THE EFFECTS OF BIOCHAR ON TRACE ELEMENT AND NUTRIENT SOLUBILITY IN THE PRESENCE OF LOW-MOLECULAR-WEIGHT ORGANIC ACIDS

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For Mum and Dad

Abstract

Low-molecular-weight organic acids (LMWOAs) secreted by plant roots play an essential role in regulating the phytoavailability of soil-borne trace elements and nutrients. Biochar is widely considered as a suitable soil remediation option, having shown an efficacy to retain both anions and cations. This study aims to unravel the roles of LMWOAs in affecting the functioning of biochar in aqueous and soil systems. Twelve biochar were characterised and rice husk biochar was selected for use. Firstly, aqueous batch experiments examined the biochar-driven immobilisation of trace elements and nutrients with and without LMWOAs. Then, batch studies tested if biochar would reduce the LMWOA-driven solubilisation of trace elements and nutrients in soil. Later, rice husk biochar and LMWOA-activated rice husk biochar were incorporated in greenhouse investigations to study the growth parameters of edible plant species and trace element uptake by pea (*Pisum sativum*). Solution sorption studies found that LMWOAs impeded the biochar-driven removal of cadmium and lead but enhanced nitrate removal. Biochar did not affect phosphate removal. Biochar inhibited the LMWOA-driven solubilisation of soil-borne cadmium and zinc. Nitrate immobilisation was not enhanced but biochar reduced the immobilisation of the added nitrate overall. Phosphate immobilisation was observed but was caused by the precipitation of practically insoluble phosphate minerals using biochar as a source of soluble calcium. Seven out of eleven trace elements accumulated in pea roots were reduced either by biochar and LMWOA-activated biochar but the uptake was variable with biochar type. The data obtained indicate LMWOAs complicate biochar functioning in solution and soil under the studied conditions. Based on the results, protonation of the biochar materials in acidic conditions impeded cation immobilisation whilst enhancing nitrate immobilisation. Acid neutralisation by the biochar materials likely reduced the LMWOA-solubilisation of trace elements which would affect trace element uptake by plants such as pea, within biochar-amended soils.

CHAPTER 1

Introduction

1.1 Research context

Soil is one of the most critical yet overlooked resources on our planet. Not only is it the medium in which we grow food to sustain ourselves, but it also filters water, sequesters carbon dioxide and supports biodiversity (Banwart et al., 2012; Ockenden, Deasy, Quinton, Surridge, & Stoate, 2014). As a result, it is essential to consider soil health in policymaking and industrial practice to sustain life and development. Despite this, soil contamination is a growing global concern that poses a severe risk to human and ecological receptors (Ahmad et al., 2014; Järup, 2003). Anthropogenic activity such as mining activities, inadequate waste disposal and atmospheric deposition from industry has caused increased soil contamination (Banwart et al., 2012). Commonly found contaminants are heavy metals and metalloids (e.g., cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr) and arsenic (As)), inorganic compounds (e.g., nitrates, phosphates, ammonium) and organic compounds (e.g., hydrocarbons, pesticides, and pharmaceuticals) (Ahmad et al., 2014).

These contaminants are commonly reported as the total metal content, to quantify soil contamination. This encompasses all of the metals within the soil matrix (including the inert and inactive fractions) (Gupta, Vollmer & Krebs, 1999). As such, total metal content is often an indiscriminate method to assess the risk of exposure to contaminants, compared to a measure of free ion activity or element solubility (Gray & Mclaren, 2006). Within soils, it is those soluble or readily exchangeable trace elements which become available to plants (Robinson, Bolan, Mahimairaja, & Clothier, 2006). This research focuses on the effect of biochar on trace element and nutrient solubility in the presence of low-molecular-weight organic acids (LMWOAs). Trace element solubility is largely affected by soil factors such as pH, redox potential, soil type and soil organic matter (SOM) but also by biogeochemical processes such as dissolution and precipitation (Robinson, Bolan, Mahimairaja, & Clothier, 2006; Young, 2010). As noted by Chuan, Shu and Liu (1996) and later others (see Adriano, 2001; Kabata-Pendias, 2004), acidic and reducing soil conditions are more favourable for metal solubilisation, with soil pH being reported as the major influencing factor for the solubility of trace elements (Robinson et al. 2006). A general guiding principle was postulated by Kabata-Pendias (2004) that in oxidising acidic soils (pH < 3) trace elements such as cadmium, zinc, copper and nickel are more soluble, therefore more likely to be utilised by plants. However in oxidising soils, if the pH was likely to increase to around 5 or greater, the solubility of trace elements would be reduced. Under reducing conditions in neutral (pH 7) or alkaline soils (pH > 7) the solubility of these elements is greatly reduced and at higher pH levels tend to precipitate out of soil solution (Adriano, 2001; Hindersmann & Mansfeldt, 2014).

