boron accumulation by maize grown in acidic soil amended with coal combustion products. Fuel 78: 179-185. doi: http://dx.doi.org/10.1016/S0016-2361(98)00133-1.

Clemente R, Walker DJ, Pardo T, Martínez-Fernández D, Bernal MP (2012) The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions. Journal of Hazardous Materials 223–224: 63-71. doi: http://dx.doi.org/10.1016/j.jhazmat.2012.04.048.

Clough T, Condron L, Kammann C, Müller C (2013) A Review of Biochar and Soil Nitrogen Dynamics. Agronomy 3: 275-293.

Gaskin JW, Steiner C, Harris K, Das KC, Bibens B (2008) Effect of Low-Temperature Pyrolysis Conditions on Biochar for Agricultural Use. 51.

Himmelbauer ML, Loiskandl AW, Kastanek AF (2004) Estimating length, average diameter and surface area of roots using two different Image analyses systems. Plant and Soil 260: 111-120. doi: 10.1023/b:plso.0000030171.28821.55.

ISO (2009) Soil quality Determination of particle size distribution in mineral soil material Method by sieving and sedimentation.

Jones DL, Rousk J, Edwards-Jones G, DeLuca TH, Murphy DV (2012) Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biology and Biochemistry 45: 113-124. doi: 10.1016/j.soilbio.2011.10.012.

Karami N, Clemente R, Moreno-Jiménez E, Lepp NW, Beesley L (2011) Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. Journal of Hazardous Materials 191: 41-48. doi: 10.1016/j.jhazmat.2011.04.025.

Kidd P, Barceló J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S, Clemente R, Monterroso C (2009) Trace element behaviour at the root–soil interface: Implications in phytoremediation. Environmental and Experimental Botany 67: 243-259. doi: http://dx.doi.org/10.1016/j.envexpbot.2009.06.013.

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota – A review. Soil Biology and Biochemistry 43: 1812-1836. doi: 10.1016/j.soilbio.2011.04.022.

Lin Q, Shen K-L, Zhao H-M, Li W-H (2008) Growth response of Zea mays L. in pyrene–copper co-contaminated soil and the fate of pollutants. Journal of Hazardous Materials 150: 515-521. doi: http://dx.doi.org/10.1016/j.jhazmat.2007.04.132.

Lozano-Rodriguez E, Luguera M, Lucena JJ, Carpena-Ruiz R (1995) Evaluation of two different acid digestion methods in closed systems for trace element determinations in plants. Quimica Analitica 14: 27-30.

Marschner H, Römheld V (1983) In vivo Measurement of Root-induced pH Changes at the Soil-Root Interface: Effect of Plant Species and Nitrogen Source. Zeitschrift für Pflanzenphysiologie 111: 241-251. doi: http://dx.doi.org/10.1016/S0044-328X(83)80083-X.

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27: 31-36. doi: http://dx.doi.org/10.1016/S0003-2670(00)88444-5.

Onwubuya K, Cundy A, Puschenreiter M, Kumpiene J, Bone B, Greaves J, Teasdale P, Mench M, Tlustos P, Mikhalovsky S, Waite S, Friesl-Hanl W, Marschner B, Müller I (2009) Developing decision support tools for the selection of "gentle" remediation approaches. Science of The Total Environment 407: 6132-6142. doi: http://dx.doi.org/10.1016/j.scitotenv.2009.08.017.

Prendergast-Miller MT, Duvall M, Sohi SP (2011) Localisation of nitrate in the rhizosphere of biochar-amended soils. Soil Biology and Biochemistry 43: 2243-2246. doi: 10.1016/j.soilbio.2011.07.019.

Prendergast-Miller MT, Duvall M, Sohi SP (2013) Biochar–root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability. European Journal of Soil Science: n/a-n/a. doi: 10.1111/ejss.12079.

Revell KT, Maguire RO, Agblevor FA (2012) Influence of Poultry Litter Biochar on Soil Properties and Plant Growth. Soil Science. doi: 10.1097/SS.0b013e3182564202.

Ruiz JM, Rivero RM, Garcia PC, Baghour M, Romero L (1999) Role of CaCl2 in nitrate assimilation in leaves and roots of tobacco plants (Nicotiana tabacum L.). Plant Science 141: 107-115. doi: http://dx.doi.org/10.1016/S0168-9452(98)00230-1.

Sukartono, Utomo WH, Kusuma Z, Nugroho WH (2011) Soil fertility status, nutrient uptake, and maize (Zea mays L.) yield following biochar and cattle manure application on sandy soils of Lombok, Indonesia. Journal of Tropical Agriculture 49: 47-52.

TMECC (2002) Test Methods for the Examination of Composting and Compost. US Composting Council, Bethesda, MD.

Vázquez S, Moreno E, Carpena R (2008) Bioavailability of metals and As from acidified multicontaminated soils: Use of white lupin to validate several extraction methods.

Environmental Geochemistry and Health 30: 193-198. doi: 10.1007/s10653-008-9143-3.

Wenzel W (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant and Soil 321: 385-408. doi: 10.1007/s11104-008-9686-1.

Witters N, Mendelsohn R, Van Passel S, Van Slycken S, Weyens N, Schreurs E, Meers E, Tack F, Vanheusden B, Vangronsveld J (2012a) Phytoremediation, a sustainable remediation technology? II: Economic assessment of CO2 abatement through the use of phytoremediation crops for renewable energy production. Biomass and Bioenergy 39: 470-477. doi: http://dx.doi.org/10.1016/j.biombioe.2011.11.017.

Witters N, Mendelsohn RO, Van Slycken S, Weyens N, Schreurs E, Meers E, Tack F, Carleer R, Vangronsveld J (2012b) Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement. Biomass and Bioenergy 39: 454-469. doi: http://dx.doi.org/10.1016/j.biombioe.2011.08.016.

Zucconi F, Pera A, Forte M, de Bertoldi M (1981) Evaluating toxicity of immature compost. Biocycle 22: 54-57.

3

Effect of biochar amendment on mercury soil pore water concentrations in soil contaminated by mercury mining activities

3.1 Introduction

Mercury has been recognised as a globally significant pollutant, as signified by the recent Minimata Convention in 2013, which bans mercury mining and imposes limits on mercury use in industry. It also makes the clean-up of mercury affected soils and waters a priority, as per Article 12 of the treaty (UNEP, 2013).

Mercury (Hg) mining activities have left a legacy of severe mercury contamination in certain parts of the world, notably the Almadén region in Spain, the world's largest and oldest mercury mining area. Mercury in this region is in the form of cinnabar (HgS), which is generally poorly mobile, although availability to plants and biota of cinnabar contaminated soils has been observed (Barringer et al., 2013; Navarro, 2008). Phytostabilisation is one low impact approach for minimising the spread of mercury contamination by reducing soil erosion and potentially reducing mercury mobility (Esteban et al., 2008; Moreno-Jiménez et al., 2006; Rocio et al., 2013; Rodriguez et al., 2003).

Mercury, particularly the positively charged methylmercury, can form complexes with anions such as chloride, nitrate and sulphate that enhance its solubility in water and potentially increase methylation of insoluble mercury, depending on the concentrations of these anions (Barringer et al., 2013; Gilmour et al., 1992; Navarro, 2008). Soil organic matter reduces Hg availability in porewater through sorption and increases in dissolved organic carbon (DOC) has been shown to be positively correlated with increases in soluble mercury (Barringer et al., 2013; Navarro, 2008; Schelker et al., 2011).

Biochar may enhance plant growth (Lehmann et al., 2011) and reduce the toxicity of mercury contamination to plants. However, there is no data available on biochar interactions with mercury contaminated soil. Activated carbon (AC) has recently been used to remediate of Hg contaminated sediments (Gilmour et al., 2013) and Hg contaminated soil (Bessinger and Marks, 2010) in controlled laboratory experiments. Another study assessed different biochars in addition to activated carbons for its mercury immobilisation potential in a sorption study simulating sediment porewater (Gomez-Eyles et al., 2013). Although results from this study demonstrated superior results for AC over biochar for inorganic mercury, biochar was shown to be just as effective as AC in methylmercury (MeHg) removal from the modelled sediment scenario, possibly explained by affinity between dissolved organic carbon and methylmercury. The study concluded that not enough is known about the sorption mechanisms governing Hg and MeHg for biochars or AC to exclude either one from further study, especially considering the lower cost of biochars (due to cost and resource use involved in standard AC activation by steam or phosphoric acid).

This chapter presents data from the first ever trial to look at the effects of biochar amendment on Hg concentrations in soil porewater and on seed germination in Hg contaminated soil. No data currently exists for the interactions between biochar and mercury porewater in soil. The hypothesis was that biochar would reduce the amount of available Hg in soil porewater (measured as total soluble Hg). The effects of biochar amendment on soluble Hg, dissolved organic carbon (DOC), chloride, nitrate and sulphate was assessed by analysing soil porewater over a five week period. A seed germination assay was carried out to determine the effect of amendment on germination success.

3.2 Materials and Methods

3.2.1 Experiment set up

Soil from the Almáden region of Spain, was obtained from a research institute within the Spanish Ministry for the Environment (CIEMAT) and sieved to <2mm before use. Soil characteristics were as follows and are expressed as mean (+/-SE, n=3): 8707 mg total Hg kg^{-1} (+/-281); 17.5% (+/- 0.03) organic matter; pH 7.7 (+/-0.1) and EC 235 µS cm^{-1} (+/-19). Analysis procedures are described in section 3.2.2.

To put the total mercury content of this soil into context, simulation with the CLEA model (CLEA v1.06 software, Environment Agency) suggest a guideline value of 1340 mg kg^{-1} inorganic mercury in soil with the same organic matter content and pH, assuming no vegetables or soil are consumed by the receptor (female).

Two biochars, derived from the slow pyrolysis of pine woodchip (PB) and olive tree pruning (OB), were used to amend the contaminated soil and were lightly crushed and sieved to between 0.5 and 2mm. Details of how the biochars were produced, the analytical methods used to characterise them and properties of the biochars are in Chapter 2 (Section 2.2.3 and Table 2.1).

Thirty grams of Hg-contaminated soil (sieved to particle size <2mm) was amended with 3% (i.e. 0.9 g) PB or OB on a dry weight basis (thoroughly mixed by end-over-end shaking) and transferred to sealed 50 mL centrifuge tubes. A control scenario consisted of Hg-contaminated soil with no amendment added. Samples were prepared in triplicate for each of the three scenarios: Hg-contaminated soil with no amendment (S), Hg-contaminated soil amended with 3% PB (PB) and Hg-contaminated soil amended with 3% OB (OB). Soil was maintained at 60% of its predetermined water holding capacity, adjusted every two days by weighing samples and adding water as required.

Samples were incubated in the dark at 20°C for the five week duration of the experiment.

Fig 3.1 *Sampling of porewater, which took place on weeks 1, 2, 3 and 5 of the experiment*

Rhizon samplers consist of porous polyethersulphone tubing (Eijkelkamp Agrisearch Equipment, The Netherlands) which filters samples to 0.15 μm upon collection with a vacuum tube (Fig 3.1). One rhizon sampler was inserted into the cap of each sample tube at the beginning of the experiment, and porewater samples were extracted on weeks one, two, three and five in order to obtain four sampling fractions. Once collected, samples were approximately 3 mL in volume. Extracts were acidified immediately with 10 μL HNO_3 and stored/maintained at 4°C until analysis.

At the end of the five week period, pH and EC were determined for each sample. A germination assay using *Lolium perenne*, perennial ryegrass, was carried out to determine the effect of biochar amendment on germination success in the contaminated soil. The germination assay involved transferring the soil samples from the centrifuge tubes to petri dishes (standard 90 mm diameter) and planting with 20

perennial ryegrass seeds. After one week, the number of germinated seeds was counted and % germination for each treatment was calculated.

3.2.2 Analytical procedures

Total mercury in soil was determined following digestion of 1g soil in 12 mL aqua regia by microwave assisted extraction (n=3), following manufacturer recommendations (MARS, CEM). Analysis was carried out by atomic fluorescence spectroscopy (Millennium Merlin, PS Analytical) following serial dilution with nanopure water and according to manufacturer recommendations for mercury analysis. Soil porewater was analysed as above following dilution by a factor of 10.

Porewater samples were analysed for chloride (Cl^-), nitrate (NO_3^-) and sulphate (SO_4^{2-}) by ion chromatography (Dionex). pH and electrical conductivity (EC) of the soil and biochar treatment groups were determined in a water extract after stirring a soil: water 1:5 (w/v) mixture mechanically for 30 mins and a settling period of 10 mins. Organic matter content (OM) was determined by loss on ignition at 550^oC for soil. Water holding capacity was determined according to the procedures described by Rothamsted Research (Grace et al., 2006). Biochar analysis procedures are fully described in Chapter 2 (Section 2.2.3).

3.2.3 Quality control

Internal quality control was monitored by preparing all samples in triplicate. Duplicate injections were performed randomly where there was enough sample to ensure analysis precision. Analytical limit of detection (LOD) for Hg was calculated from the mean plus three times the standard deviation of seven blanks: 10 ng L^{-1}. Analytical blanks were below detection limits for all analytes. Experimental accuracy for Hg analysis was monitored by analysis of a certified reference material, with a recovery of 102% (+/- 0.45 SE) (n=3). Percentage recovery was calculated by dividing the

analytical value by the reference value and multiplying by 100. Random samples were also spiked to test recovery. Recovery for spiked samples was within acceptable limits of 100% +/- 10% SE (n=3). Sample duplicate variation did not exceed 16% relative standard deviation.

3.2.4 Statistical analysis

Statistical analyses were carried out on statistical software IBM® SPSS® Statistics Version 21. Data were checked to fit the hypothesis of normality and homoscedasticity (homoscedastic data have population distributions of equal variance and homoscedasticity is an assumption for ANOVA). All data were normally distributed and were tested with a one-way ANOVA. Tukey's post-hoc test was used for mean comparisons of the homoscedastic data. Games-Howell's test was used for the comparisons of non-homoscedastic data.

3.3 Results and discussion

3.3.1 Effect of biochar on mercury (Hg) concentrations in porewater

Total soluble mercury was measured using the porewater from the rhizon samplers, which would be composed of soluble inorganic and organic (methyl) mercury forms. No appreciable change occurred in the control S over the four fractions sampled in the experiment. PB significantly increased Hg in porewater between weeks 1 and 3, but by week 5 there was no difference observed compared to week 1. OB significantly increased between weeks 1 and 2, but there was no difference in weeks 3 and 5 compared to week one (Fig. 3.2).

In terms of differences between treatments at each sampling fraction, PB or OB amendment did not alter porewater concentrations compared to S except in week 3, where Hg concentrations in PB were significantly higher than S. By week 5,

concentrations had stabilised and no differences were observed between S and PB or OB.

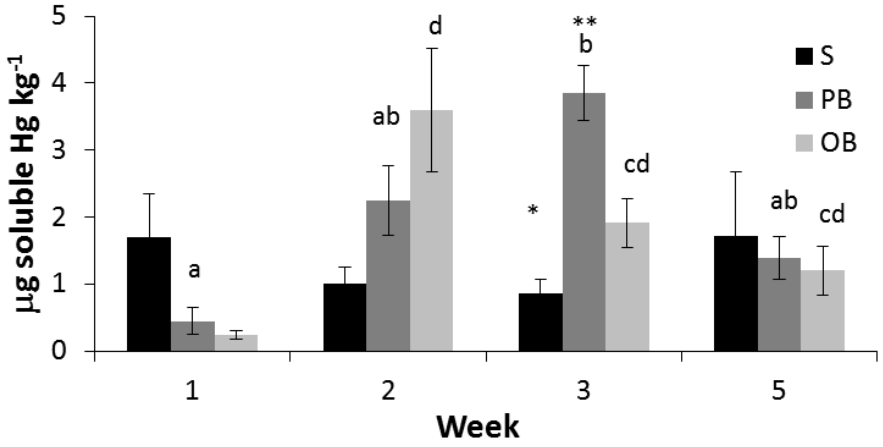


Fig. 3.2 *Porewater Hg in control (S) and biochar amended soils (PB and OB) taken after 1, 2, 3 and 5 weeks. Mean +/- SE (n=3). The letter grouping a-b indicates significant differences (p<0.05) between sampling fractions for PB while the c-d grouping represents OB sampling fractions. S data were non-significant. The asterisks (*, **) indicate differences between groups within a given sampling fraction (* vs. ** p<0.05).*

3.3.2 Effect of biochar on pH, EC, DOC and anions in porewater

Biochar amendment did not affect pH or EC compared to the unamended soil. S had a pH of 7.7 (+/-0.13 SE) and an EC of 235 µS cm^{-1} (+/-0.04 SE). PB had a pH of 7.2 (+/-0.04 SE) and an EC of 289 µS cm^{-1} (+/-3.5 SE) and OB had a pH of 7.3 (+/-0.05 SE) and an EC of 285 µS cm^{-1} (+/-15 SE). DOC did not differ significantly across treatments within sampling fractions and did not differ within treatments across sampling fractions; for the former, the lack of difference is probably attributable to high sample variability for this parameter (Fig. 3.3). One tentative observation is that the biochars reduced DOC fluxes in the soil, which had a naturally high organic matter content (17.5%).

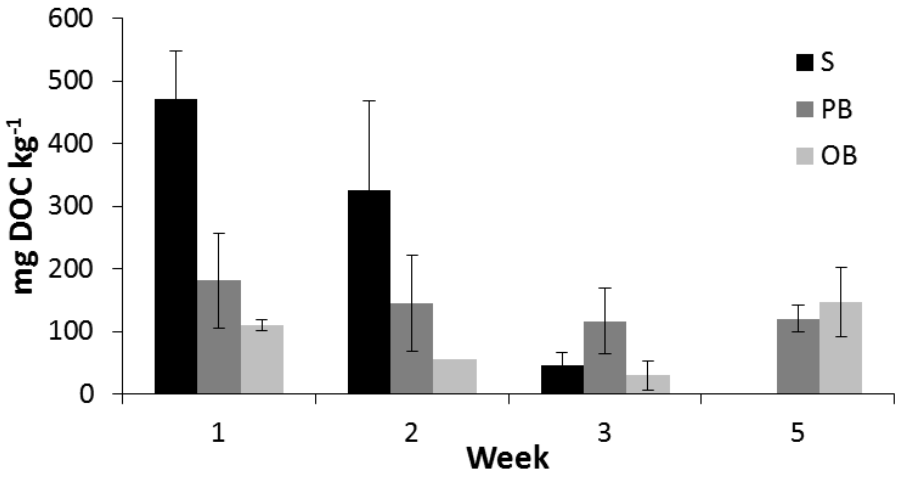


Fig. 3.3 *Dissolved organic carbon (DOC) in porewater in control (S) and biochar amended soils (PB and OB) taken after 1, 2, 3 and 5 weeks. Mean +/- SE (n=2-3), where error bars or data points are absent, n=1.*

Chloride was not altered by amendment for any of the sampling fractions (Fig 3.4a). S and PB did not change over time. OB significantly decreased chloride in porewater in weeks 3 and 5 relative to OB in week 1. Nitrate was not altered by amendment for weeks 1, 3 and 5, but in week 2, nitrate significantly increased in both PB and OB compared to S. None of the treatments changed significantly over the course of the sampling period. Sulphate did not vary across treatments within sampling fractions, nor did it vary for treatments over time.