Alongside trace elements, this thesis also examines nitrate and phosphate behaviour in various systems. The aforementioned general principles are not explicitly applicable to either nitrate or phosphate. Nitrate has a weak affinity to soil colloids and therefore is soluble under most soil conditions (Heaney, Mamman, Tahir, Al-Gharib & Lin, 2018). Losses in the soil matrix are generally due to denitrification processes, commonly present in anaerobic soils. Inorganic phosphate is readily soluble, however, the solubility of phosphate is affected by primarily by pH and soil type which affect presence of free oxides and hydroxides of aluminium and iron and the presence of calcium (Jensen, 2010; Rodríguez & Fraga, 1999). In more acidic soils, phosphate ions are likely to complex with iron and aluminium hydroxides, reducing phosphate solubility. Whereas in alkaline soils, phosphate ions bind with available calcium and magnesium to form insoluble complexes (Jensen, 2010). Regardless of the contaminant type, once soluble, contaminants become physically mobile within the soil and are readily available for plant uptake. Therefore, solubility governs the mobility of contaminants within the environment.

The risk from soluble harmful contaminants presents an ongoing challenge for the environmental practitioner. The different properties of each compound vary as does the soil properties from one site to the next, making generalised remediation practices difficult (Khan, Husain, & Hejazi, 2004). Likewise, traditional excavation techniques are both expensive and time-consuming. In the European Union, there are approximately 340,000 contaminated sites, which require remediation at an estimated cost of six billion euro per year to remediate (Domínguez, Madrid, Marañón, & Murillo, 2009; Van Liedekerke, Prokop, Rabl-Berger, Kibblewhite, & Louwagie, 2014). The number of contaminated sites, which exist, and the cost to remediate them is driving a search for an economically practical and sustainable remediation technology. The use of organic amendments such as composts and biosolids for soil remediation is a preferred and attractive alternative to traditional remediation methods in terms of cost and sustainability (Beesley et al., 2011; Janus et al., 2015; Mohan, Sarswat, Ok, & Pittman, 2014).

Biochar is a carbonaceous organic soil amendment which has demonstrated an ability to remediate inorganic and organic contaminants because of its favourable physicochemical characteristics for contaminant adsorption such as a high specific surface area and negatively charged surface (Ahmad et al., 2014; Park et al. 2015). However, the ability to immobilise contaminants is a function of the production parameters and original biomass choice, which markedly affect the physicochemical properties of biochar (Aller, 2016). Furthermore, biochar is reportedly cheaper than and as effective as activated carbon, suggesting that it can meet the criteria for efficacy and sustainability without the costs of traditional and similar soil remediation techniques (Paz-Ferreiro, Lu, Fu, Méndez, & Gascó, 2014). As a result, a considerable literature has been produced in the past few years. At the time of writing, there are 9137 papers found to include, 'biochar' or 'bio-char' and 'soil' indexed on the Web of Science® Core Collection from 2000 to 2019 with nearly 25 % of these papers being produced in 2018.

With such an increase in related publications, replication has taken place and research progression delayed due to the many production parameters available to create biochar. To date, despite the increased information about biochars' effects of contaminant behaviour in soils, there is still insufficient understanding to allow for the generalisation of biochar functions regarding their uses for environmental remediation. Firstly, most publications focus on limited numbers of environmental contaminants with a limited number of biochar types. Systematic examination of biochar's performance is rare yet required if we are to extrapolate biochar's potential for soil remediation. Secondly, how biochar applications affect the different biogeochemical processes in the soil is still misunderstood. This shortfall in current knowledge has possibly led to an overestimation in biochar's ability to remediate soils in real-world scenarios. Biochar's effects on different biogeochemical processes within soils must be examined further. The work in this thesis uses biochar produced using a standardised procedure. By choosing reproducible and standardised biochar, biochar-contaminant dynamics under a variety of environmental conditions can be examined.

Previous research has suggested that the release of LMWOAs by plants could solubilise biochar-borne trace elements, increasing phytotoxicity (Vause, Heaney, & Lin, 2018). Furthermore, Alozie, Heaney, & Lin (2018) demonstrated LMWOA-driven protonation of biochar materials favoured the retention of anionic contaminants over cationic contaminants suggesting that LMWOAs may significantly affect the functioning of biochar in soil environments. However, the role of LMWOAs in affecting trace element and nutrient solubility in biochar-amended soils have been overlooked. So far, there are no systematic investigations that unveil the roles of LMWOAs in complicating the biochar functioning in the amended soils. This represents a significant knowledge gap that needs to be filled to enhance the understanding of how biochar improves soil conditions for plant growth and reduces food safety risk from the accumulation of toxic elements in agricultural products. This thesis provides an original contribution to knowledge by its systematic examination of how LMWOAs affect biochar-contaminant dynamics, which until now has been overlooked.