A Pearson correlation matrix found that nitrate was negatively correlated to DOC ($r=-0.53$, $p<0.01$) but not to any other parameter (Hg, chloride, sulphate). No other correlations were observed. It had been hypothesised that the Hg in solution would correlate with the DOC or some of the anions, due to the strong relationship between Hg and DOC observed elsewhere (Bessinger and Marks, 2010; French et al., 2014; Schelker et al., 2011) and the role of sulphate mobilising mercury (Gilmour et al., 1992). The small sample size of the experiment and high sample variability for DOC and anions could have obscured this correlation.

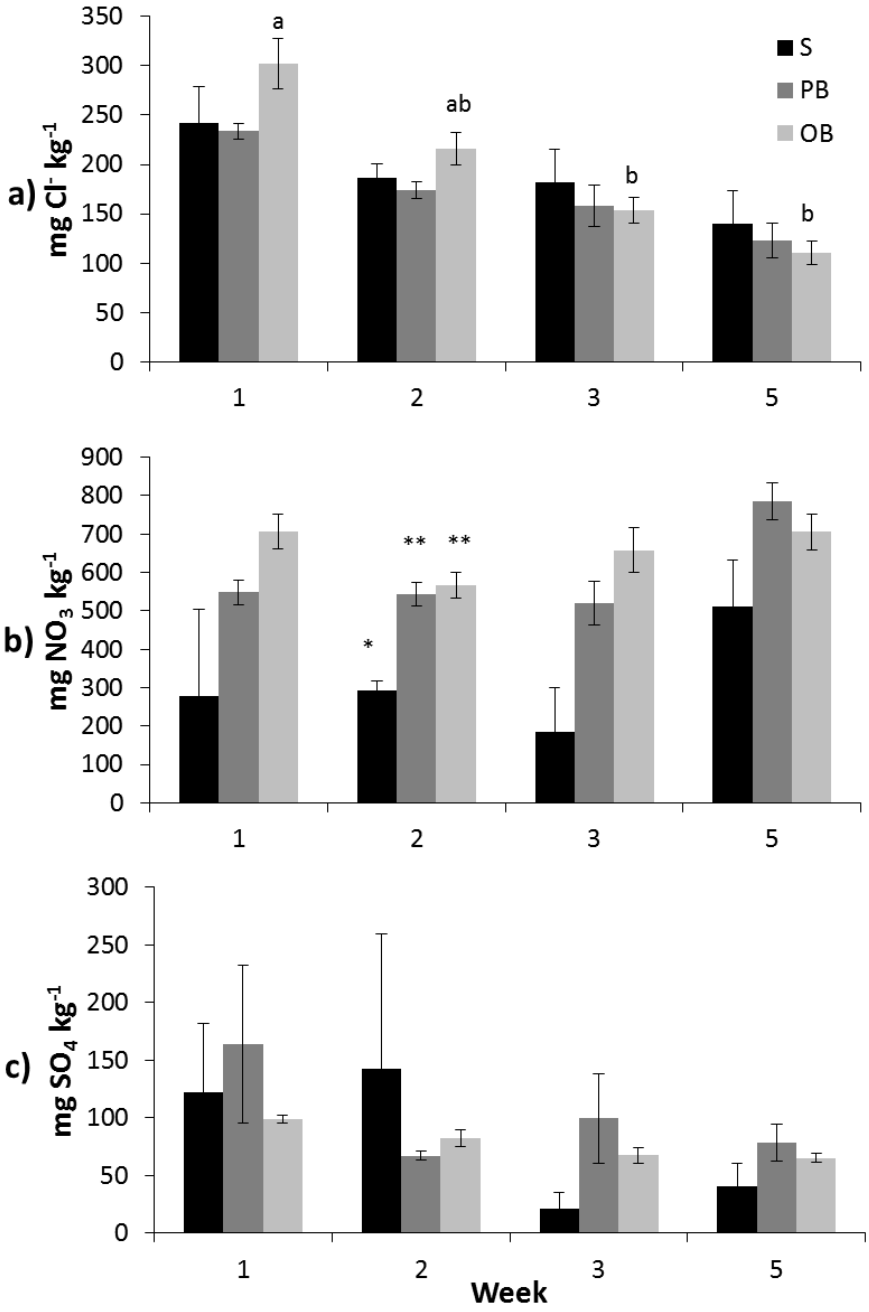


Fig. 3.4 *Anions Cl^-, NO_3^-, SO_4^{2-} in porewater of control (S) and biochar amended soils (PB and OB) taken after 1, 2, 3 and 5 weeks. Mean +/- SE (n=3). The letter grouping a-b indicates significant differences (p<0.05) between sampling fractions for OB. S and PB data were non-significant between sampling fractions. The asterisks (*, **) indicate differences between groups within a given sampling fraction (* vs. ** p<0.05).*

3.3.3 Effect of biochar on germination success

Hg toxicity to plants is thought to be more significant than for other metals (Mishra and Choudhuri, 1999; Munzuroglu and Geckil, 2002). Despite the ambiguous effects of

amendment on Hg in porewater compared to the control (S) observed for both biochars, OB significantly increased the germination rate of ryegrass in the contaminated soil. PB amendment had no effect on germination compared to S (Fig. 3.5). Although the lettuce and cress germination percentages calculated for each biochar are comparable (Chapter 2, Table 2.1), this simple germination test with the contaminated substrate illustrates how different biochars affect plant response in the presence of a phytotoxic contaminant. One possible explanation may lie in the differing bulk densities of the two biochars where OB bulk density is approximately twice that of PB (Table 2.1). This may have caused a dilution effect which led to the improved germination, although it must be noted that this possible effect was not consistently observed for the other analytical parameters and as such may not explain the improved germination in the OB amended soil. Another possible explanation may be that OB significantly reduced chloride in solution (Fig. 3.4a), the soluble mercury in the soil used in this experiment may have been associated with the chloride anion and its reduction in the OB amended soil led to a reduction in toxicity to the seeds. Further analysis, particularly speciation, would be required to evaluate this possible explanation, particularly considering the recent findings of Gomez-Eyles et. al (2013) where biochar was more effective at reducing methyl mercury than inorganic mercury.

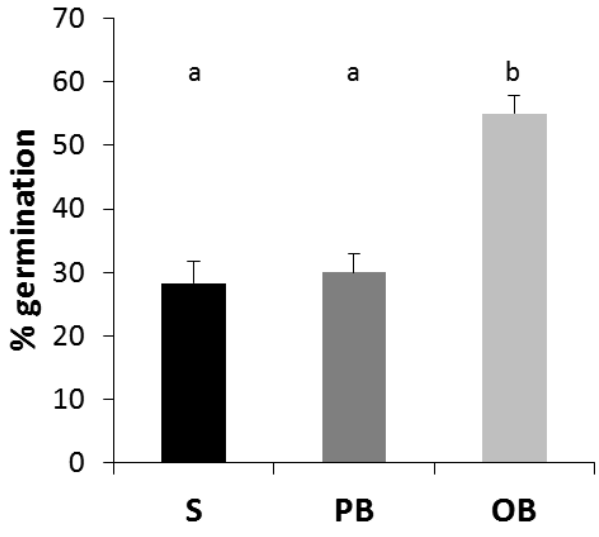


Fig. 3.5 *% Lolium perenne germinated after 7 days for S, PB and OB. Mean +/- SE (n=3). Different letters indicate statistical significance (p<0.05).*

3.4 Conclusions

OB showed the most promise as an amendment for mercury contaminated soils due to the fact itenhanced seed germination in the mercury contaminated soil. Although no effect on Hg in porewater was observed, chloride reduction in OB amended soil suggests mercury speciation may have been changing in the OB amended soil. This experiment was conducted on the micro-scale, potentially obscuring differences between unamended and amended soils. Therefore, scope exists for a larger scale laboratory trial and/or greenhouse trial that examines parameters such as porewater, dissolved organic carbon fluxes, anions and in particular, soluble mercury speciation. Such studies are required to elucidate the potential role of biochars in reducing Hg in porewater to a meaningful extent and aiding the re-establishment of plant growth in mercury contaminated soil. With the signing of the Minimata Convention in 2013, proven low impact techniques are an important tool to reduce the risks posed by mercury contaminated sites across the world.

3.5 Acknowledgements

The award of a Short Term Scientific Mission for A.B. funded by EU COST Action TD1107 is acknowledged for enabling the experimental portion of this work to be undertaken at the Universidad Autónoma de Madrid, Spain. Biochar samples were supplied by José A. Albuquerque. Maria José Sierra Herraiz from CIEMAT, Madrid, is acknowledged for supplying the mercury impacted soil used in the experiment, Sesugh Ande from the University of Strathclyde's Chemistry department is thanked for digesting the total mercury samples and Peter Anderson at Scottish Environmental Technology Network (SETN) is acknowledged for the IC analysis.

3.6 References

Barringer, J.L., Szabo, Z., Reilly, P.A., 2013. Occurrence and Mobility of Mercury in Groundwater. Current Perspectives in Contaminant Hydrology and Water Resources Sustainability.

Bessinger, B.A., Marks, C.D., 2010. Treatment of mercury-contaminated soils with activated carbon: A laboratory, field, and modeling study. Remediation Journal 21, 115-135.

Esteban, E., Moreno, E., Peñalosa, J., Cabrero, J.I., Millán, R., Zornoza, P., 2008. Short and long-term uptake of Hg in white lupin plants:Kinetics and stress indicators. Environmental and Experimental Botany 62, 316-322.

French, T.D., Houben, A.J., Desforges, J.-P.W., Kimpe, L.E., Kokelj, S.V., Poulain, A.J., Smol, J.P., Wang, X., Blais, J.M., 2014. Dissolved Organic Carbon Thresholds Affect Mercury Bioaccumulation in Arctic Lakes. Environmental Science & Technology 48, 3162-3168.

Gilmour, C.C., Henry, E.A., Mitchell, R., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environmental Science & Technology 26, 2281-2287.

Gilmour, C.C., Riedel, G.S., Riedel, G., Kwon, S., Landis, R., Brown, S.S., Menzie, C.A., Ghosh, U., 2013. Activated Carbon Mitigates Mercury and Methylmercury Bioavailability in Contaminated Sediments. Environmental Science & Technology 47, 13001-13010.

Gomez-Eyles, J.L., Yupanqui, C., Beckingham, B., Riedel, G., Gilmour, C., Ghosh, U., 2013. Evaluation of Biochars and Activated Carbons for In Situ Remediation Of Sediments Impacted With Organics, Mercury, And Methylmercury. Environmental Science & Technology 47, 13721-13729.

Grace, C., Hart, M., Brookes, P.C., 2006. Laboratory Manual of the Soil Microbial Biomass Group. Rothamsted Research, http://www.rothamsted.ac.uk/aen/smbweb1/methdnld.html. (cited 5th June 2014)

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biology and Biochemistry 43, 1812-1836.

Mishra, A., Choudhuri, M.A., 1999. Monitoring of Phytotoxicity of Lead and Mercury from Germination and Early Seedling Growth Indices in Two Rice Cultivars. Water, Air, and Soil Pollution 114, 339-346.

Moreno-Jiménez, E., Gamarra, R., Carpena-Ruiz, R.O., Millán, R., Peñalosa, J.M., Esteban, E., 2006. Mercury bioaccumulation and phytotoxicity in two wild plant species of Almadén area. Chemosphere 63, 1969-1973.

Munzuroglu, O., Geckil, H., 2002. Effects of Metals on Seed Germination, Root Elongation, and Coleoptile and Hypocotyl Growth in Triticum aestivum and Cucumis sativus. Archives of Environmental Contamination and Toxicology 43, 203-213.

Navarro, A., 2008. Review of characteristics of mercury speciation and mobility from areas of mercury mining in semi-arid environments. Reviews in Environmental Science and Bio/Technology 7, 287-306.

Rocio, M., Elvira, E., Pilar, Z., María-José, S., 2013. Could an abandoned mercury mine area be cropped? Environmental Research 125, 150-159.

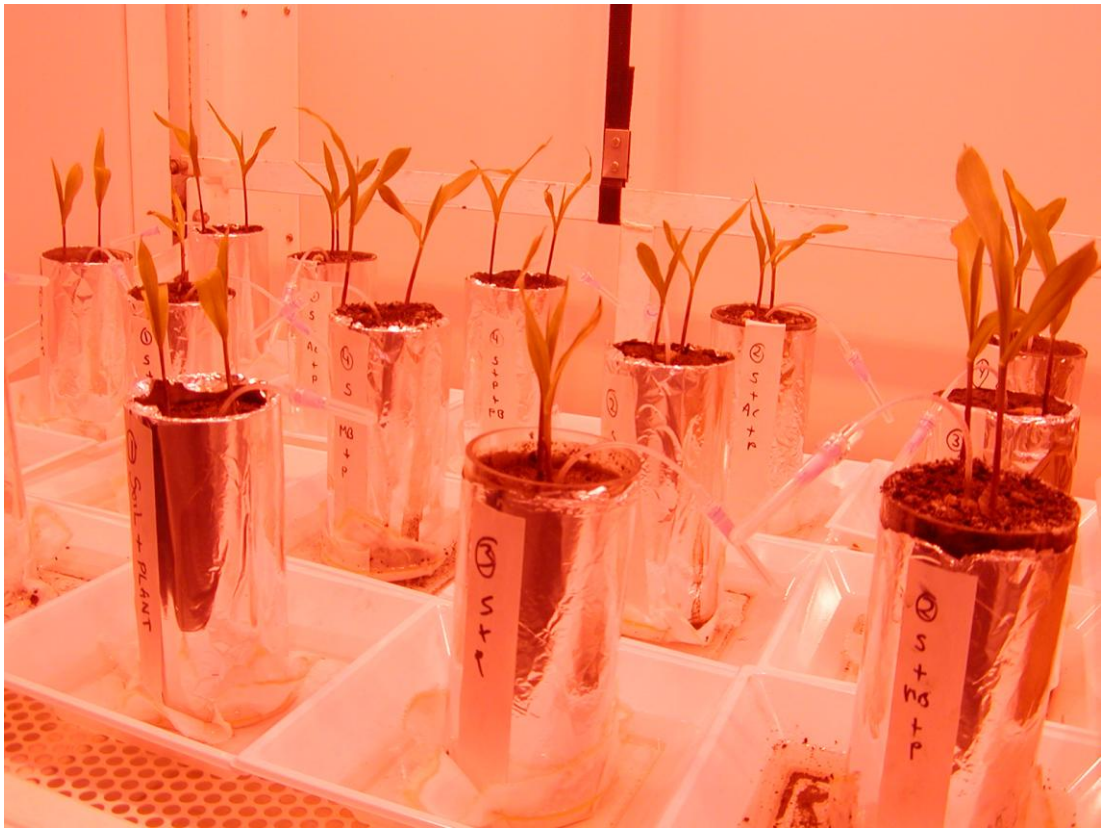
Rodriguez, L., López-Bellido, F., Carnicer, A., Alcalde-Morano, V., 2003. Phytoremediation of mercury-polluted soils using crop plants. Fresenius Environmental Bulletin 12, 967-971.

Schelker, J., Burns, D.A., Weiler, M., Laudon, H., 2011. Hydrological mobilization of mercury and dissolved organic carbon in a snow-dominated, forested watershed: Conceptualization and modeling. Journal of Geophysical Research: Biogeosciences 116, G01002.

UNEP, 2013. Minimata Convention on Mercury- Text and Annexes. United Nations Environment Programme (UNEP), Geneva, Switzerland, p. 69.

4

Effects of biochar and activated carbon amendment on maize growth and the uptake and measured availability of polycyclic aromatic hydrocarbons (PAHs) and potentially toxic elements (PTEs)



4.1. Introduction

Contamination arising from industrial and other anthropogenic activities has led to widespread contamination of soils with both inorganic and organic contaminants. This situation has the potential to affect entire ecosystems as well as to pose risk to human health. Recent advances in the understanding of contaminant behaviour in soils have driven a greater focus on bioavailable fractions of contaminants: how to assess contaminant availability and how to reduce the bioavailable fraction.

The use of carbonaceous sorbents as soil amendments has the potential to reduce contaminant bioavailability (Ahmad et al., 2014; Beesley et al., 2011; Denyes et al., 2013; Hale et al., 2012; Karami et al., 2011; Marchal et al., 2014). This trend comes from a greater understanding of sorption dynamics and organic contaminant relationships with carbonaceous fractions in soils and sediments (Cornelissen et al., 2005; Luthy et al., 1997; Pignatello and Xing, 1995). Both activated carbon and biochar amendments have demonstrated positive results. Plant establishment can be enhanced by amendment and contaminant availability can be reduced (Fellet et al., 2014; Jakob et al., 2012), but results vary widely because of the heterogeneous nature of different biochars.

The environmental impact of the sorbents themselves is another important consideration if remediation practices are to be ultimately sustainable. A life cycle assessment (LCA) study on the use of activated carbons (AC) for sediment remediation found that coal derived AC had a higher environmental footprint than biomass derived AC (coconut waste) when energy and resource use were factored into the analysis (Sparrevik et al., 2011). If the activation step is removed from the process (e.g. steam or phosphoric acid activation to increase porosity and surface area), biochars are also of a lower cost than activated carbons, US$51 - 386 per tonne for biochars (Meyer et al.,

2011) compared to around US$2200 per tonne for activated carbon (Ghosh et al., 2011), although prices are highly dependent on market fluxes. These LCA and cost factors highlight the potential for biochar use in remediation, if its efficacy can be established.