1.2 Research aim and hypotheses

The broad aim of this research is to examine the effect of biochar on trace element and nutrient solubility in the presence of LMWOAs. To achieve this aim, a series of batch experiments and microcosm experiments under different environmental conditions was carried out. Five research hypotheses are proposed:

1. LMWOAs will inhibit the biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems.
2. LMWOAs will facilitate the biochar-driven immobilisation of nitrate and phosphate in aqueous systems.
3. Biochar will reduce the LMWOA-driven solubilisation of trace elements and nutrients in soils.
4. Biochar and LMWOA-activated biochar will:
    a) Increase the germination percentage of different edible plant species.

b) Increase the fresh biomass of different edible plant species.

c) Increase the shoot height of different edible plant species.

d) Increase the root length of different edible plant species.

5a) Biochar will reduce trace element uptake by a selected plant species.

5b) LMWOA-activated biochar will have no effect on trace element uptake by a selected plant species.

1.3 Thesis outline

The thesis has six chapters. Chapter 1 has introduced the research theme and presented the research aim and hypotheses. Chapter 2 includes an introduction to soil remediation and biochar by way of a concise literature review covering the complex relationships that occur in soil-plant-biochar systems. Chapter 3 presents the methods used throughout this thesis. In Chapter 4, the results for each study are presented. In Chapter 5, the major findings are critically discussed. Finally, Chapter 6 presents a summary of the key research findings, limitations, contributions and future research recommendations. A flow chart showing the thesis layout is presented in Figure 1.1.