Carbonaceous sorbent amendment may assist phytostabilisation as part of an integrated *in situ* remediation approach. Biochar research in the agriculture domain has shown that biochar has the capacity to alter soil physical and chemical properties , leading to potentially beneficial effects on plant establishment and growth (Atkinson et al., 2010; Lehmann et al., 2011). Phytomanagement of degraded soils aims to establish plant cover that primes ecosystem succession and concomitantly reduces soil erosion and contaminant mobility on a degraded site. Biomass crop generation on degraded sites is a proposed solution for deriving commercial benefit from a phytomanagement approach (Houben et al., 2013; Van Slycken et al., 2013). Maize (*Zea mays*) is a potential crop choice due to its quick growth cycle and high biomass production, having previously been used to investigate contaminant impact on plant health and growth (Lin et al., 2008). However, a greater mechanistic understanding of the effects of amendment on contaminant availability and plant establishment, as well as interactions between contaminants, plants and soils is required before full scale field application.

In this paper, we present results from a 21 day pot trial growing maize in an experiment designed to compare the efficacy of two different biochars and a commercial activated carbon in reducing the negative effects of soil contaminants on plant establishment. Based on the hypothesis that the carbonaceous sorbents would reduce contaminant availability to plants and in the soil and improve plant growth

overall, polycyclic aromatic hydrocarbon (PAH) and potentially toxic element (PTE) concentrations were assessed in the soil, soil porewater and plants across treatments.

4.2. Methods

4.2.1 Experimental set up

Soil was obtained from a former manufactured gas plant site in the UK and from an abandoned mine site in Spain. Both soils were air dried, sieved to 4 mm and mixed together in the ratio 1:1 in order to obtain a soil with both organic and inorganic contaminants. The resulting soil was classified as a loam (43% sand, 47% silt and 10% clay), with a pH of 7.1 and 7.1% organic matter content. The soil was contaminated with As, Cu and Zn (3604, 276 and 2226 mg kg^{-1}, respectively) and moderate levels of 13 USEPA priority PAHs (those with three or more benzene rings, 68.6 mg kg^{-1}). Analysis procedures for the above measuresd parameters are detailed in Section 4.2.2.

Two biochars, derived from the slow pyrolysis of pine woodchip (PB) and maize stubble (MB), were used to amend the contaminated soil in order to investigate feedstock differences and were lightly crushed and sieved to 0.5 - 2mm. Biochars were produced in a pilot plant at 450 °C by the University of León, with a 15 minute residence time in the reactor (Natural Resources Institute, Spain). Biochar properties are summarised in Table 4.1. Methods used for characterising the biochar properties are fully described in Chapter 2 (Section 2.2.3). The activated carbon (AC) used in the experiments was in granular form and branded as Norit® GAC 1240 (Norit, USA), with the following properties: bulk density 0.49 g cm^{-3}, specific surface area 1175 m^2 g^{-1},pH 10.3, effective particle size 0.65mm (range 0.42mm-1.7mm) (data provided by manufacturer).

Table 4.1 Characteristics (on a dry weight basis) of the two biochars (PB: pine woodchip biochar, MB: maize stubble biochar).

Parameters	PB	MB
Bulk density (g cm^{-3})	0.63	0.24
Liming equivalence (g $CaCO_3$ kg^{-1})	7.4	61.6
pH	7.52[a]	9.81[a]
Electrical conductivity (µS cm^{-1})	256[a]	2945[a]
Organic matter (g kg^{-1})	982	794
C (g kg^{-1})	837	686
N (g kg^{-1})	3.6	7.9
P (mg kg^{-1})	148	2981
K (mg kg^{-1})	1708	22331
Zn (mg kg^{-1})	42	99
Cu (mg kg^{-1})	134	41
As (mg kg^{-1})	1.7	n.d.[b]
∑13 EPA PAH (mg kg^{-1})[c]	18.5	14.5
Specific surface area (m^2 g^{-1})	288	240
Germination index (lettuce, %)	92	66
Germination index (cress, %)	117	92
Cation exchange capacity (cmol kg^{-1})	12.6	52.3
Water-soluble fractions		
Water-soluble organic C (WSC, mg kg^{-1})	920	2919
Water-soluble inorganic C (mg kg^{-1})	122	1817
Water-soluble N (WSN, mg kg^{-1})	10	41
WSC/WSN	90	71
Water-soluble P (mg kg^{-1})	6	489
Water-soluble K (mg kg^{-1})	256	7632

[a] water extract 1:10 (w/v) [b] n.d. not determined [c]PAHs from flourene to benzo (g,h,i) perylene

Four soil treatments were prepared: contaminated soil only (C), soil plus 3% PB (PB), soil plus 3% MB (MB) and soil plus 3% AC (AC). Each pot was prepared with 500 g (+/- 0.5 g) of soil plus 15 g (+/- 0.01 g) biochar or AC in the relevant treatments and mixed thoroughly manually together and then with 100 g (+/-0.1 g) of pre-cleaned pebbles (size range 20-25mm). The pebbles were added in order to give the soil structure and minimise compaction and anoxic conditions during the experiment. Pebbles were pre-

cleaned in bulk by sequential washing, first soaking in 5% HCl, then in deionised water, then rinsed three times. Each mixture was then added to a plant pot containing 100 g of pebbles at the base, watered to 60% of its water holding capacity (WHC), weighed and left to equilibrate for one week before planting.

Eight replicates for each soil treatment were prepared, resulting in a total of 32 plant pots. Within the eight replicates for each soil treatment, four were planted with maize germinants (+P), and four left unplanted (-P) to in order to compare differences between planted and unplanted soil treatment scenarios. As such, results are discussed according to the following treatment groups: C-P, C+P, PB-P, PB+P, MB-P, MB+P, AC-P, AC+P.

Maize seeds were washed and pre-germinated before planting to ensure only viable seeds were used. They were washed by sonicating in 10% sodium hypochlorite for 30 minutes and then in deionised water for 30 minutes. They were then placed on tissue paper moistened with deionised water and several drops of calcium sulphate (1.5 mM) and incubated at 28°C for 72 hours for germination (Clark et al. 1999).

After one week, four pots from each of the treatment scenarios were planted with two maize germinants per pot and all pots were moved to a controlled growth chamber for a 21 day period (with a day/night cycle of 13/11 hours, temperature/relative humidity 25°C/40% by day and 20°C/60% by night and light intensity 520µmol m^{-2} s^{-1}). 60% WHC was maintained in the pots throughout the experiment by weighing and adjusting water content as necessary.

4.2.2 Sampling regime and methods

4.2.2.1 Soil analysis procedures

Soil particle size distribution was determined using standard method ISO 11277:2009 (ISO 2009). pH and electrical conductivity (EC) of the soil samples were determined in the water extract 1:5 (w/v) after stirring the mixture mechanically for 2 hours. Organic matter content (OM) was determined by loss on ignition at 550^{o}C. Water holding capacity was determined according to the procedures described by Rothamsted Research (Grace et al., 2006).

4.2.2.2 Plant extraction and analysis

Shoots were cut 1cm above the soil surface. Roots were carefully removed from the soil, shaken gently to remove excess soil and then cleaned by rinsing and then sonicating in deionised water and gently patting dry with tissue. Plant shoots and roots were weighed for fresh and dry biomass before and after freeze drying. Freeze dried samples were extracted and analysed for PTEs and PAHs according to the methods described in sections 4.2.2.3 and 4.2.2.4.

Fresh shoot material was analysed for chlorophyll content. A 5mL solution of 80% acetone was added to 0.1g shoot tissue and ground with a mortar and pestle, which was then filtered into a 15mL centrifuge tube and the process repeated twice more. Chlorophyll *a*, chlorophyll *b* and total carotenoids were then determined by UV spectrophotometry at 663nm, 645nm and 480nm. (Wellburn, 1994)

4.2.2.3 Polycyclic aromatic hydrocarbons (PAHs)

At the end of the experiment all soil and amended soil samples were sieved to < 2 mm prior to extraction and analysis. Total and freely dissolved PAH concentrations were determined at the end of the experiment for all samples. Total were determined by hexane-acetone extraction (Gomez-Eyles et al., 2011) while freely dissolved

concentrations were determined by aqueous equilibrium experiments using polyoxymethylene (POM) samplers (Jonker and Koelmans, 2001).

For total extractions, 4g of soil or soil + amendment with surrogate solution added (fluorene-D10, phenanthrene-D10, fluoranthene-D10, chrysene-D12) was extracted twice with 10mL 1:1 hexane-acetone for 2 hours per extraction on an orbital shaker at 20°C (Gomez-Eyles et al., 2011). The extractant was filtered with Whatman filter paper grade GF/F. Each vial was then rinsed twice with 10mL hexane-acetone, the resulting 40 mL was evaporated to 2mL under a gentle stream of nitrogen, exchanged to cyclohexane and cleaned up with a silica gel column topped with sodium sulphate (after EPA method 3630C). A 1mL aliquot of the resulting eluate was analysed by GC-MS following addition of internal standards (1-fluoronaphthalene, p-terphenyl-D14, benzo(a)pyrene-D12). GC-MS conditions were as follows: Trace Ultra GC coupled with DSQ II (Thermo Scientific); splitless mode; column DB-5MS 30m x 0.25mm x 0.25µm; initial temperature 45°C, hold 2 min, ramp 2°C per min to 80°C, then ramp 4°C per min to 320°C, hold 5 min.

Aqueous equilibrium experiments were used to measure freely dissolved fractions of PAHs in the soil at the end of the experiment. Polyoxymethylene (POM) passive samplers in strips 76 µm thick (POM-76) (CS Hyde, IL, USA) were shaken with soil aliquots slurried with 40 mg L^{-1} sodium azide solution for 30 days (Gomez-Eyles et al., 2011; Jonker and Koelmans, 2001). After 30 days, POM samplers were cleaned with damp tissue, phenanthrene-D10 surrogate standard was added and the POM was extracted three times with 20mL 1:1 hexane-acetone solution for 24:2:2 hours. The resulting 60mL solution was concentrated to 2mL under nitrogen and cleaned (after EPA method 3630C). The resulting eluate was concentrated to 1mL, at which point internal standard for GC-MS analysis was added as for totals extractions. K_{POM} values

used for calc` ulating C_w (where $C_w = C_{POM}/K_{POM}$) were taken from literature derived values for POM-76 (Endo et al., 2011).

Root and shoot samples were extracted three times by sonicating approximately 0.1g of tissue with surrogate solution added (as for total soil extractions) in 20mL 1:1 hexane: acetone for 2, 0.5 and 0.5 hours. Samples were then cleaned and analysed as for totals in soil and POM extractions.

Pure biochar (PB, MB) samples were extracted in triplicate by accelerated solvent extraction (Dionex ASE 350) at 100°C by sequential extraction. 1 g biochar sample was ground to a fine powder, mixed with diatomaceous earth into a 5 mL cell and extracted twice with toluene. Toluene has previously been shown to be a suitable extraction solvent for these materials (Hilber et al., 2012). Surrogate recovery was monitored by the addition of phenanthrene-D10, anthracene-D10, and chrysene-D12. In-cell clean-up was performed using 2g activated silica gel (Sigma Aldrich) at the bottom of the ASE extraction cell in addition to a glass fibre filter (Dionex). Extracts were evaporated under a gentle stream of nitrogen to 1 mL, filtered to 0.2 μm with glass syringes using PTFE syringe filters and analysed by GC-MS as described above.

Percentage surrogate recovery was calculated by dividing the analytical value by the reference value and multiplying by 100. Surrogate recovery exceeded 62% for all total soil extractions data presented (median 98%, mean 91%, rsd 18%). For POM-76 extractions, surrogate recovery exceeded 73% (median 100%, mean 99%, rsd 7%). For plant extractions, recovery exceeded 64% (median 88%, mean 92%, rsd 27%). Biochar recovery exceeded 72% (median 89 %, mean 84 %, rsd 12 %)

4.2.2.4 Potentially toxic elements (PTEs)

Following autoclaving (Lozano-Rodriguez et al., 1995) and ammonium sulphate extraction (Vázquez et al., 2008), pseudo-total and extractable As in the treatments

were determined by atomic fluorescence spectroscopy (Millennium Excalibur, PS Analytical). Pseudo-total and extractable Cu and Zn were determined by atomic absorption spectroscopy (AA800, Perkin Elmer).

For pseudo-total soil concentrations, 0.5 g of soil was transferred into 50 ml autoclave bottles to which 6 ml of MilliQ water, 6 ml of 65% HNO_3 and 4 ml of 33% H_2O_2 were added. The autoclave was set at pressure 1.5 kg cm^{-2} (147kPa) and at temperature 125°C for 30 minutes, samples were left to cool, then filtered and made up to 50 mL (Lozano-Rodriguez et al., 1995).

Total plant concentrations were determined by weighing 0.1 g dried plant tissue into 20 ml autoclave bottles to which 2 ml of MilliQ water, 1.5 ml of 65% HNO_3 and 1 ml of 33% H_2O_2 were added. The samples were then autoclaved under the conditions described in the previous paragraph, cooled, then filtered and made up to 5 mL.

Extractable PTEs in the soils were determined by extracting 1.5 g of soil with 15 ml of $(NH_4)_2SO_4$ 0.1M in 50 ml tubes and shaking for four hours at 180 rpm. The samples were then filtered and 0.1 ml of HNO_3 was added (Vázquez et al., 2008).

4.2.3 Statistical and data analysis

Statistical analyses were carried out on statistical software IBM® SPSS® Statistics Version 21. Data were checked to fit the hypothesis of normality and homoscedasticity; log transformation was applied to data as necessary (homoscedastic data have population distributions of equal variance and homoscedasticity is an assumption for ANOVA). Hypotheses were tested with ANOVA. Tukey's post-hoc test was used for mean comparisons of the homoscedastic data. Games-Howell's test was used for the comparisons of non-homoscedastic data.

BSAFs (biota-soil accumulation factor) were calculated for the PAH concentrations in maize shoots by use of the following equation: $C_{PAH\ shoot}/(C_{PAH\ soil}*f_{OM})$, where shoot PAH concentrations for each treatment were divided by the soil PAH concentrations (from the control soils) normalised to the soil organic matter (OM) fraction (for each treatment) (Jakob et al., 2012).

In order to predict root values from POM data, K_{lip} and K_{ch} values were taken from the SI section of Gomez Eyles et al (2011). Lipid and carbohydrate fractions used (1.1% for lipids and 15.3% for carbohydrates in the roots of wheat plants) were taken from Li et al (2005). The equation used for calculating predicted data was taken from Zhang and Zhu (2009):

$$C_{root\text{-}predicted} = C_{free}(f_{lip}K_{lip} + f_{ch}K_{ch})$$

where $C_{root\text{-}predicted}$ is the predicted root concentrations, C_{free} is the freely dissolved calculation measured by POM, f_{lip} is the lipid fraction (0.011 in this study) K_{lip} is the lipid partitioning coefficient, f_{ch} is the carbohydrate fractions (0.153 in this study) and K_{ch} is the carbohydrate partitioning coefficient.

4.3. Results and discussion

4.3.1 Soil PAH concentrations

PAHs were grouped according to the number of benzene rings in their structure, due the similar statistical patterns observed from analysis of the individual compounds: 3 ring PAHs (fluorene, phenanthrene, anthracene), 4 ring PAHs (fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)flouranthene, benzo(k)flouranthene) and 5/6 ring PAHs (benzo(a)pyrene, dibenz(a,h)anthracene, indeno(a)pyrene, benzo(ghi)perylene). Hexane-acetone extracted concentrations are presented in Fig 4.1. Observed reductions in the amended soils compared to the unamended soil are considered to represent the sorbent bound PAHs, due to the higher black carbon

content of carbonaceous sorbents, which affected PAH extractability by hexane-acetone and has also been noted for other solvents (n-heptane) (Beesley et al., 2010; Hale et al., 2012). As such, total PAHs in the soil are considered to be the total derived from the unamended soil extraction plus the PAHs native to the biochars and activated carbon for the relevant amendments, although this sorbent PAH input is not significant.

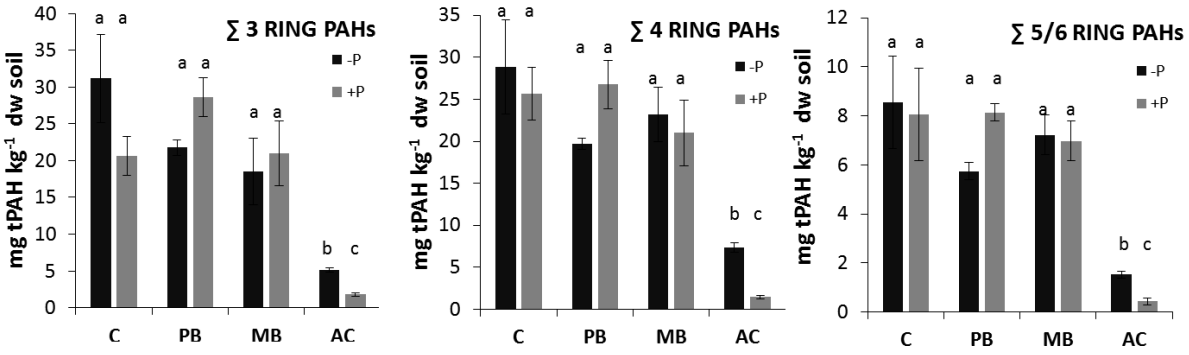


Fig. 4.1 *Hexane-acetone extractable concentrations of PAHs in planted (+P) and unplanted (-P) contaminated soil with different biochar treatments, C: control, PB: pine woodchip biochar amended soil, MB: maize husk biochar amended soil, AC: activated carbon amended soil. Mean ± SE (n=3-4). Different letters signify differences between groups at p<0.001. Data were log transformed to fit homoscedasticity for post hoc tests.*

4.3.2 Effect of sorbent amendment on PAH bioavailability and plant uptake

POM extractions suggested there were no difference in porewater PAH concentrations between unplanted and planted replicates within amendment groupings (Fig. 4.2), apart from the 5/6 ring PAH class where MB-P had significantly higher porewater PAHs than MB+P. The results for the 3 and 4 ring PAHs is in contrast with the findings by Marchal et al (2014), where the unplanted soil had higher anthracene, fluoranthene and pyrene values than the planted soil, while phenanthrene did not differ between the two scenarios. No data is available from this study for the differences between unplanted and planted amended soils for comparison. A number of possible reasons could account for the differences observed in our study, from the use of spiked soil in

the cited study versus the field contaminated soil used in our study, to the different timescales employed, 60 days in the cited study versus 21 days in the current study.