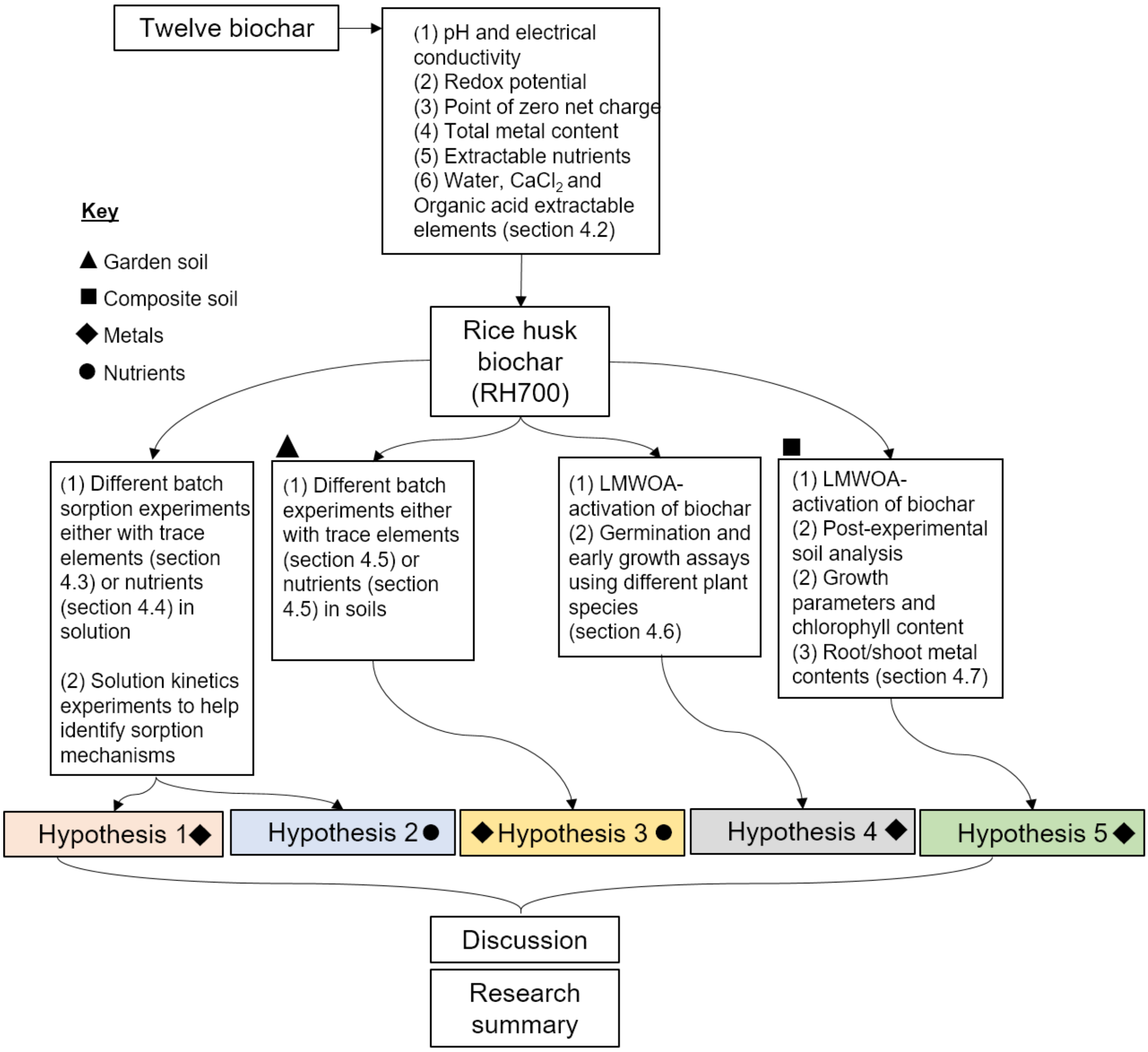

Figure 1.1 A flow chart showing the organisation of the thesis, the theme of each chapter and the relationship between each chapter and the research hypotheses

CHAPTER 2

Literature Review

2.1 Introduction

Most of the research concerning biochar soil amendment is limited to the interactions with bulk-soils. However, biochar is likely to encounter LMWOAs present in soils, especially in rhizospheric soils (Alozie et al., 2018). Distinctly, rhizospheric soils are those at the soil-plant interface, whereas bulk soil is soil void of plant roots. In soils, LMWOAs are primarily derived from exudation of plant roots. These root-secreted carboxylic acids play a central role in the solubilisation of soil-borne contaminants and nutrients (Guppy, Menzies, Moody, & Blamey, 2005; Jones, 1998; Violante, Cozzolino, Perelomov, Caporale, & Pigna, 2010). The reported concentrations of LMWOAs in soils range from 1 to 100 mmol kg^{-1}, depending on soil type, plant type and other factors (Ash et al., 2016; Jones, 1998). Different kinds of LMWOAs exist in the rhizosphere with citric, malic, oxalic acids being amongst the most common LMWOA species (Chen, Dou, & Xu, 2018). LMWOAs may modify the characteristics of biochar surfaces and consequently affect biochar-contaminant interactions in amended soils (Alozie et al., 2018). However, the potential role of LMWOAs in complicating biochar functioning in such systems has so far been overlooked.

Several studies have indicated that biochar can reduce the bioavailability of heavy metals in contaminated soils (Beesley & Marmiroli, 2011; Houben, 2013; Park, Choppala, Bolan, Chung, & Chuasavathi, 2011). Key characteristics such as a large surface area, a negative surface charge and functional groups are related to biochars’ ability to immobilise contaminants. However, such characteristics are primarily dependent on pyrolysis temperature, feedstock type and environmental conditions. Recent literature reviews demonstrate the large volumes of publications that examine soil remediation potential using different biochar materials, e.g. Ahmad et al. (2014); O'Connor et al. (2018); Yue et al. (2019). It has conclusively been shown that biochar production parameters affect remediation ability (Aller, 2016). There is, therefore, an interest in biochar characterisation studies and the standardisation of biochar production procedures to create a ‘standard biochar’.

In 2009, the UK Biochar Research Centre (UKBRC) was founded, and one of their objectives was to ‘efficiently and effectively address the many uncertainties that exist around the development of biochar technologies and potential products, providing a robust evidence base’ (UK Biochar Research Centre, 2013). One of the outcomes was the production of twelve biochar materials from six common feedstock materials. The products were highly reproducible and extensively characterised, making them appropriate for use in further research that can allow for the generalisation of biochar-contaminant interactions to be made.

Despite the availability of UKBRC standard biochar materials, few published studies have utilised the products. Those studies that have used UKBRC standard biochar materials (e.g. Buss, Graham, MacKinnon, & Mašek, 2016; Mašek, Brownsort, Cross, & Sohi, 2013; Shen, Zhang, Jin, McMillan, & Al-Tabbaa, 2017) often focus on different aspects of biochar-related research such as carbon sequestration or production. For instance, the highly cited review article by Ahmad et al. (2014) did not review a single publication that used UKBRC biochar. Although this is not surprising given the large number of biochar publications generated annually, this underpins the lack of biochar production standardisation within the scientific community. Furthermore, only two published papers found in the literature review have examined biochar-contaminant interactions in the presence of LMWOAs using UKBRC standard biochar (Alozie et al., 2018; Vause et al., 2018). This reiterates the lack of understanding regarding biochar-contaminant interactions in rhizospheric soils.

Organic contaminants behave very differently from inorganic trace elements and nutrients owing to their different chemical characteristics. This is expected to hold for the scenarios in the co-presence of biochar and LMWOAs (Ahmad et al., 2014; Cao, Ma, Gao, & Harris, 2009; Chen & Chen, 2009; Chen, Zhou, Xu, Wang, & Lu, 2015). It is acknowledged that coexistence between inorganic and organic contaminants in heavily contaminated soils is possible. However, research should first, establish the biochar-trace element and nutrient dynamics in rhizospheric soils due to inorganic ions natural presence in geological materials such as rocks, ores, soils, sediments and water opposed to synthesised compounds. For that reason, it was beyond the scope of this review to investigate biochar-organic contaminant related themes.

This chapter aims to establish a framework for the current research. Firstly, background information regarding soil biogeochemistry, contaminant behaviour and an introduction to biochar are provided. Then, a critical synthesis of the available literature on the effect of biochar on contaminant solubility and subsequent mobility in varying environmental mediums was completed. Also, an attempt to establish how LMWOAs may affect biochars' remediation potential is included. The remaining part outlines specific knowledge gaps and the research hypotheses that will be tested to fulfil the research aim.

2.2 Soil contamination

2.3 Definitions of soil contamination

Certain amounts of elements occur naturally within soils, derived from the weathering and mineralisation of local rock materials. The normal background value is dependent on the parent rock material and historical land use, so it is highly variable. In instances where the concentrations of potentially toxic substances are elevated compared to normal background levels, the terminology 'contaminated' or 'polluted' is often used and in many of the publications used throughout this thesis, these terms are used interchangeably. The UK Government defines contaminated land as:

'any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land that –

> (a) significant harm is being caused, or there is a significant possibility of such harm being caused; or (b) significant pollution of controlled waters is being caused, or there is a significant possibility of such pollution being caused,' (Environmental Protection Act 1990 s. 78A).

From a legal standpoint, land cannot be classified as contaminated or polluted until a whole source-pathway-receptor relationship is established (Strange & Langdon, 2008). Therefore, it is vital to understand the different sources that are polluting soil, the receptors which could be at risk and the numerous pathways linkages between them before any attempts at remediation is carried out. Sources of soil contamination can either be by point-source or diffusion. In either case, sources are primarily anthropogenic such as poor waste disposal, mining practices and energy production. Often cases of historical contaminants are still causing problems today due to the scale and persistence of some contaminants found within the environment.

2.3.1 Inorganic contaminants and usage

Differentiation of contaminants in water and soil environments depends whether they are organic or inorganic. Organic contaminants are carbon-containing organic compounds such as petroleum hydrocarbons, BTEX and various pesticides. Inorganic contaminants refer to compounds without a C-H bond such as metals, metalloids, and forms of nitrogen and phosphorus. It is acknowledged that contaminated sites are often sites containing a mixture of inorganic and organic contaminants. The work in this thesis focuses on inorganic contaminants only. The following section will concentrate on the model contaminants used in this work only.

2.3.1.1 Cadmium, Lead and Zinc

Three model heavy metals have been selected for use in this thesis, cadmium, lead and zinc. Cadmium (Cd) (A_r = 112.41, density = 8.65 g cm^{-3}) is a non-essential divalent heavy metal from Group 12 of the periodic table. Cadmium exists naturally in the lithosphere in relatively small quantities (around 0.53 mg kg^{-1}) and the natural cycling of materials by weathering is a major source of cadmium into the environment (Kabata-Pendias & Pendias, 1992; Khan, Khan, Khan, & Alam, 2017). Anthropogenic sources of cadmium include alloys and nickel-cadmium batteries and their inadequate disposal and application of phosphate-based fertilisers (Järup, 2003).

Lead (Pb) (A_r = 207.2, density = 11.34 g cm^{-3}) is a post-transition metal that belongs to Group 14 of the periodic table. Like cadmium, it is a non-essential metal (Khan et al., 2017). Naturally occurring concentrations of lead range from 2 to 200 mg kg^{-1} (Alloway, 1968, cited in Ahmad, Najeeb, & Zia, 2015). Humans have used lead for millennia, resulting in widespread historic contamination. In addition, smelting activities and shooting ranges are often cited as the leading sources of lead anthropogenic inputs to soil (Juhasz, Weber, & Smith, 2011).

The final model metal, zinc (Zn) ($A_r = 65.38$, density = 7.14 g cm^{-3}) is a transition metal that belongs to Group 12 of the periodic table. It is also an essential micronutrient (Ishimaru, Bashir, & Nishizawa, 2011). Typical lithogenic zinc concentrations range between 10 and 300 mg kg^{-1} (Kiekens, 1995, cited in Ahmad et al., 2015). Non-ferric metal mining and smelting activities are usually cited as being responsible for the anthropogenic inputs of zinc into soils (Cuypers et al., 2013). However, zinc is also included in fertilisers, and as a result, is a concern in agricultural soils (Alloway, 2008; Sturikova, Krystofova, Huska, & Adam, 2018).