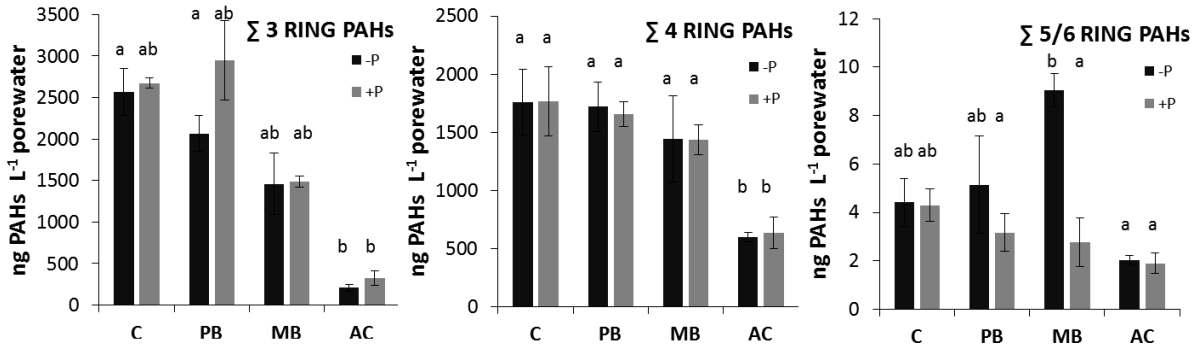


Fig. 4.2 *Porewater concentrations of PAHs in planted (+P) and unplanted (-P) contaminated soil with different biochar treatments, C: control, PB: pine woodchip biochar amended soil, MB: maize husk biochar amended soil, AC: activated carbon amended soil. Mean ± SE (n=4).Different letters signify statistical differences between treatments at p<0.05.*

Assessing porewater PAHs according to amendment type, biochar had no effect on porewater concentrations for 3 and 4 ring PAHs, while AC showed a significant reduction in porewater concentrations compared the control. For 5/6 ring PAHs, none of the studied amendments reduced the porewater concentrations. Indeed, the MB-P demonstrated a significant increase in porewater concentrations compared to the controls (Fig 4.2). While this increase may partly be accounted for by the native PAHs in the MB biochar (Table 4.1, 14.5 mg kg^{-1} ∑13 EPA PAH), it is unlikely (Freddo et al., 2012). The observed increase is likely to have been caused by other factors and this increase is no longer observed when plants are in the system (see MB-P vs. MB+P in Fig. 4.2). Possible factors are increased dissolved organic carbon fluxes with biochar addition or 5/6 ring PAH mobilisation due to interactions with inorganic or organic co-contaminants.

The AC results reflect findings from other short term studies where rapidly desorbing fraction of lower molecular weight PAHs bound quickly to the studied GAC amendment

compared to the unamended control soil (Brändli et al., 2008), while the heavier 5/6 ring PAHs showed limited differences between controls and amended soils in the short term. The cited study had similar contact times to the current study. Longer contact times using field amended soils have previously highlighted effective reduction of freely dissolved heavier PAHs by GAC (Oen et al., 2011). Sorbent particle size is another potential factor for the biochar and AC carbon results in this study, as powdered activated carbon (PAC) has been shown to be more effective in the short term to mid-term reduction of porewater PAHs (Brändli et al., 2008; Hale et al., 2012). Nonetheless, in the longer term GAC and biochars may be more beneficial for overall effects on plant growth and soil biota, perhaps partially due to the larger particle sizes, although this merits further study (Gomez-Eyles et al., 2013; Jakob et al., 2012; Lehmann et al., 2011).

Root PAH concentrations were not significantly altered by PB, MB or AC (Fig 4.3). PAH shoot uptake was significantly reduced by MB and AC for all PAH classes, but not by PB (Fig 4.3). It is not clear exactly why PAH shoot uptake was reduced in MB amended soils and not PB amended soils, and demonstrates that shoot uptake may be explained by differences in biochar properties creating differences in soil conditions. Differences in EC, CEC, soluble NPK, bulk densities (Table 4.1) may be contributing factors, but the influence of parameters not measured, such as particle size distributions, oxygen contents cannot be ruled out (Atkinson et al., 2010). This trend in shoot uptake was supported by the BSAF data, which showed significant reductions in BSAF for MB and AC compared to the control. PB reduced BSAF by 33% (+/-5%) for 3 ring PAHs ($p=0.063$), 25% (+/-9%) for 4 ring PAHs ($p=0.202$), 27% (+/-7%) for 5 ring PAHs ($p=0.138$). MB reduced BSAF by 58% (+/-5%) for 3 ring PAHs ($p<0.01$), 57% (+/-7%) for 4 ring PAHs ($p<0.05$), 65% (+/-7%) for 5 ring PAHs ($p<0.001$). AC reduced BSAF by 42% (+/-4%) for 3 ring PAHs ($p<0.05$), 44% (+/-14%) for 4 ring PAHs ($p<0.05$), 58% (+/-6%) for 5 ring PAHs ($p<0.001$). These findings demonstrate the heterogeneous

results produced by biochars from different feedstocks and the activated carbon data support the results of other studies where BSAFs of bio-relevant PAHs were reduced (Jakob et al., 2012).

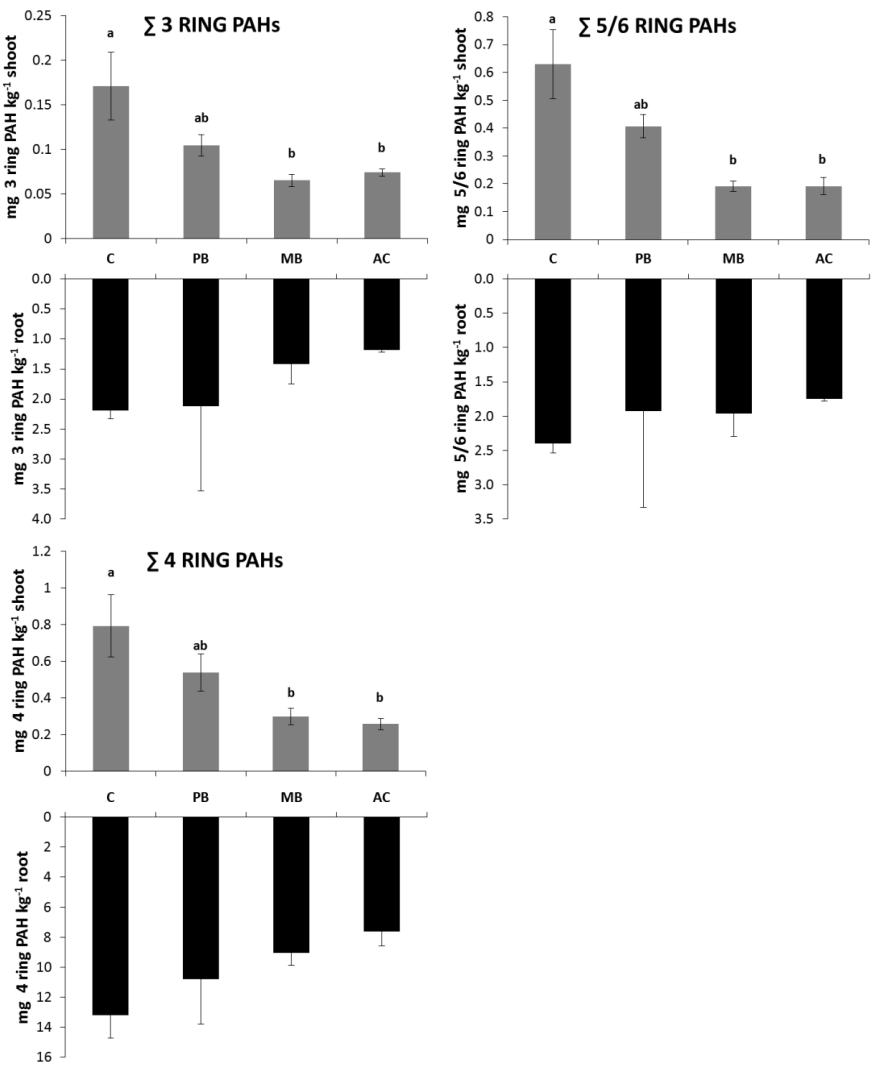


Fig. 4.3 *PAH concentrations in shoots and roots of maize plants growing contaminated soils with different biochar treatment, C: control, PB: pine woodchip biochar amended soil, MB: maize husk biochar amended soil, AC: activated carbon amended soil. Mean ± SE (n=2-4, 2 reps in the case of C and PB root data, 3-4 reps for all other data). Different letters mean statistical differences between shoot groups at p<0.05, no root data showed statistically significant differences.*

Fig 4.4 explores the relationship between actual root uptake (Table 4.2) and predicted values using POM-derived data. A sorption prediction model proposed by Zhang and

Zhu (2009) that accounts for both carbohydrate and lipid PAH partitioning to plant roots was assessed for its efficacy in predicting sorption to the plants used in the current experiment. Gomez-Eyles et al. (2011) used POM-derived porewater PAH concentrations to apply the model and the same POM approach was used here. However, lipid and carbohydrate fractions were not determined for the maize plants used in this experiment and so lipid and carbohydrate fractions of wheat roots and shoots (Li et al., 2005) were used for the predictions presented. Despite this, the POM derived data provides a fairly accurate assessment of root uptake in the current study with all data falling within one order of magnitude on the log scale. AC PAH uptake to root is slightly under-predicted and PB data is variable (Fig 4.4).

Table 4.2 *Averaged values for individual PAH compounds in porewater (POM) and roots (n=3-4 POM, n=2-4 roots). Individual data points from +P data were used to compare root predictions using POM to actual root data.*

	FLU	PHE	ANT	FLUA	PYR	BaA	CHR	BbF	BkF	BaP	IdP	DbA	BghiP
Freely dissolved concentrations in planted pots (± SE, expressed as ng L^{-1})													
C+P	560 ± 79.2	1542 ± 91.7	572 ± 42.2	1071 ± 173	644 ± 120	27.5 ± 3.04	18.4 ± 3.47	6.62 ± 1.01	1.55 ± 0.22	3.82 ± 0.65	0.19 ± 0.05	0.053 ± 0.014	0.237 ± 0.05
PB+P	741 ± 172	1631 ± 232	576 ± 83.8	1011 ± 64.2	579 ± 37.3	38.1 ± 14.7	22.3 ± 7.41	4.58 ± 1.16	1.17 ± 0.3	2.48 ± 0.6	0.24 ± 0.06	0.074 ± 0.008	0.366 ± 0.13
MB+P	293 ± 27	904 ± 63.4	291 ± 21.7	853 ± 70.6	548 ± 48.8	17.0 ± 4.22	14.4 ± 2.18	4.00 ± 1.48	0.993 ± 0.37	2.25 ± 0.82	0.205 ± 0.08	0.067 ± 0.028	0.241 ± 0.07
AC+P	30.9 ± 9.66	213 ± 56.3	77 ± 21.8	372 ± 82.6	241 ± 49.1	10.7 ± 2.25	8.20 ± 2.00	2.72 ± 0.62	0.681 ± 0.15	1.52 ± 0.35	0.153 ± 0.03	0.052 ± 0.014	0.180 ± 0.03
Root concentrations (± SE, expressed as µg kg-1)													
C+P	173 ± 10.8	1551 ± 93.4	465 ± 33.8	5113 ± 819	3677 ± 562	1324 ± 55.7	1388 ± 5.2	1195 ± 31	522 ± 55	955 ± 14.8	567 ± 55	228 ± 51	645 ± 41.2
PB+P	211 ± 162	1412 ± 824	498 ± 322	4091 ± 976	3139 ± 822	1174 ± 358	1441 ± 332	881 ± 351	383 ± 159	744 ± 368	494 ± 283	182 ± 100	505 ± 258
MB+P	145 ± 59.6	967 ± 199	304 ± 74	3003 ± 263	2367 ± 179	1262 ± 19.7	1050 ± 162	955 ± 152	418 ± 47.6	769 ± 134	483 ± 60.1	195 ± 0.8	515 ± 42.8
AC+P	88.7 ± 5.05	787 ± 3.3	305 ± 26.3	2601 ± 283	1945 ± 208	885 ± 85	955 ± 166	877 ± 146	379 ± 69.7	700 ± 83.7	429 ± 74.1	171 ± 37.3	446 ± 75

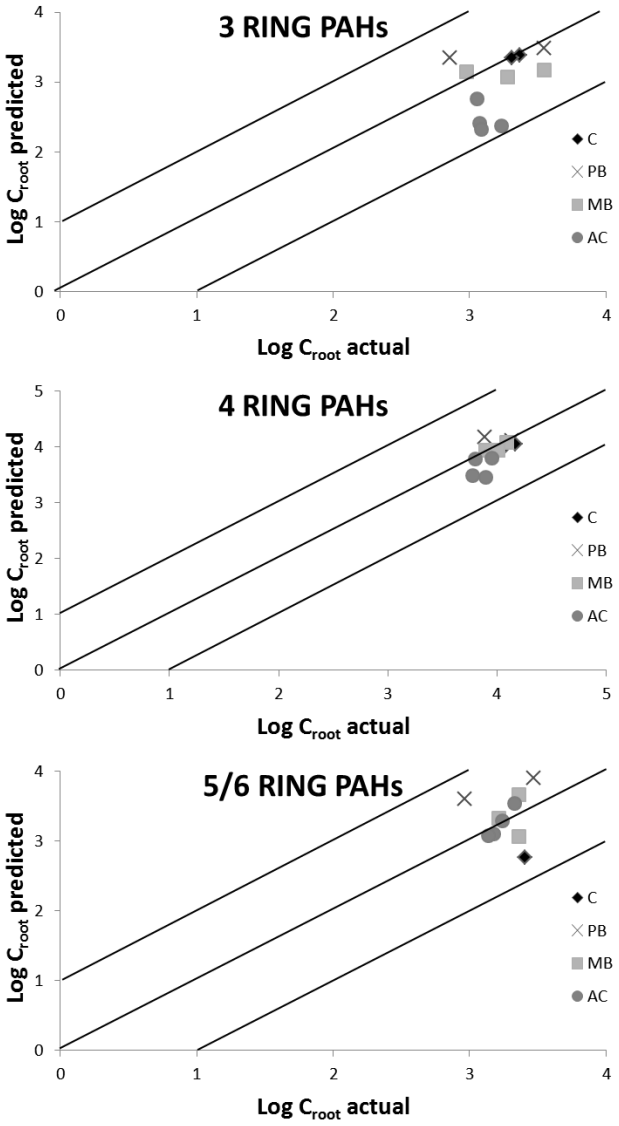


Fig. 4.4 *Predicting root concentrations using POM. Middle line indicates a 1:1 relationship while the lines on either side represent one order of magnitude either way.*

AC showed the greatest decrease in porewater concentrations, yet had similar PAH uptake to roots and shoots as MB (Figs 4.2 and 4.3). It is not clear why this occurred, as previous studies investigating PAH uptake to plants have demonstrated the importance of water soluble fractions in PAH root uptake and subsequent translocation to shoots (Gao et al., 2011; Gao and Collins, 2009). As suggested by other authors (Gomez-Eyles et al., 2011; Yoshitomi and Shann, 2001) interactions with root exudates may affect uptake and in the current study, differences in root exudate production among

treatments may have affected uptake, although this would need to be confirmed by further study. As we have shown (Fig 4.4), measuring PAHs in soil porewater and comparing to PAH plant uptake may contribute to further understanding of the mechanisms behind PAH uptake to plants, particularly with regards to amended soils. Even if is this does not prove to be the case, using POM remains an inexpensive and straightforward method for monitoring changes in freely dissolved PAH concentrations.

Taking both PAH porewater data and PAH plant uptake data into account, AC displayed consistent improvements compared to controls. Nonetheless, MB proved effective at reducing PAH shoot uptake and no detrimental effect on porewater concentrations was observed in the planted MB soils. PB appears unsuitable for addressing problems with PAH contamination, at least in the short term.

4.3.3 Effect of sorbent amendment on PTE extractability and plant uptake

Similarly to the PAH data, the ammonium sulphate extractions (Fig 4.5) highlighted no differences in PTE mobility between unplanted and planted replicates of each treatment group. Across C, PB, MB and AC amendment groups, Cu and As exhibited significant differences in some cases. Amendment had no statistical effect on Zn behaviour in the soil. Cu and As in unplanted PB, MB and AC did not differ significantly to the unplanted control. Similarly for the planted replicates, Cu and As were unaffected by any of the amendments compared to the control. However, when comparing differences to the control across planted and unplanted replicates, planted MB and AC significantly reduced Cu and As compared to the unplanted control. Unplanted PB had significantly higher concentrations of Cu and As compared to the planted control (Fig 4.5).

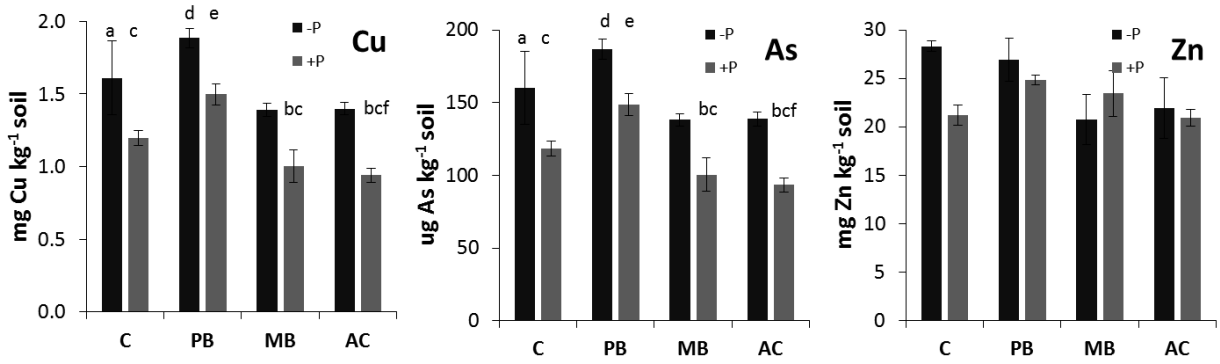


Fig. 4.5 *Ammonium sulphate-extractable Cu, As and Zn in planted (+P) and unplanted (-P) contaminated soil with different biochar treatments, C: control, PB: pine woodchip biochar amended soil, MB: maize husk biochar amended soil, AC: activated carbon amended soil. Mean ± SE (n=4).Letters signify statistical differences between treatments at p<0.05 and are divided into independent group pairs, a vs. b, c vs. d, e vs. f, where no letters are indicated, no differences are observed.*

pH did not change across treatments in this study (data not shown), similar to previous work (Chapter 2, published as Brennan et al., 2014), and may explain the small changes in extractability observed. Studies that observed increases in soil pH with biochar amendment also observed increases in porewater As (Beesley et al., 2013) and decreases in porewater Cu linked to increase in amended soil alkalinity over time (Karami et al., 2011). The differences observed compared to our study may be a result of the different amendment approaches used (3% w/w basis in our study compared to a volumetric approach), as well as the properties of the different soils used. To our knowledge, no data is available on interactions of AC and PTEs in contaminated soils, despite widespread use of AC for metal removal in the water filtration industry.