2.3.1.2 Nitrogen and Phosphorus

Nitrogen (N) and phosphorus (P) are essential nutrients for plant growth. However, these elements are naturally depleted in soils as part of their geochemical cycles (Y. Zhang, Xie, Ni, & Zeng, 2019). Significant loss pathways include surface runoff, leaching through subsurface soils into groundwaters and volatilisation into gaseous forms (Y. Zhang et al., 2019). Agricultural applications of nitrogen and phosphorus-based fertilisers are a common practice as crops are often deficient in nitrogen and phosphorus due to the anionic nature of their bioavailable ions (NO_3^-, $H_2PO_4^-$ and HPO_4^{2-}). Nitrate compounds have a weak affinity to soil colloids, and as a result, are easier to mobilise under most soil conditions (Barber, 1995; Dickinson & Murphy, 2008). Due to their lack of retention to soil particles but the necessity for improved crop yield, repeated and excessive application of inorganic fertilisers often occurs to meet crop demands (Gul & Whalen, 2016). However, such excessive application is now a global concern. For instance, leaching of nitrates from soils weakens the capacity of soils to supply nutrients for plant growth and increases the level of nitrogen in receiving water environments, which could contribute to eutrophication in the affected aquatic ecosystems (Beeckman, Motte, & Beeckman, 2018; Camargo & Alonso, 2006; Di & Cameron, 2002). Although phosphorus does not have the same solubility as nitrates through soils owing to its affinity to iron hydroxides, it too, in part, contributes to nutrient losses via erosion and leaching which can also contribute to eutrophication (Wang et al., 2005).

2.3.2 Associated health effects

The existence of contaminants within soils does not necessarily result in the ill health of those nearby. Instead, the exposure to labile soil-borne contaminants poses a risk. Exposure to contaminants can occur through different pathways. The three primary pathways are direct dermal contact with contaminated soil, ingestion or the inhalation of particulate matter (Luo et al., 2012). Numerous studies have documented the associated health risks concerning heavy metals and metalloids exposure. A well-cited review of the associated health risks of heavy metal exposure from the environment suggested exposure to metals caused headaches, central nervous system effects and gastrointestinal symptoms (Järup, 2003). A more recent review and meta-analysis by Chowdhury et al. (2018) corroborated that exposure to arsenic, cadmium and copper is associated with an increased risk of cardiovascular disease and coronary heart disease.

Regarding nitrogen exposure, excessive consumption or exposure to nitrates is reportedly carcinogenic and may cause congenital disabilities, goitre, diabetes and changes to the heart muscles (Taneja, Labhasetwar, & Nagarnaik, 2019). In the same vein, phosphorus exposure has been linked to endocrine dysfunction and both kidney and cardiovascular disease (Uribarri & Calvo, 2017).

What is clear is how the exposure or accumulative exposure to specific amounts of labile inorganic contaminants can be detrimental to human health, which emphasises the importance of sufficient soil remediation strategies for contaminated sites. It is essential to state exposure is dependable on the biogeochemical processes at work in the soil, which governs the fate and transport of inorganic contaminants in soils and ultimately, the risk of exposure.

2.4 Biogeochemistry of contaminated soils

2.4.1 Distribution and availability of inorganic ions in soils

Anthropogenic contaminants tend to be more mobile in soils, primarily existing within the first 30 cm of soil where biogeochemical processes are likely to liberate contaminants more easily, increasing availability for successful contaminant pathways to flora and fauna receptors (Adriano, Wenzel, Vangronsveld, & Bolan, 2004; Wuana & Okieimen, 2011). Bioavailability was first used to define the total available concentration of metals in bulk soil that pose a risk to receptors (Adriano et al., 2004). However, using bioavailability to define the mobile fraction of metal ions available for uptake by biota, known as the bioavailable fraction, is a more effective definition (Cuypers et al., 2013; Zhang et al., 2013). Various factors such as soil pH, redox conditions, and ionic strength of soils govern the solubility and therefore, the bioavailability of contaminants. For example, the solubility of free cations decreases with increasing pH and is reversed for anionic species (e.g. chromium and arsenic oxyanions) (Antoniadis et al., 2017; Tack, 2010). However, to understand the biogeochemical processes at work, it is vital to understand the chemical distribution of species within soils.