Root concentrations of Cu and Zn were not significantly affected by amendment (Fig 4.6); no data are available for root As concentrations due to insufficient root material for arsenic analysis. All amendments (PB, MB and AC) significantly reduced Cu in maize shoots compared to the control. Shoot As was significantly reduced in MB compared to the control, but not in PB or AC. Shoot Zn concentrations were statistically unaffected by amendment.

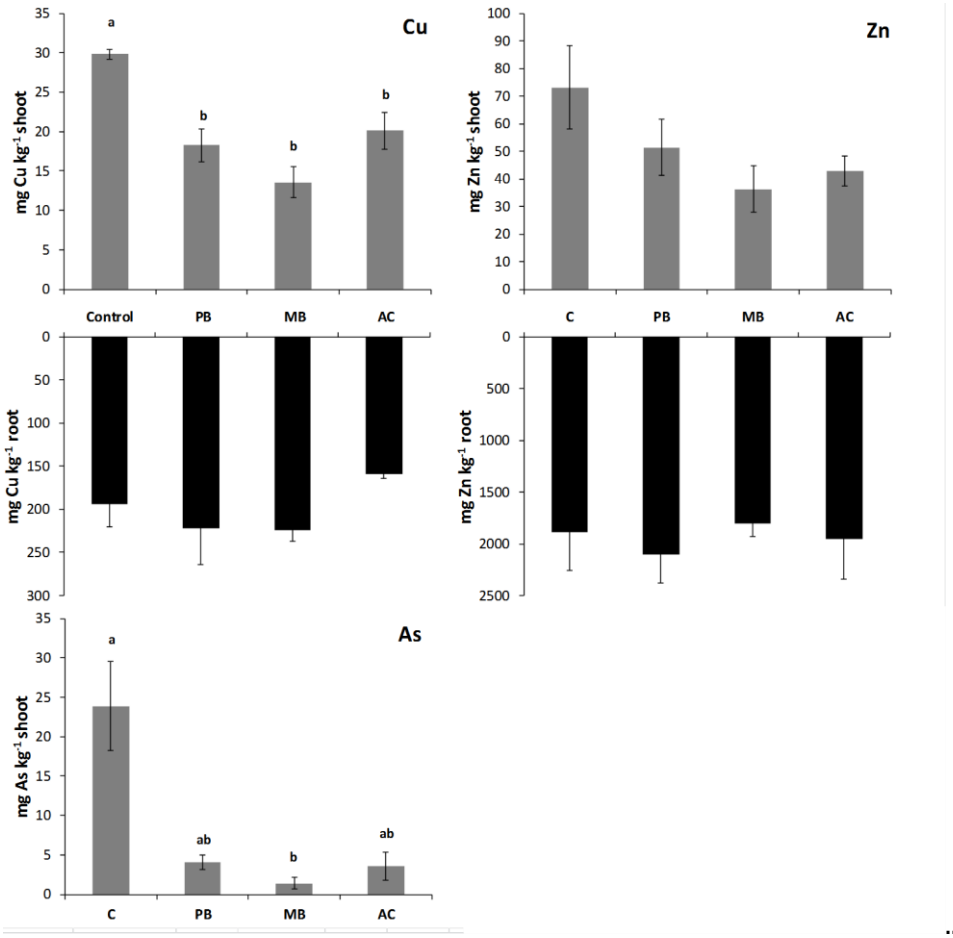


Fig. 4.6 *Cu, As and Zn concentrations in shoots and roots (insufficient sample for root As analysis) of maize plants growing on contaminated soils with different biochar treatment, C: control, PB: pine woodchip biochar amended soil, MB: maize husk biochar amended soil, AC: activated carbon amended soil. Mean ± SE (n=3-4). Different letters mean statistical differences between groups at p<0.05, where there are no letters, no differences were observed.*

The zinc data overall is in agreement with other studies with a similar level (<5%) of sorbent amendment (Waqas et al., 2014) while studies with higher biochar quantities observed reductions in zinc availability and plant uptake (Beesley et al., 2010). Reductions in copper extractability and uptake are commonly observed (Karami et al., 2011; Waqas et al., 2014); the reductions in uptake were observed in this study for all amendments but extractability data was more ambiguous. Interestingly, ammonium sulphate extractable As did not increase with amendment in this study. Increases in porewater As have been observed occasionally elsewhere (Beesley et al., 2013) and this

is likely related to experiment-specific conditions such as biochar quantity and feedstock properties, as well as changes in soil pH and dissolved organic matter fluxes.

4.3.4 Plant parameters as affected by sorbent amendment application to contaminated soil

Maize root biomass (dry wt.) was unaffected by PB, MB or AC amendment. However, maize shoot biomass significantly increased ($p<0.05$) for MB and AC compared to the control. This increase in shoot biomass then led to higher shoot: root ratio for these treatments ($p<0.05$) (Fig 4.7), which follows a similar pattern to the contaminant uptake data. This pattern similarity could be due to different factors for MB and AC amended soils. The physicochemical properties of MB (Table 4.1) compared to PB, particularly differences in soluble NPK, may have contributed to the improved shoot growth for MB. Meanwhile, the capacity for AC to bind contaminants in soils reduced contaminant availability (as indicated by shoot uptake- Fig 4.3) in a way not accounted for by the POM extractions (Fig 4.2), thereby leading to improved shoot growth.

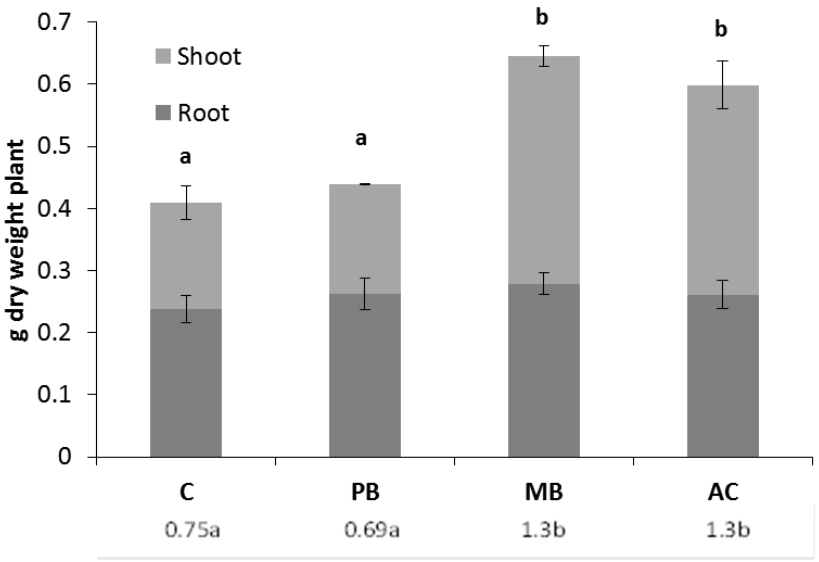


Fig. 4.7 *Plant biomass (g of dry weight per maize plant) in the contaminated soil with different biochars, C: control, PB: pine woodchip biochar amended soil, MB: maize husk biochar amended soil, AC: activated carbon amended soil. Mean ± SE (n=3-4). The shoot: root ratio was calculated and shown on the bottom of the x axis. Different letters indicate statistical differences between groups at p<0.05.*

Chlorophyll *a* has previously been used as a biomarker to assess photosynthesis ability in plants and the presence of both PAHs and PTEs has been shown to inhibit photosynthesis (Kummerová et al., 2006; Oleszczuk, 2008; Wang et al., 2013). Chlorophyll *a* content increased with PB and MB amendment ($p<0.05$) compared to the control, but not with AC amendment. Chlorophyll *b* and total carotenoids were unaffected by amendment. When taken as a total of the different components, the pattern for total chlorophyll was as for chlorophyll *a* (Fig 4.8). Compared with the contaminant data, where PB has no effect on any PAHs or As compared to the control, this data suggests that chlorophyll content is less affected by reduction in PAH/As availability and PAH/As plant uptake than by the reduction in copper uptake and extractability (see PB data in Figs 4.5 and 4.6). Nonetheless, other factors related to differences in PB, MB and AC properties cannot be ruled out.

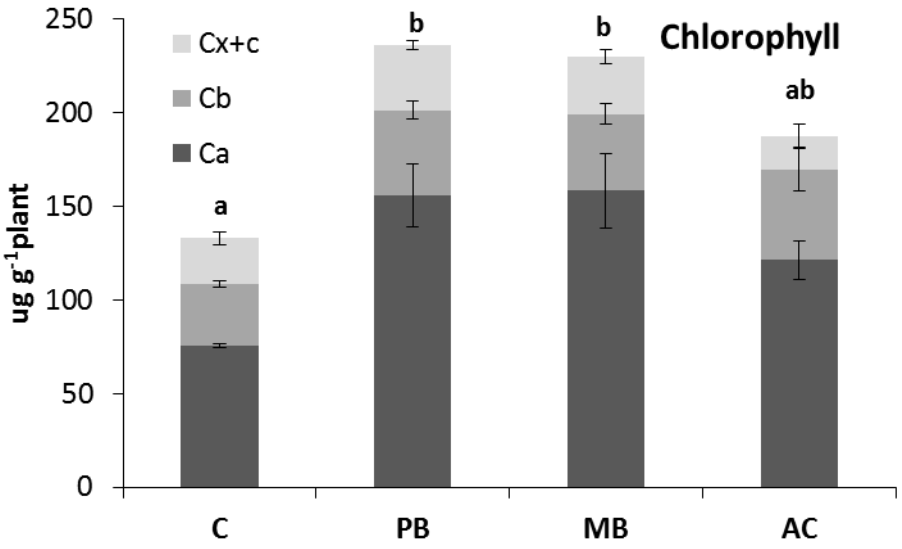


Fig. 4.8 *Chlorophyll a, chlorophyll b and total carotenoids expressed in ug g^{-1}. Mean +/-SE (n=3-4). Soil treatments correspond to C: control, PB: pine woodchip biochar amended soil, MB: maize husk biochar amended soil, AC: activated carbon amended soil. Different letters indicate statistical differences between groups at p<0.05.*

4.3.5 Implications for using carbonaceous sorbent amendment on contaminated soils

Our findings show how carbonaceous sorbent amendment leads to an overall improvement in the condition of contaminated soils and are supported by data from other studies (Beesley et al., 2010; Fellet et al., 2014; Waqas et al., 2014). However, the short term effects noted in this study are unlikely to reflect sorption kinetics in the longer term, particularly for the most hydrophobic organic contaminants and this should be considered in future studies. Sorbent amendment improved measured plant health parameters and reduced contaminant uptake and extractability to varying extents. Although biomass in PB did not change significantly compared to the controls, plants had higher chlorophyll contents and reduced Cu uptake. MB increased plant biomass parameters and chlorophyll content, consistently reduced contaminant uptake to plants and metal extractability but had ambiguous effects on PAHs in porewater. MB reduced BSAF to the greatest extent. AC improved plant biomass production but did not increase chlorophyll levels, while consistently reducing organic and inorganic contaminant bioavailable fractions and measured uptake to plants.

4.3.6 Conclusions

Having examined the effect of sorbents in the early stages of plant growth, both biochar and AC warrant further investigation as part of an integrated phytomanagement approach for contaminated sites. Taking LCA considerations into account, these further investigations would benefit from comparisons of coconut shell-derived AC to different biochars in addition to coal-derived ACs. Our results illustrate the suitability of certain types of biochar for aiding plant establishment in degraded soils, giving comparable results to commercial AC. Biochars from different feedstock did produce different results; nonetheless, no detrimental effect was observed as a result of its addition to the soil. Activated carbon is an industry standard product but the choice over which amendment to use, if at all, is likely to be based on site-specific requirements, cost

considerations and the need for result consistency. Given the heterogeneous behaviours of the different sorbents with regards to both plant growth and how they affect the mobility of organic and inorganic contaminants, this study highlights the necessity of treatability studies prior to using biochar or activated carbon in the field, in order to fully understand amendment effects prior to field deployment.

4.4 Acknowledgements

Part of this work was carried out as part of a STSM awarded to the A.B. by EU COST Action TD1107 *Biochar as an option for sustainable resource management*. E. Moreno and J.A. Albuquerque are co-authors in the published version of this work. We are grateful to Nik Johnson at ERS Remediation Ltd for supplying the PAH impacted soil and J. Gomez-Eyles for initial discussions on the use of POM.

4.5 References

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S., 2014. Biochar as a sorbent for contaminant management in soil and water: A review. Chemosphere 99, 19-33.

Atkinson, C., Fitzgerald, J., Hipps, N., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant and Soil 337, 1-18.

Beesley, L., Marmiroli, M., Pagano, L., Pigoni, V., Fellet, G., Fresno, T., Vamerali, T., Bandiera, M., Marmiroli, N., 2013. Biochar addition to an arsenic contaminated soil increases arsenic concentrations in the pore water but reduces uptake to tomato plants (Solanum lycopersicum L.). Science of The Total Environment 454–455, 598-603.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution 158, 2282-2287.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B., Sizmur, T., 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environmental Pollution 159, 3269-3282.

Brennan, A., Moreno Jiménez, E., Puschenreiter, M., Alburquerque, J.A., Switzer, C., 2014. Effects of biochar amendment on root traits and contaminant availability of maize plants in a copper and arsenic impacted soil. Plant and Soil, 1-10.

Brändli, R.C., Hartnik, T., Henriksen, T., Cornelissen, G., 2008. Sorption of native polyaromatic hydrocarbons (PAH) to black carbon and amended activated carbon in soil. Chemosphere 73, 1805-1810.

Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., van Noort, P.C.M., 2005. Extensive Sorption of Organic Compounds to Black Carbon, Coal, and Kerogen in Sediments and Soils: Mechanisms and Consequences for Distribution, Bioaccumulation, and Biodegradation. Environmental Science & Technology 39, 6881-6895.

Clark RB, Zeto SK, Ritchey KD, Baligar VC (1999) Boron accumulation by maize grown in acidic soil amended with coal combustion products. Fuel 78: 179-185.

Denyes, M.J., Rutter, A., Zeeb, B.A., 2013. In situ application of activated carbon and biochar to PCB-contaminated soil and the effects of mixing regime. Environmental Pollution 182, 201-208.

Endo, S., Hale, S.E., Goss, K.-U., Arp, H.P.H., 2011. Equilibrium Partition Coefficients of Diverse Polar and Nonpolar Organic Compounds to Polyoxymethylene (POM) Passive Sampling Devices. Environmental Science & Technology 45, 10124-10132.

Fellet, G., Marmiroli, M., Marchiol, L., 2014. Elements uptake by metal accumulator species grown on mine tailings amended with three types of biochar. Science of The Total Environment 468–469, 598-608.

Freddo, A., Cai, C., Reid, B.J., 2012. Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. Environmental Pollution 171, 18-24.

Gao, Y., Cao, X., Kang, F., Cheng, Z., 2011. PAHs Pass Through the Cell Wall and Partition into Organelles of Arbuscular Mycorrhizal Roots of Ryegrass All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. J. Environ. Qual. 40, 653-656.

Gao, Y., Collins, C.D., 2009. Uptake Pathways of Polycyclic Aromatic Hydrocarbons in White Clover. Environmental Science & Technology 43, 6190-6195.

Ghosh, U., Luthy, R.G., Cornelissen, G., Werner, D., Menzie, C.A., 2011. In-situ Sorbent Amendments: A New Direction in Contaminated Sediment Management. Environmental Science & Technology 45, 1163-1168.

Gomez-Eyles, J.L., Beesley, L., Moreno-Jimenez, E., Ghosh, U., Sizmur, T., 2013. The potential of biochar amendments to remediate contaminated soils, in: Ladygina, N., Rinea, F. (Eds.), Biochar and Soil Biota. CRC Press, p. 278.

Gomez-Eyles, J.L., Jonker, M.T.O., Hodson, M.E., Collins, C.D., 2011. Passive Samplers Provide a Better Prediction of PAH Bioaccumulation in Earthworms and Plant Roots than Exhaustive, Mild Solvent, and Cyclodextrin Extractions. Environmental Science & Technology 46, 962-969.

Grace, C., Hart, M., Brookes, P.C., 2006. Laboratory Manual of the Soil Microbial Biomass Group. Rothamsted Research, http://www.rothamsted.ac.uk/aen/smbweb1/methdnld.html. (cited 5th June 2014)

Hale, S.E., Elmquist, M., Brändli, R., Hartnik, T., Jakob, L., Henriksen, T., Werner, D., Cornelissen, G., 2012. Activated carbon amendment to sequester PAHs in contaminated soil: A lysimeter field trial. Chemosphere 87, 177-184.

Hilber, I., Blum, F., Leifeld, J., Schmidt, H.-P., Bucheli, T.D., 2012. Quantitative Determination of PAHs in Biochar: A Prerequisite To Ensure Its Quality and Safe Application. Journal of Agricultural and Food Chemistry 60, 3042-3050.

Houben, D., Evrard, L., Sonnet, P., 2013. Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (Brassica napus L.). Biomass and Bioenergy 57, 196-204.

Jakob, L., Hartnik, T., Henriksen, T., Elmquist, M., Brändli, R.C., Hale, S.E., Cornelissen, G., 2012. PAH-sequestration capacity of granular and powder activated carbon amendments in soil, and their effects on earthworms and plants. Chemosphere 88, 699-705.

Jonker, M.T.O., Koelmans, A.A., 2001. Polyoxymethylene Solid Phase Extraction as a Partitioning Method for Hydrophobic Organic Chemicals in Sediment and Soot. Environmental Science & Technology 35, 3742-3748.

Karami, N., Clemente, R., Moreno-Jiménez, E., Lepp, N.W., Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. Journal of Hazardous Materials 191, 41-48.

Kummerová, M., Barták, M., Dubová, J., Tříska, J., Zubrová, E., Zezulka, Š., 2006. Inhibitory Effect of Fluoranthene on Photosynthetic Processes in Lichens Detected by Chlorophyll Fluorescence. Ecotoxicology 15, 121-131.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biology and Biochemistry 43, 1812-1836.

Li, H., Sheng, G., Chiou, C.T., Xu, O., 2005. Relation of Organic Contaminant Equilibrium Sorption and Kinetic Uptake in Plants. Environmental Science & Technology 39, 4864-4870.