Inorganic contaminants exist in soils in three pools, the soluble pool, the labile pool and the non-labile pool (Adamo & Zampella, 2008) (Figure 2.1). Firstly, the soluble pool relates to ions which exist within soluble forms in pore waters at equilibrium. Adsorption refers to contaminants that are physically or chemically bound to the soil matrix. Likewise, desorption is the liberation of bound species due to dissolution. These processes are dependent on soil conditions but govern the movement between the soluble and exchangeable labile pool. Solution phase species include free ions existing in soil solutions such as Cd^{2+}, Pb^{2+}, and Zn^{2+}. Some metals exist in soil solutions as inorganic complexes bound to chloride ions for example. In contrast, some metallic species can bind to organic ligands such as LMWOAs or bind to suspended clay particles and organic matter (see Table 2.1). Weakly bound complexes with lower affinities to soil colloids are more likely to undergo desorption mechanisms governed by decreasing pH, increasing their solubility and availability for liberation (Tack, 2010).

The labile pool can be separated into either the exchangeable or non-exchangeable phase. In the exchangeable phase, contaminants are liable to undergo solubilisation or fixation. Non-exchangeable species are fixed within the soil matrix as they are bound to soil colloids. If solubilisation occurs, contaminants can re-enter the exchangeable phase of the labile pool. Lastly, those species that are within the non-labile pool are insoluble and are fixed within the soil unless mobilisation or mineralisation takes place, for example, insoluble metal oxides. Together, such biogeochemical processes like adsorption-desorption, precipitation-mineralisation and mobilisation can determine the solubility and transport of contaminants within soils (Comerford, 2005).

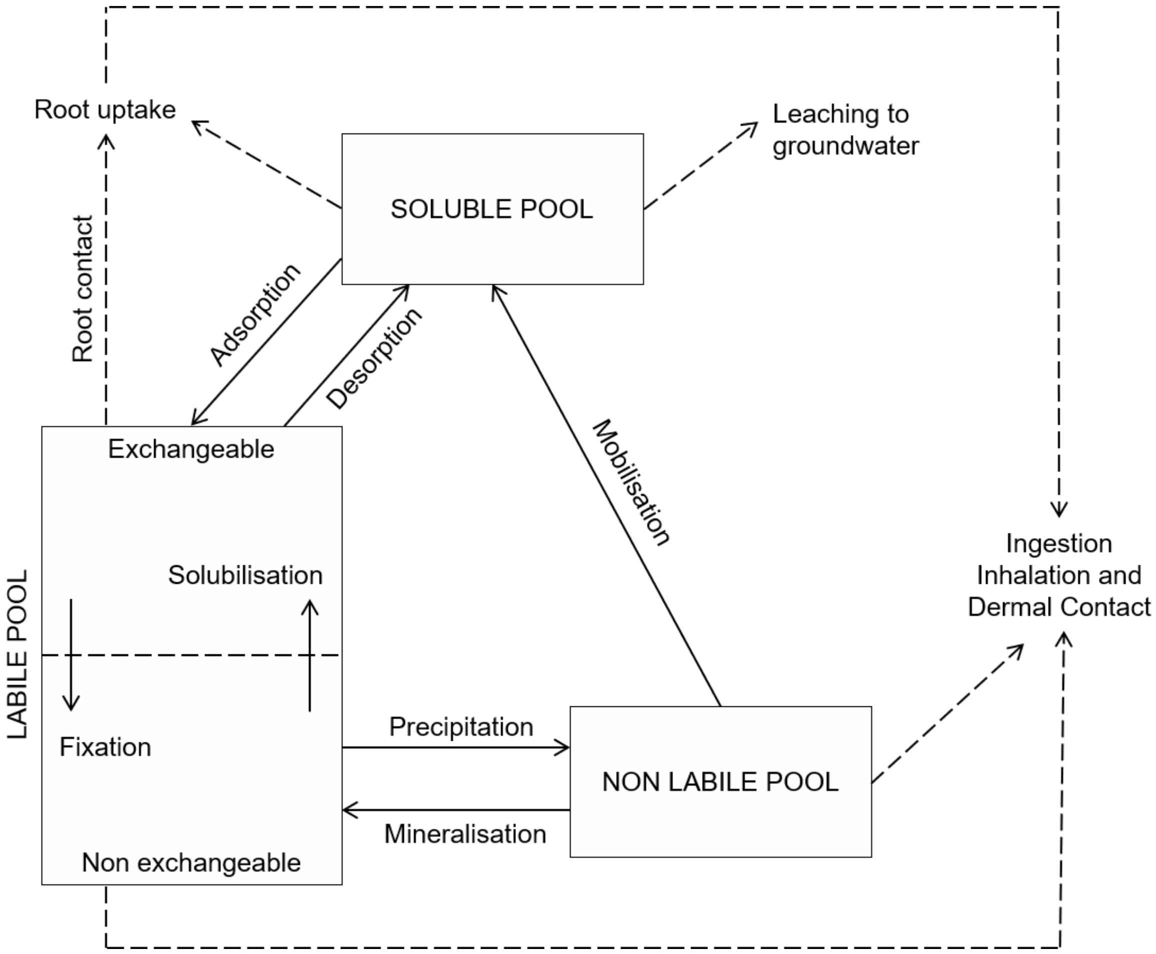

Figure 2.1 A schematic of the different contaminant pools and the associated biogeochemical processes that may affect contaminant solubility in the soil matrix. Adapted from Adamo and Zampella (2008).