Lin, Q., Shen, K.-L., Zhao, H.-M., Li, W.-H., 2008. Growth response of Zea mays L. in pyrene–copper co-contaminated soil and the fate of pollutants. Journal of Hazardous Materials 150, 515-521.

Lozano-Rodriguez, E., Luguera, M., Lucena, J.J., Carpena-Ruiz, R., 1995. Evaluation of two different acid digestion methods in closed systems for trace element determinations in plants. Quimica Analitica 14, 27-30.

Luthy, R.G., Aiken, G.R., Brusseau, M.L., Cunningham, S.D., Gschwend, P.M., Pignatello, J.J., Reinhard, M., Traina, S.J., Weber, W.J., Westall, J.C., 1997. Sequestration of Hydrophobic Organic Contaminants by Geosorbents. Environmental Science & Technology 31, 3341-3347.

Marchal, G., Smith, K.E.C., Mayer, P., Wollesen de Jonge, L., Karlson, U.G., 2014. Impact of soil amendments and the plant rhizosphere on PAH behaviour in soil. Environmental Pollution 188, 124-131.

Meyer, S., Glaser, B., Quicker, P., 2011. Technical, Economical, and Climate-Related Aspects of Biochar Production Technologies: A Literature Review. Environmental Science & Technology 45, 9473-9483.

Oen, A.M.P., Beckingham, B., Ghosh, U., Kruså, M.E., Luthy, R.G., Hartnik, T., Henriksen, T., Cornelissen, G., 2011. Sorption of Organic Compounds to Fresh and Field-Aged Activated Carbons in Soils and Sediments. Environmental Science & Technology 46, 810-817.

Oleszczuk, P., 2008. Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotoxicology and Environmental Safety 69, 496-505.

Pignatello, J.J., Xing, B., 1995. Mechanisms of Slow Sorption of Organic Chemicals to Natural Particles. Environmental Science & Technology 30, 1-11.

Sparrevik, M., Saloranta, T., Cornelissen, G., Eek, E., Fet, A.M., Breedveld, G.D., Linkov, I., 2011. Use of Life Cycle Assessments To Evaluate the Environmental Footprint of Contaminated Sediment Remediation. Environmental Science & Technology 45, 4235-4241.

Van Slycken, S., Witters, N., Meers, E., Peene, A., Michels, E., Adriaensen, K., Ruttens, A., Vangronsveld, J., Du Laing, G., Wierinck, I., Van Dael, M., Van Passel, S., Tack, F.M.G., 2013. Safe use of metal-contaminated agricultural land by cultivation of energy maize (Zea mays). Environmental Pollution 178, 375-380.

Vázquez, S., Moreno, E., Carpena, R., 2008. Bioavailability of metals and As from acidified multicontaminated soils: Use of white lupin to validate several extraction methods. Environmental Geochemistry and Health 30, 193-198.

Wang, P., Luo, L., Ke, L., Luan, T., Tam, N.F.-Y., 2013. Combined toxicity of polycyclic aromatic hydrocarbons and heavy metals to biochemical and antioxidant responses of free and immobilized Selenastrum capricornutum. Environmental Toxicology and Chemistry 32, 673-683.

Waqas, M., Khan, S., Qing, H., Reid, B.J., Chao, C., 2014. The effects of sewage sludge and sewage sludge biochar on PAHs and potentially toxic element bioaccumulation in Cucumis sativa L. Chemosphere 105, 53-61.

Wellburn, A.R., 1994. The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. Journal of Plant Physiology 144, 307-313.

Yoshitomi, K.J., Shann, J.R., 2001. Corn (Zea mays L.) root exudates and their impact on 14C pyrene mineralization. Soil Biology and Biochemistry 33, 1769-1776.

Zhang, M., Zhu, L., 2009. Sorption of Polycyclic Aromatic Hydrocarbons to Carbohydrates and Lipids of Ryegrass Root and Implications for a Sorption Prediction Model. Environmental Science & Technology 43, 2740-2745.

5

Assessing the effects of carbonaceous sorbent amendment on *Lolium multiflorum* growth and the uptake and measured availability of polycyclic aromatic hydrocarbons (PAHs)

5.1 Introduction

Previous chapters have introduced the potential role of carbonaceous sorbents in assisting contaminated site recovery, under controlled temperature and moisture conditions. This chapter looks at amendment behaviour in semi-field conditions (outdoors but contained in pots) in order to determine whether or not the general trends observed in previous chapters and in the literature are observed.

Previous chapters have explored the establishment of biomass crops on contaminated sites. Finding cost effective solutions for reducing the risk posed by contaminated sites where biomass crops are not suitable is also of interest. Phytomanagement in the form of assisted phytostabilisation, as discussed in previous chapters (3 and 4), is still of interest in sites of low commercial value in order to stabilise soil structure, prevent erosion and leaching and encourage ecosystem succession. Carbonaceous sorbents (activated carbon or biochar) may help assist plant re-establishment during early stages of growth by reducing contaminant availability to plants (Karami et al., 2011). Understanding differences between activated carbon and biochar behaviour will inform decisions for how to best treat a degraded site. Fertilisation of carbonaceous sorbent amended soils is deemed desirable from a plant growth perspective (Lehmann et al., 2011) but effects of fertilisation on contaminant availability are largely unknown, apart from some inorganic contaminants (Beesley et al., 2013; Hossain et al., 2010).

Predicting PAH sorption to plants using bioavailability measurements has previously been done by developing a carbohydrate and lipid normalised sorption model using Italian ryegrass (Gomez-Eyles et al., 2011; Zhang and Zhu, 2009). This species was used in the experiment described here for two reasons. Because of its use in developing the sorption model, its ability to predict uptake in amended soils could be assessed. Also, it is a hardy perennial that is widely available and commonly grows in the UK.

In order to investigate whether contaminant mobility was increased or decreased by fertiliser amendment in addition to carbonaceous sorbent amendment, results are presented from a 90 day outdoor pot trial growing Italian ryegrass (*Lolium multiflorum*) in a PAH impacted soil amended with maize stover biochar and a commercial activated carbon. The hypothesis was based on observations in Chapter 4 and aimed to investigate the reproducibility of these observations with a different plant species and under uncontrolled temperature and moisture conditions. As such, the primary hypotheses for this experiment were that the carbonaceous sorbents would reduce contaminant uptake to plants, reduce soil porewater concentrations and improve plant growth overall. A secondary hypothesis was that fertiliser addition would enhance the effect of the sorbents. PAH concentrations were assessed in the soil, soil porewater and plants across treatments.

5.2 Materials and Methods

5.2.1 Experimental set up

A loamy sand soil (81% sand, 16% silt, 3% clay), with pH 4, EC 30 μS cm^{-1} and organic matter content 2%, was obtained from an exposed field site approximately one hour north-west of Glasgow, air-dried and sieved to 11 mm before use. PAH levels in the soil were below detection limits. The soil was mixed with commercial grade coal tar (Koppers UK Ltd, Scunthorpe) at the rate of 3g kg^{-1} to obtain a moderately hydrocarbon contaminated soil prior to ageing (~250 mg kg^{-1} of the ∑13 EPA PAHs analysed in this study, see 5.2.3 for a list of the compounds analysed). Batches of soil were mixed in a clean cement mixer, to which coal tar dissolved in acetone was gradually added (100ml, mixed for 10 minutes, another 100mL added and so on until desired contamination level was obtained). The whole process took approximately two hours. This soil was watered and left to age for two weeks in a well-ventilated space. To put this level of

contamination into context, Dutch environmental regulations have published an intervention value of 40 mg kg^{-1} for 10 priority PAHs (as for the USEPA 16, excepting acenaphthene, acenaphthylene, fluorene, pyrene, benzo(b)fluoranthene, diben(a,h)anthracene), although values across countries vary widely (Carlon, 2007) and in the UK is determined by using a source-pathway-receptor model to derive guideline values, taking into account soil organic matter.

Maize stover biochar (MB) and a commercial activated carbon (AC) were used to amend the contaminated soil. Biochar production, properties and methods used for characterising the biochars are fully described in Chapters 2 and 4. The activated carbon (AC) used in the experiments was in granular form and branded as Norit® GAC 1240 (Norit, USA), with the following properties: bulk density 0.49 g cm^{-3}, specific surface area 1175 m^2 g^{-1},pH 10.3, effective particle size 0.65mm (range 0.42mm-1.7mm) (data provided by manufacturer).

A total of 45 pots were prepared. 2kg soil was transferred into each 2L plant pot. One third of the pots had 1% w/w MB (unsieved, <20mm particle size) mixed throughout by hand, one third had 1% w/w AC mixed throughout by hand, and the final one third were unamended controls. Pots were watered and left for one week in a ventilated space before planting, to equilibrate sorbents with soil prior to plant addition. Five pots from each treatment were watered with NPK (15:15:18.5 kg ha^{-1}) solution so that each treatment had fertilised replicates. After one week, two thirds of the pots were planted with 2g *Lolium multiflorum* per pot.

This led to a total of nine treatment scenarios, each with five replicates (Fig 1). Controls (C) consisted of unplanted control (C-P), planted control (C+P) and fertilised planted control (C+F). Maize biochar amended soils (MB) consisted of unplanted MB (MB-P), planted MB (MB+P) and fertilised MB (MB+F). Activated carbon amended soils (AC)

were made up of unplanted AC (AC-P), planted AC (AC+P) and fertilised AC (AC+F). Pots were moved outdoors for field exposure for 12 weeks from mid-August to mid-November 2013. A wire mesh enclosure protected the pots from birds.

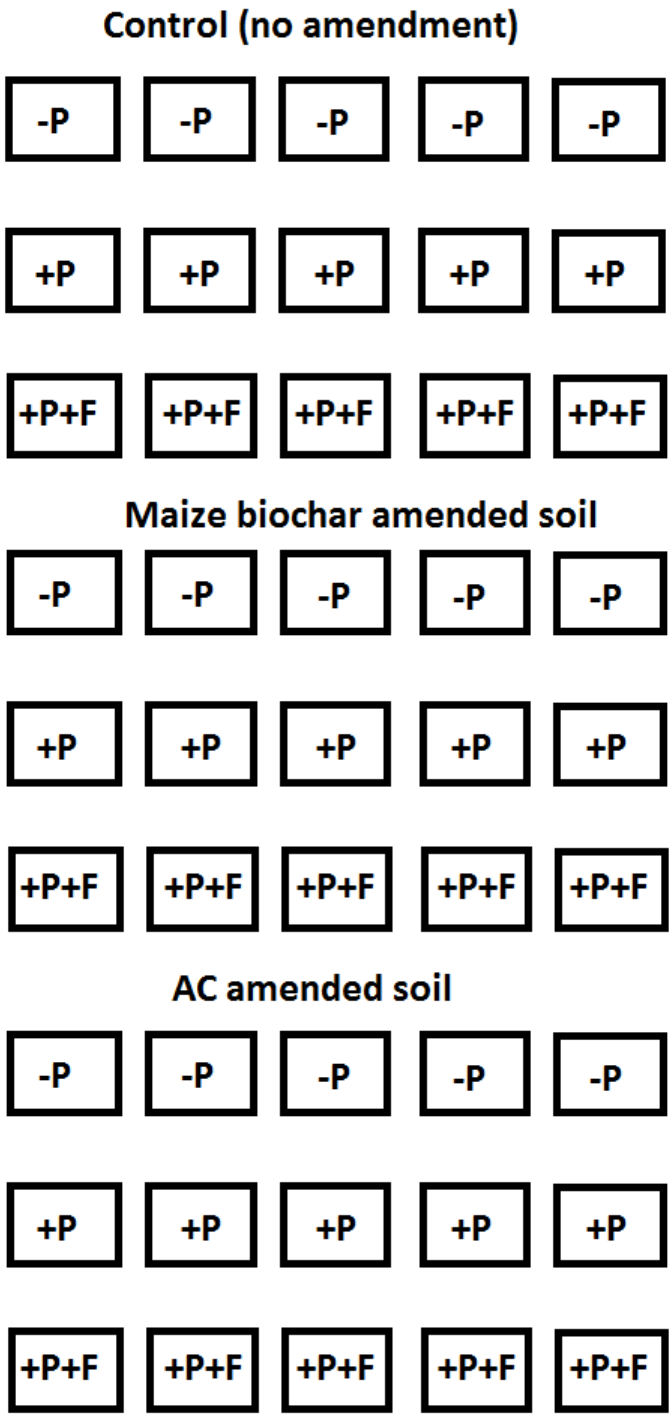


Fig. 5.1 *Experimental set up*

5.2.2 Sampling regime and methods

5.2.2.1 Plant extraction and analysis

Roots were cleaned by rinsing and sonicating in deionised water and gently patting dry with tissue. Plant shoots and roots were weighed for fresh and dry biomass before and after freeze drying. Freeze dried samples were extracted and analysed for PAH contents according to the methods described in the following sections.

5.2.2.2 Polycyclic aromatic hydrocarbons (PAHs)

At the end of the experiment all soil and amended soil samples were sieved to < 2 mm prior to extraction and analysis. Total and freely dissolved PAH concentrations were determined at the end of the experiment for all samples. Total were determined by sequential hexane-acetone and toluene extraction while freely dissolved concentrations were determined by aqueous equilibrium experiments using polyoxymethylene (POM) samplers (Jonker and Koelmans, 2001).

Total PAH in the soil was determined by accelerated solvent extraction (ASE) at 100°C by sequential extraction. 2g soil mixed with diatomaceous earth was first extracted with 1:1 hexane-acetone (HA) and then toluene. Surrogate recovery was monitored by the addition of phenanthrene-D10, anthracene-D10, and chrysene-D12. In-cell clean-up was performed using 2g activated silica gel (Sigma Aldrich) at the bottom of the ASE extraction cell in addition to a glass fibre filter (Dionex). A 1mL aliquot of the resulting eluate was analysed by GC-MS following addition of internal standards (1-fluoronaphthalene, p-terphenyl-D14, benzo(a)pyrene-D12). GC-MS conditions were as follows: Trace Ultra GC coupled with DSQ II MS (Thermo Scientific), splitless mode; column DB-5MS 30 m x 0.25 mm x 0.25 µm; initial temperature 40°C, hold 2 min, ramp 2°C per min to 80°C, then ramp 4°C per min to 320°C, hold 5 min.

Aqueous equilibrium experiments were used to measure bioavailable fractions of PAHs in the soil at the end of the experiment. Polyoxymethylene passive samplers in strips 76 µm thick (POM-76) (CS Hyde, IL, USA) were shaken with soil aliquots for 30 days (Gomez-Eyles et al., 2011; Jonker and Koelmans, 2001). After 30 days, POM samplers were cleaned with damp tissue, surrogate standard (phenanthrene-D10, anthracene-D10, chrysene-D12 and dibenz(a,h)anthracene-D14) was added and the POM was extracted three times with 20mL 1:1 HA solution for 24: 2: 2 hours. The resulting 60mL solution was concentrated to 2mL under nitrogen and cleaned (after EPA method 3630C). The resulting eluate was concentrated to 1mL, at which point internal standard for GC-MS analysis was added as for totals extractions. K_{POM} values used for calculating C_w (where $C_w = C_{POM}/K_{POM}$) were taken from literature derived values for POM-76 (Endo et al., 2011).

Root and shoot samples were extracted by microwave assisted extraction (MARS, CEM), approximately 0.1g of tissue with surrogate solution added (containing phenanthrene-D10, anthracene-D10, chrysene-D12, dibenzanthracene-D14) in 30mL 1:1 HA for 35 mins at 120°C. Samples were filtered with filter paper grade GF/F (Whatman). Each vial was then rinsed twice with 10mL solvent. Samples were then cleaned and analysed as described above.

For totals extractions, data were corrected to surrogate recovery values. For POM-76 extractions, surrogate recovery exceeded 76% (median 103%, mean 101%, relative standard deviation 17%) and data were used without alteration. For plant extractions, data were corrected to surrogate recovery values. GC-MS quantification was carried out using the internal standard method and was based on compound retention time and mass.

5.2.2.3 pH, dissolved organic carbon (DOC) and electrical conductivity (EC)

pH, dissolved organic carbon (DOC) and electrical conductivity (EC) were determined for each treatment at the end of the experiment in the ratio 1:5 soil: water. DOC was analysed following 0.45um filtration by a TOC analyser (Tekmar).

5.2.3 Data analysis

Statistical analyses were carried out on SPSS. Data were checked to fit the hypothesis of normality and homoscedasticity; log transformation was applied to data as necessary. Data were tested for significant differences with ANOVA. Tukey's post-hoc test was used for mean comparisons of the homoscedastic data. Games-Howell's test was used for the comparisons of non-homoscedastic data. Student's *t* test was used to compare populations sampled at different time points.

PAHs were grouped according to the number of benzene rings in their structure, due the similar statistical patterns observed from analysis of the individual compounds: three ring PAHs (fluorene, phenanthrene, anthracene), four ring PAHs (fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)flouranthene, benzo(k)flouranthene) and five/six ring PAHs (benzo(a)pyrene, dibenz(a,h)anthracene, indeno(a)pyrene, benzo(ghi)perylene).

5.3 Results and Discussion

5.3.1 pH, EC, DOC

pH did not differ significantly across treatments at the end of the experiment (Table 5.1). EC was significantly higher for MB than C or AC ($p<0.001$). Dissolved organic carbon (DOC) was significantly higher in MB than C ($p<0.05$) and AC ($p<0.01$).

Table 5.1 *pH, EC and DOC. Mean (+/- SE). Letters denote statistical significance. *= p<0.05, ***= p<0.001.*

	C	MB	AC	Sig.
pH	5.95 (0.08)	6.43 (0.05)	6.03 (0.07)	n.s.
EC	6.56 (0.5) a	11.81 (0.49) b	6.8 (0.32) a	***
DOC	7.02 (0.89) a	13.1 (1.5) b	4.8 (0.35) a	*

5.3.2 Shoot and root biomass

Fig. 5.2 presents shoot and root biomass data. Root biomass in the unfertilised control was significantly lower than fertilised AC ($p<0.05$), but not to any other treatment. Unfertilised MB had a significantly lower roots biomass to unfertilised AC and all fertilised replicates ($p<0.05$). Unfertilised AC had a significantly lower root biomass than fertilised AC.

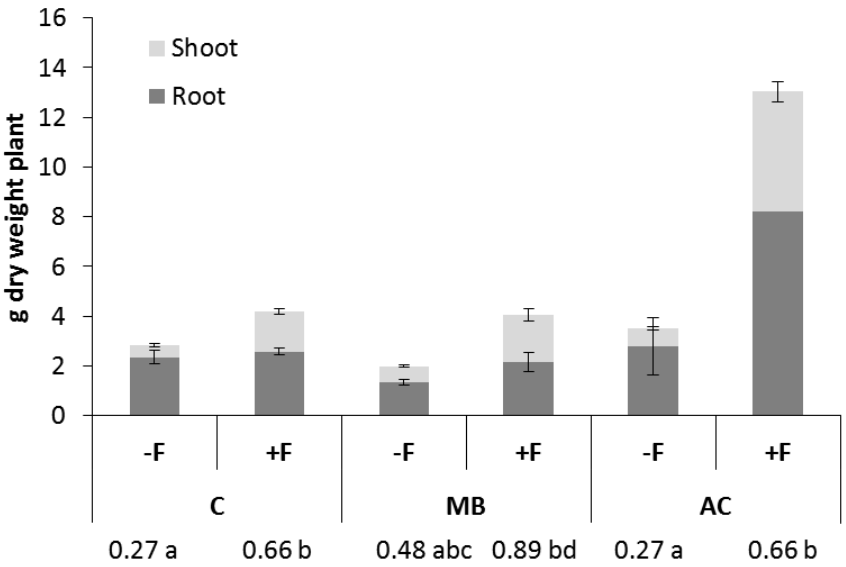


Fig. 5.2 *Plant biomass (g of dry weight per maize plant) in the contaminated soil, C: control, MB: maize stover biochar amended soil, AC: activated carbon amended soil, -F: unfertilised, +F: fertilised. Mean ± SE (n=5), where absent, error bars fall within symbols. The shoot: root ratio was calculated and shown on the bottom of the x axis. Different letters (a-d) indicate statistical differences between ratios at p<0.05.*

Shoot biomass was significantly lower in the unfertilised treatments (C, MB, AC) than in the fertilised treatments (C, MB, AC). Shoot biomass in fertilised AC was significantly higher compared to all other treatments ($p<0.001$). There was no statistical difference in shoot biomass between fertilised C and MB, but they were significantly higher than the other treatments ($p<0.01$), excepting fertilised AC ($p<0.001$).

Shoot: root ratio was unaltered by AC amendment compared to the control, unfertilised or fertilised, despite the biomass increase, indicating shoot and root biomass increased

at the same rate as the controls with AC amendment. Fertilised MB had a significantly higher shoot: root ratio than the unfertilised replicates ($p<0.05$ when compared to unfertilised MB, and $p<0.001$ when compared to unfertilised C and AC), suggesting MB amendment in conjunction with fertilisation increased shoot biomass to a greater extent than sorbent amendment alone. However, these results illustrate how the shoot: root ratio can be misleading. The fertilised AC treatment vastly increased plant biomass (Fig. 5.1). From a plant establishment and soil stabilisation perspective, this increase is a highly desirable outcome. By contrast, the MB amendments have a relatively minimal impact on plant establishment. These data also illustrate the beneficial effects of fertilisation in the early stages of plant biomass production.

5.3.3 Total soil PAH concentrations (tPAH)

Fig. 5.3 shows tPAH values across treatment groups, representing the sum of the two extractions (HA + toluene). No differences were observed for the four ring PAHs across treatments. Significant reduction in tPAH compared to the unplanted control was observed for five/six ring PAHs in both unfertilised C and unfertilised AC, but was not significant for any other treatment. For three ring PAHs, unplanted C did not differ significantly to any other treatment while unfertilised C was significantly higher than unplanted MB and fertilised MB, but significantly lower than unfertilised MB and unplanted AC.

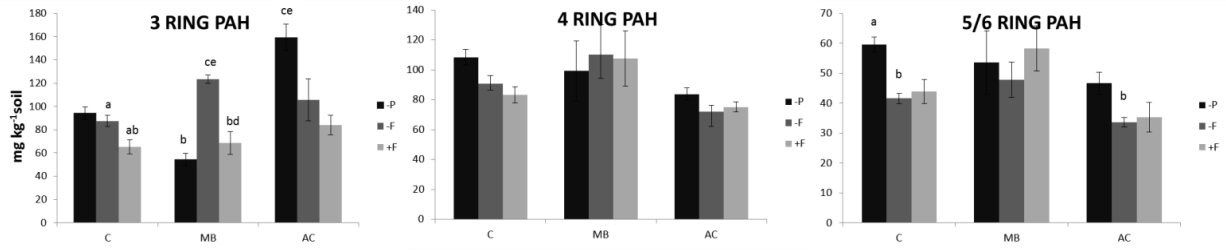


Fig. 5.3 *Total PAH concentrations in contaminated soil at end of experiment with different sorbent amendments, C: control, MB: maize stover biochar amended soil, AC: activated carbon amended soil, -P: unplanted, -F: planted, unfertilised, +F: planted, fertilised. Mean ± SE (n=5). Different letters signify statistical differences between treatments at p<0.05. Where no letters are indicated, data from a given treatment do not differ significantly to any other treatment.*

The second toluene extraction had no effect on soil or biochar extractions but for activated carbon amendments it was more effective than HA extraction alone. HA extraction showed PAH decreases with AC amendment, likely representing the strongly sorbed fractions that were then extracted by the toluene extraction.

While this study was not designed to examine PAH degradation, these results show that total PAHs are essentially unaffected by amendment, planting or fertilisation, apart from the exceptions above. It is difficult to attribute sample variation to microbial degradation of PAHs without further analysis. These data indicate the extent of sorption to AC. The toluene extraction accounted for less than 0.5% of PAHs extracted from C and MB on average while it accounted for 41% (+/-8%) of the PAHs extracted from AC. Previous studies have shown, either by aggressive solvent extractions (Hilber et al., 2012) or by acknowledging the non –exhaustiveness of their chosen solvent (Hale et al., 2012), that both AC and biochar amended soils require more aggressive extraction in order to recover true total values. Given the heterogeneity encountered with different biochars both as a result of feedstock and intra-biochar differences (Bucheli et al., 2014; Zhao et al., 2013), this is not surprising. Whether or not these more aggressive extractions should be done comes down to specific study research

questions. In many cases, the non-exhaustive extraction values obtained with HA extraction or similar gives sufficient information to inform other results.

5.3.4 Porewater PAH concentrations

Three ring PAHs at the experiment midway point (Fig. 5.4a) highlighted that for the control samples, porewater PAH concentrations in the soil were unaffected by planting (-P vs. –F, +F) or planting plus fertilisation (-P, -F vs. +F). For the MB amended soils, MB+F significantly decreased porewater PAH concentrations compared to MB-F, but MB-P did not differ significantly to either of the planted replicates. None of the AC treatments differed to one another but were significantly lower in porewater PAHs than all treatments of C and MB ($p<0.001$). MB-P and MB-F did not differ to any of the control treatments, but MB+F was significantly lower than C-F.

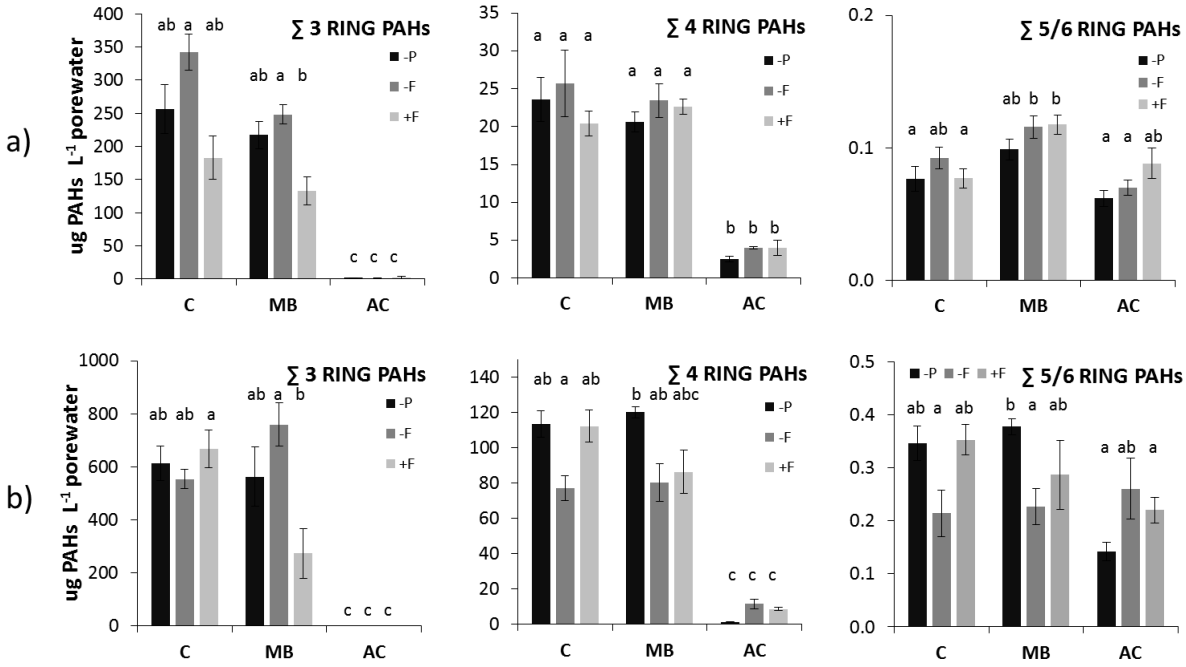


Fig. 5.4 *Porewater concentrations of PAHs in contaminated soil with different sorbent amendments at the experiment midway point (a) and at the end of the experiment (b), C: control, MB: maize stubble biochar amended soil, AC: activated carbon amended soil, -P: unplanted, -F: planted, unfertilised, +F: planted, fertilised. Mean ± SE (a: n=5, b: n=3-5). Different letters signify statistical differences between treatments at p<0.05.*

For four ring PAHs, all AC treatment (AC-P, AC-F, AC+F) were significantly lower to all other treatments ($p<0.001$). None of the MB treatments were significantly different to any C treatment, while planting or fertilisation had no effect on porewater concentrations within C, MB and AC treatment groups.

Five/six ring PAHs in porewater were unaffected by planting or fertilisation within groups. MB-F and MB+F had significantly higher porewater concentrations than those of C-P, C+F, AC-P and AC-F. No differences were observed between C and AC treatment groups.

Porewater concentrations at the end of the experiment (Fig. 5.4b) followed a similar pattern with a few exceptions. Porewater concentrations were statistically unaffected by planting or fertilisation for C and AC treatment groups for all PAH structures. Three ring PAHs in porewater for all AC treatments were significantly lower than all C and MB. Concentrations in MB+F were significantly lower than C+F and MB-F. Four ring PAHs were significantly lower in all AC groups than all C groups and MB-P, MB-F. MB+F did not differ significantly to any AC. MB-P was significantly higher than C-F but not its C-P counterpart. Five/six ring PAHs were significantly higher in MB-P than C-F, MB-F, AC-P, and AC+F. No other differences were observed in this PAH class.

While biochar seems to have a generally negligible effect on PAHs in porewater, fertilisation (i.e. MB+F) generally led to lower concentrations of PAHs in solution, showing significant reductions in three ring PAHs in particular between MB-F and MB+F. Meanwhile, AC was as effective at reducing PAHs in porewater whether or not soil was planted or fertilised.

Differences in porewater concentrations between the midway point (Fig. 5.4a) and the end of the experiment (Fig. 5.4b) were assessed by the Student's *t* test. Three ring PAHs

significantly increased for all C ($p<0.01$) and MB ($p<0.05$) treatments between the two points while no difference was observed for any of the AC treatments ($p=0.341$). A similar pattern was observed for four ring PAHs, where C ($p<0.001$) and MB ($p<0.05$) significantly increased while no difference was observed for AC ($p>0.05$). For five/six ring PAHs, C, MB and AC all increased significantly between the two time points ($p<0.001$, 0.05 and 0.05 respectively).

Where the differences were significant PAHs in porewater approximately doubled for 3 and five/six ring PAHs and approximately tripled for four ring PAHs. The most likely explanation for this increase is the extremely wet weather conditions experienced in the period between the two sampling points (early October to mid-November 2013) which led to saturated soil conditions and may have increased leaching of soil PAHs into solution. However, a more systematic sampling approach over a longer time period would be required to support this possible explanation. Little long term data are available on porewater PAHs in biochar/AC amended soils as the POM-SPE technique is still in early stages in soil research and only a few studies have looked at amendment effects on porewater/leachate in field exposed soils contaminated with PAHs (Hale et al., 2012).

5.3.5 Shoot and root uptake

Despite differences between MB and AC amendment observed in porewater concentrations, MB and AC behave in a similar fashion when it comes to plant uptake (Fig. 5.5). Amendment did not significantly affect shoot uptake of three ring PAHs; however, amendment and fertilisation (MB+F, AC+F) significantly decreased shoot uptake compared to the unfertilised control (C-F) for four and five/six ring PAHs. No differences were observed between fertilised and unfertilised replicates in the same treatment group for any of the PAH classes.

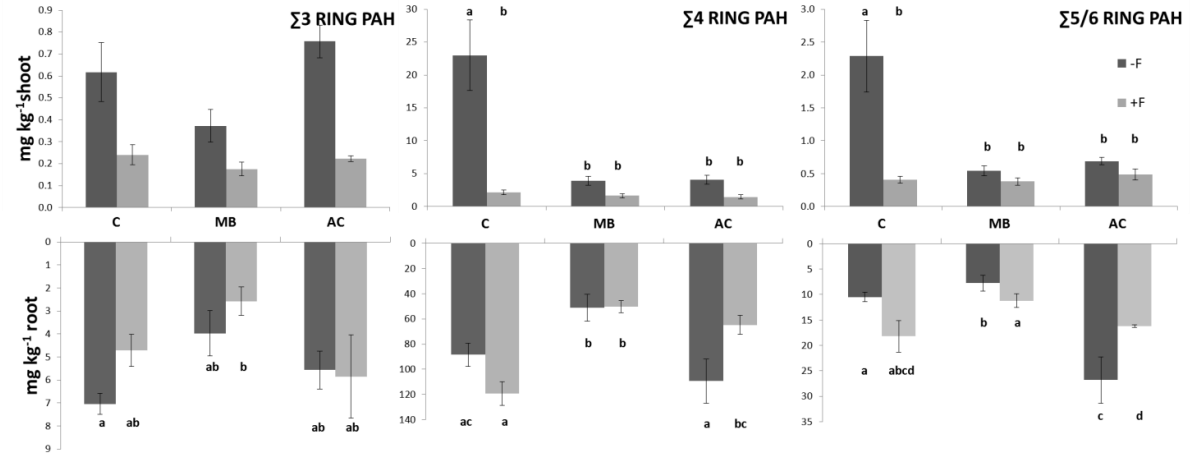


Fig. 5.5 *PAH concentrations in shoots and roots of ryegrass growing in contaminated soil with different sorbent amendment, C: control, MB: maize stover biochar amended soil, AC: activated carbon amended soil, -F: planted, unfertilised, +F: planted, fertilised. Mean ± SE (n=3-5). Different letters mean statistical differences between groups at p<0.05. Where no letters are indicated, data from a given treatment do not differ significantly to any other treatment.*

Root uptake of three ring PAHs was, in general, unaffected by amendment or fertilisation, with the exception of MB+F which had significantly lower concentrations of three ring PAHs compared to C-F. four ring PAH uptake was unaffected by fertilisation in the control soil, while amendment significantly reduced root uptake compared to C-F and C+F. AC-F uptake did not differ significantly to C-F or C+F but was significantly higher than MB-F, MB+F and AC+F. AC+F uptake was significantly lower than C-F, C+F and AC-F, but did not differ significantly to MB-F or MB+F.

Five/six ring PAH root uptake was unaffected by fertilisation in the control soil, while MB amendment significantly decreased root uptake compared to C-F, but not when compared to C+F. MB+F had significantly higher uptake than MB-F, while both types of AC amendment led to significantly higher uptake compared to C-F and MB-F and MB+F, but not when compared to C+F (no difference). AC+F had significantly lower root uptake than AC-F.

5.3.6 Use of sorption prediction models to assess biochar and AC remediation

A sorption prediction model proposed by Zhang and Zhu (2009) that accounts for both carbohydrate and lipid PAH partitioning to plant roots was assessed for its efficacy in predicting sorption in plants grown in semi-field conditions. Gomez-Eyles et al. (2012) used POM-derived porewater PAH concentrations to apply the model and the same POM approach was used here. Fig. 5.6 shows how the model under-predicted root uptake in AC amended soils by more than an order of magnitude for three ring PAHs, while slight under-predictions were observed for the control and MB amended soils, close to the 1:1 relationship line and mostly within one order of magnitude. Four and five/six ring PAH uptake was well predicted for C and MB, but was slightly under-predicted for AC, within one order of magnitude.

Fig. 5.6 suggests there are limitations to modelling PAH uptake based on porewater concentrations in the carbonaceous sorbent amended soils. As has been observed for metals (Degryse et al., 2009), once plant roots uptake PAHs, newly available PAHs may be resupplied into solution. New techniques using silicon O-rings to measure diffusive fluxes in situ may help improve these predictions (Mayer et al, 2011; Marchal et al, 2014). Soil PAHs may be mobilised by interaction with root exudates which may affect plant PAH uptake (Gomez-Eyles et al., 2011; Yoshitomi and Shann, 2001).

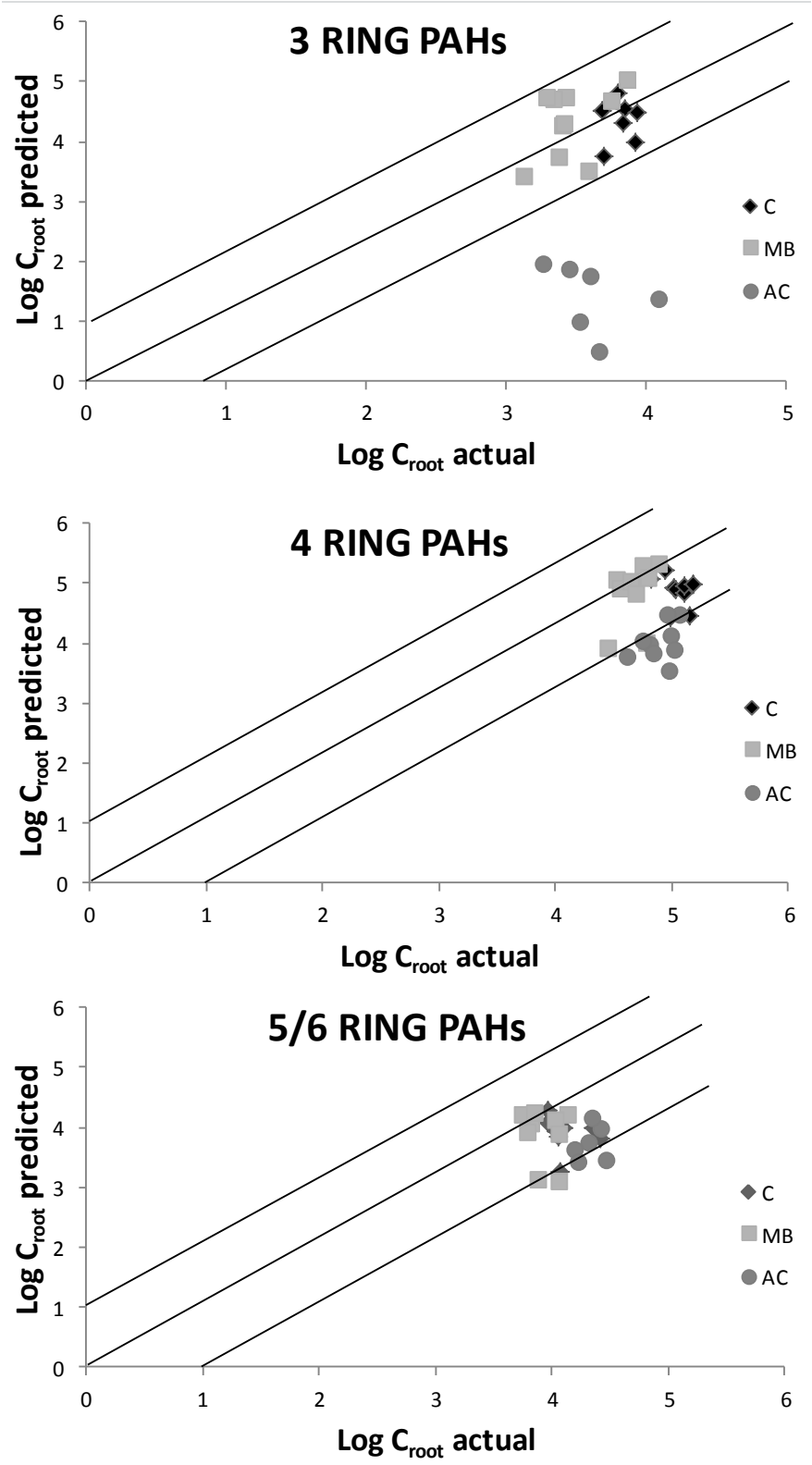


Fig. 5.6 *Predicting shoot and/or root concentrations using POM. Middle line indicates a 1:1 relationship while the lines on either side represent one order of magnitude either way. Data was calculated using equations and K_{ow} values derived from the literature (Gomez-Eyles et al., 2011); (Zhang and Zhu, 2009)*

5.3.7 The role of carbonaceous sorbent amendment in contaminated soil management

Short term experiments under controlled moisture and temperature conditions (<1 month), such as those described in Chapters 3 and 4, have shown more comparable results between biochar and AC. Conducting a three month outdoor experiment gives a clearer picture of what may happen with these amendments in the longer term, where in our study AC vastly outperformed biochar in terms of limiting PAH contaminant mobility (porewater concentrations). An even longer timescale is desirable in order to get a clear picture of carbonaceous sorbent effects on soil biota. Sediment and freshwater studies (Beckingham et al., 2013; Cornelissen et al., 2008; Ghosh et al., 2011) are much better developed but are generally limited to AC observations only. Results for an AC trial (Hale et al., 2012) gives an idea of longer term effects for AC amended soils, but long term data for biochar in this context are non-existent. Several challenges remain when it comes to biochar amendment, principally to heterogeneous behaviour of biochars in soil depending on their feedstock (Chapters 3 and 4; Fellet et al., 2014). Particle size may be another aspect affecting contaminant mobility and was not considered in this study. MB was used as received for convenience (closely resembling what would happen in the field), which may partly explain the differences between MB (<20mm particle size) and AC (<0.6 mm particle size). However, similar patterns in porewater PAHs were observed in Chapter 4 between MB and AC and in that study, MB was sieved to a particle size distribution between 0.5 mm and 2 mm. Based on the Chapter 4 results, particle size differences may remain non-significant when experiments are scaled up.

AC is the most effective amendment in terms of reducing three and four ring PAHs in porewater. Fertilised and amended MB effectively reduced porewater three ring PAHs but none of the heavier PAHs. None of the amendments were categorically better at

reducing porewater levels of five/six ring PAHs, which, although present in the smallest quantities, represent compounds such as benzo(a)pyrene and dibenz(a,h)anthracene, which are important drivers for human health risk assessment for contaminated sites. Differences in particle sizes between the two amendments, leading to different equilibration patterns may have led to the differences in reducing porewater levels while the heavier PAHs, with their higher K_{ow}, may need more time under field conditions to sorb to the amendments. and therefore, perhaps in the longer term it is likely a different profile would emerge as the heavier PAHs (high K_{ow}) became sorbed to the biochar and AC matrices (Oen et al, 2011; Hale et al, 2012).

Fertilisation had a negligible effect on porewater PAH concentrations within treatment groups but had a highly significant effect when it came to reducing shoot concentrations of four to six ring PAHs between fertilised and unfertilised controls. Amendment combined with fertilisation had a negligible effect on shoot uptake of PAHs for both MB and AC.

In terms of differences in plant biomass, fertilisation vastly improved plant establishment between AC-F and AC+F but led to a negligible improvement between C-F and C+F, MB-F and MB+F. While three and four ring PAH availability seems to be vastly decreased with AC amendment, nutrient limitation in the soil seemed to inhibit plant growth until the addition of fertiliser to the AC amended soil allowed more rapid and widespread plant establishment. Even if differences in porewater concentrations between fertilised and unfertilised replicates were largely absent, the better the plant establishment, the less likely soil erosion and consequent particulate dispersion off-site would be to occur. In addition, in the long term, better plant establishment may mean contaminants are less likely to leach through the soil column to groundwater, although this aspect needs to be explored to a greater extent.

5.4 Conclusions

Although the activated carbon in conjunction with fertilisation was the best performer overall in terms of combined effects on plant establishment and PAH uptake and porewater mobility, choice over use of this particular treatment combination is site, cost and resource dependent. If rapid plant establishment is not crucial then biochar or activated carbon addition alone could be deployed to reduce PAH uptake to plants and risk to higher organisms. Fertiliser alone could be added if reduction in uptake was the sole aim, but due to fertiliser resource scarcity it is not a particularly sustainable option. Fertiliser addition did not result in a significant change in contaminant availability in the amended soils, but it aided plant establishment. The plant sorption model proved effective at predicting sorption, although some discrepancies with the activated carbon predictions (under-predictions) highlights that porewater concentrations are not the only mechanisms affecting plant uptake and this sort of assessment tool should be used cautiously.

5.5 Acknowledgments

Andrew Pape is thanked for his assistance with collecting the soil and for providing some of the soil characteristics. Eduardo Moreno-Jiménez is thanked for initial discussions on experimental design. José A. Albuquerque is thanked for supplying and characterising the biochar used in the experiment.

5.6 References

Beckingham, B., Buys, D., Vandewalker, H., Ghosh, U., 2013. Observations of limited secondary effects to benthic invertebrates and macrophytes with activated carbon amendment in river sediments. Environmental Toxicology and Chemistry 32, 1504-1515.

Beesley, L., Marmiroli, M., Pagano, L., Pigoni, V., Fellet, G., Fresno, T., Vamerali, T., Bandiera, M., Marmiroli, N., 2013. Biochar addition to an arsenic contaminated soil increases arsenic concentrations in the pore water but reduces uptake to tomato plants (Solanum lycopersicum L.). Science of The Total Environment 454–455, 598-603.

Bucheli, T.D., Bachmann, H.J., Blum, F., Bürge, D., Giger, R., Hilber, I., Keita, J., Leifeld, J., Schmidt, H.-P., 2014. On the heterogeneity of biochar and consequences for its representative sampling. Journal of Analytical and Applied Pyrolysis. in press.

Cornelissen, G., Pettersen, A., Broman, D., Mayer, P., Breedveld, G.D., 2008. Field testing of equilibrium passive samplers to determine freely dissolved native polycyclic aromatic hydrocarbon concentrations. Environmental Toxicology and Chemistry 27, 499-508.

Carlon CE (2007) Derivation methods of soil screening values in Europe. A review and evaluation of national procedures towards harmonization. European Commission, Joint Research Centre, Ispra.

Degryse, F., Smolders, E., Zhang, H., Davison, W., 2009. Predicting availability of mineral elements to plants with the DGT technique: a review of experimental data and interpretation by modelling. Environmental Chemistry 6, 198-218.

Endo, S., Hale, S.E., Goss, K.-U., Arp, H.P.H., 2011. Equilibrium Partition Coefficients of Diverse Polar and Nonpolar Organic Compounds to Polyoxymethylene (POM) Passive Sampling Devices. Environmental Science & Technology 45, 10124-10132.

Fellet, G., Marmiroli, M., Marchiol, L., 2014. Elements uptake by metal accumulator species grown on mine tailings amended with three types of biochar. Science of The Total Environment 468–469, 598-608.

Ghosh, U., Luthy, R.G., Cornelissen, G., Werner, D., Menzie, C.A., 2011. In-situ Sorbent Amendments: A New Direction in Contaminated Sediment Management. Environmental Science & Technology 45, 1163-1168.

Gomez-Eyles, J.L., Jonker, M.T.O., Hodson, M.E., Collins, C.D., 2011. Passive Samplers Provide a Better Prediction of PAH Bioaccumulation in Earthworms and Plant Roots than Exhaustive, Mild Solvent, and Cyclodextrin Extractions. Environmental Science & Technology 46, 962-969.

Hale, S.E., Elmquist, M., Brändli, R., Hartnik, T., Jakob, L., Henriksen, T., Werner, D., Cornelissen, G., 2012. Activated carbon amendment to sequester PAHs in contaminated soil: A lysimeter field trial. Chemosphere 87, 177-184.

Hilber, I., Blum, F., Leifeld, J., Schmidt, H.-P., Bucheli, T.D., 2012. Quantitative Determination of PAHs in Biochar: A Prerequisite To Ensure Its Quality and Safe Application. Journal of Agricultural and Food Chemistry 60, 3042-3050.

Hossain, M.K., Strezov, V., Yin Chan, K., Nelson, P.F., 2010. Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (Lycopersicon esculentum). Chemosphere 78, 1167-1171.

Jonker, M.T.O., Koelmans, A.A., 2001. Polyoxymethylene Solid Phase Extraction as a Partitioning Method for Hydrophobic Organic Chemicals in Sediment and Soot. Environmental Science & Technology 35, 3742-3748.

Karami, N., Clemente, R., Moreno-Jiménez, E., Lepp, N.W., Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. Journal of Hazardous Materials 191, 41-48.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biology and Biochemistry 43, 1812-1836.

Marchal, G., Smith, K.E.C., Mayer, P., Wollesen de Jonge, L., Karlson, U.G., 2014. Impact of soil amendments and the plant rhizosphere on PAH behaviour in soil. Environmental Pollution 188, 124-131.

Mayer, P., Olsen, J.L., Gouliarmou, V., Hasinger, M., Kendler, R., Loibner, A.P., 2011. A Contaminant Trap as a Tool for Isolating and Measuring the Desorption Resistant Fraction of Soil Pollutants. Environmental Science & Technology 45, 2932-2937.

Oen, A.M.P., Beckingham, B., Ghosh, U., Kruså, M.E., Luthy, R.G., Hartnik, T., Henriksen, T., Cornelissen, G., 2011. Sorption of Organic Compounds to Fresh and Field-Aged Activated Carbons in Soils and Sediments. Environmental Science & Technology 46, 810-817.

Yoshitomi, K.J., Shann, J.R., 2001. Corn (Zea mays L.) root exudates and their impact on ^{14}C-pyrene mineralization. Soil Biology and Biochemistry 33, 1769-1776.

Zhang, M., Zhu, L., 2009. Sorption of Polycyclic Aromatic Hydrocarbons to Carbohydrates and Lipids of Ryegrass Root and Implications for a Sorption Prediction Model. Environmental Science & Technology 43, 2740-2745.

Zhao, L., Cao, X., Mašek, O., Zimmerman, A., 2013. Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. Journal of Hazardous Materials 256–257, 1-9.

6

Conclusions and Recommendations for Future Work

In this thesis, the effects of biochar amendment on contaminated soils have been studied in small to medium scale laboratory and outdoor experiments. This final chapter discusses the key findings of the work and future research opportunities.

6.1 Key findings

The experiments described in this thesis were driven by investigation of the initial hypothesis that biochar amendment would reduce contaminant availability and aid plant growth. Activated carbon was used as a reference sorbent against which biochar was assessed in the two pot trials described (Chapters 4 and 5). Despite differences properties such as surface area, both substances are forms of black carbon and as such are natural sorbents. By defining the conditions in which sorbent amended soils successfully reduced contaminant bioavailability and improved plant growth, this work has obtained results that can inform future studies. In the following sections, the findings relevant to the two key criteria of plant establishment and contaminant availability are summarised. Finally, the implication of these findings for applying biochar in the field is considered.

6.1.1 Plant establishment

Prior to this work, no specific data were available on the mechanisms of root responses to biochar amendment in contaminated soils. Through a rhizobox experiment conducted with a mine spoil soil, Chapter 2 illustrated the beneficial effects of biochar amendment on root establishment, promoting root architecture, root morphology and plant resource allocation below ground, albeit with variations observed between the different biochars used.

Short term experiments ranging from <1 month to 3 months in Chapters 2, 4 and 5 has established the role of biochar and activated carbon in promoting plant biomass development in early growth stages. These results illustrate the suitability of certain types of biochar for aiding plant establishment in degraded soils, giving comparable results to commercial activated carbon. Biochars from different feedstock did produce different results; nonetheless, no detrimental effect on plant growth was observed as a result of its addition to the soil.

6.1.2 Contaminant availability

This thesis looked at contaminant availability in two ways: in terms of porewater concentrations or extractability, and in terms of actual plant uptake. The presented results for porewater/extractable concentrations offer further evidence on the heterogeneous effects of different biochars derived from different feedstocks compared to the relatively consistent effects of activated carbon. Biochars were selected for each experiment according to their differing feedstock and properties, particularly with regards to pH and cation exchange capacity properties (Tables 2.1 and 4.1). Where comparisons to activated carbon were made, different biochars outperformed the activated carbon when it came to extractable concentrations and plant uptake of the inorganic contaminants copper and arsenic (Chapter 4). For organic contaminants, while activated carbon amendment led to consistently lower porewater concentrations for PAHs overall, actual plant uptake in activated carbon amended soils was comparable to the biochar amended soils (Chapters 4 and 5). Mercury (Chapter 3), zinc (Chapter 4) and manganese (Chapter 2) were unaffected by sorbent amendment in general, although zinc extractability and uptake decreased with amendment to varying extents in Chapter 2.

6.1.3 The potential for field application of biochar to contaminated soils

Pre-screening of different biochars for the contaminants of interest in the laboratory prior to application on a particular site is essential due to the differences in performance observed for the different biochars. Choice of biochar on site will be governed by desired remediation endpoints, whether it is to reduce the uptake of contaminants into organisms or reduce contaminant mobility in porewater.

If both plant uptake and reduced porewater concentrations of contaminants are required, on balance coal-derived activated carbon demonstrated the most consistent results for the range of contaminants analysed in this thesis. The decision over whether or not to use this type of activated carbon will also have to take into consideration higher costs and the use of virgin coal resources.

6.2 Future research opportunities

Activated carbons derived from biomass (coconut shells) were not included in this study. To overcome the limitation of having to use virgin coal resources with the coal-derived activated carbons, this type of activated carbon needs to be tested for use in soil remediation alongside biochars. Interest in activated biochars for remediation has increased as alternatives to activated carbon for soil and sediment remediation. However in the soil remediation context, no information on activated biochar interactions with plants in contaminated soils is available. This represents a clear avenue for research investigating these interactions and comparing them to the interactions with ordinary biochars.

The effects of biochar amendment on root development and growth patterns needs to be explored to a greater extent in the contaminated soil context. The study presented in Chapter 2 has provided information on soil-biochar-inorganic contaminant-root interactions but data for organic contaminants are still required. Additionally,

rhizoboxes are a useful tool for looking at these patterns in early growth stages. Due to the natural restrictions imposed by rhizoboxes on root development after a certain point, studies at later stages of root growth are required in order to gain a more mechanistic understanding of the effects of biochar amendment on plant establishment underground in contaminated soils.

Innovative passive sampling techniques were used to monitor changes in porewater concentrations, rhizon samplers for inorganic contaminants and POM for organic contaminants. Porewater concentrations have been suggested as predictors of organism uptake (plants, earthworms, etc.) as the freely dissolved concentrations are considered most available for uptake. However, when correlating porewater data with actual plant uptake in the research presented in this thesis (using sorption models for PAHs), these techniques were shown to have limitations, mainly as porewater concentrations in the study did not change across unplanted and planted replicates of control and amended treatments. Activated carbon had lower concentrations of contaminants in porewater than biochars (particularly with regards to PAHs), but plant uptake was comparable, suggesting plant uptake of contaminants is governed by more than porewater concentrations.

This thesis presents research conducted in relatively short timescales. While the biochar in agriculture research community has been conducting field trials for a number of years, limited data are available on field trials for biochar amended contaminated soils. Available studies were conducted for inorganic contaminants and did not incorporate plants. This significant gap illustrates how young this research field still is. Activated carbon in amended contaminated soils is also at a relatively early stage in research. Results from just one large scale field trial (using lysimeters) for organic contaminants are available. While some parallels can be drawn between

biochar and activated carbon, the differences highlighted in Chapters 4 and 5 demonstrate the requirement for longer term studies in field conditions for both sorbents and for both organic and inorganic contaminants. While the effects of amendment on contaminant mobility and uptake are still not fully understood, the use of lysimeters or similar will enable research into biochar amendment for the phytomanagement of contaminated soils to move forward, investigating effects of sorbent amendment under environmental conditions while minimising risk of contaminant transfer.

Growing biomass crops on contaminated land offers the double benefit of re-vegetating degraded land and generating income. The role of biochar in enhancing the establishment and yield of these crops presents a clear avenue for research. Whether or not the aim of re-vegetation is to generate income, the use of low impact phytomanagement techniques in contaminated soils may well be enhanced by the addition of biochar.