Table 2.1 Categories of chemical species in the different soil solution and soil solid phase species as described by Tack (2010)

Soil solution phases species	Soil solid phases species
Free ions	Exchangeably bound to charged surfaces
Inorganic complexes	Complexed with or occluded with organic matter
Organic complexes	Adsorbed or occluded un hydrated oxides of iron and manganese
Bound to suspended colloids	Adsorbed or occluded in carbonates
	As precipitates (carbonates, phosphates, sulphides)
	As structural component in minerals

2.4.2 The rhizosphere and soil-plant interactions

The rhizosphere is the 1 to 2 mm zone that surrounds plant roots which undergoes different biogeochemical processes compared to that of plant-free or bulk soil. At the soil-plant interface, roots secrete rhizodeposits, also known as root exudates. The chemical compounds contained in root exudates include LMWOAs, sugars, phenolics and high-molecular-weight organic substances such as proteins (Mimmo et al., 2014). The pH of the rhizosphere is generally acidic credited to the release of H^+ or OH^- to maintain a cation-anion exchange balance, the secretion of organic acids, root respiration and redox-coupled processes (Seshadri, Bolan, & Naidu, 2015; Sun et al., 2019). The root-induced acidification tends to be a response to nutrient deficiency or other stress-induced factors such as aluminium toxicity. The decreased pH can increase the solubility of nutrients and trace elements, mobilising them for plant uptake. For example, increased available phosphorus in neutral and alkaline soils following root-induced acidification has been reported (Hinsinger, 2001). In contrast, LMWOAs can form insoluble complexes such as cadmium-oxalate to limit plant uptake (Lamy, Djafer, & Terce, 1991; Mulligan, Yong, & Gibbs, 2001). Interactions at the soil-plant interface are also affected by species-specific exudations, like the reported root-induced liming by certain hyperaccumulating species (Sun et al., 2019).

2.4.2.1 Plant-plant interactions and root exudation

Along with soil-plant interactions, root exudates have an essential role in plant-plant interactions and determine plant survival (Bais, Weir, Perry, Gilroy, & Vivanco, 2006). Positive plant-plant interactions include the release of phytotoxic compounds to repel predators and the synergistic release of exudates and volatiles to kill herbivores (Bais et al., 2006). In terms of negative interactions between plants, parasitic plant host invasions and chemical interferences from exudates can limit plant growth. Allelopathy is probably the most significant negative interaction between plants. It can be described as chemical-mediated plant-plant interferences, whereby exudations help plants gain an advantage over neighbouring competitors (Bais et al., 2006). Common side effects experienced by competitors include reduced seedling growth and germination depending on plant species (Ferguson, Rathinasabapathi, & Chase, 2013).

Allelopathic chemicals can also exist in soils after plant removal suggesting the effects can still influence biogeochemistry following root removal (Ferguson et al., 2013; Inderjit & Weston, 2003). One example is the case of the Black Walnut Tree (*Juglans nigra*) and the exudation of juglone ($C_{10}H_6O_3$) which is reported to persist in soils for somewhere between 6 and 12 months following removal and the reduced photosynthesis, root necrosis and respiration of plants are commonly observed (Bertin, Yang, & Weston, 2003; Inderjit & Weston, 2003).

2.4.3 Factors influencing root exudation

Exudation can be influenced by either biotic or abiotic processes, and we must achieve a deeper understanding of the effects of root exudation on contaminant solubility in soils for remediation. This section offers a view of factors which can influence exudation and potentially hinder or aid remediation efforts following influences identified by (Gupta & Mukerji, 2002). A summary is provided in Table 2.2.

2.4.3.1 Plant Factors Influencing Root Exudation

Root exudation is thought to change in quality and quantity with the three plant growth stages, seeding, flowing and fructification (Brimecombe, De Leij, & Lynch, 2007; Inderjit

& Weston, 2003; Uren, 2007). Work by Rovira (1956, 1959, cited in Hamlen, Lukezic, & Bloom, 1972) suggested species-specific enhanced exudation during initial growth stages. Similarly, Vancura and Hovadik (1965, cited in Rovira, 1969) found differences in cucumber, tomato and red pepper exudates with cucumber only excreting during early seeding compared to tomato and red pepper excreting at fruiting stage. However, some studies have found decreased exudations with increased plant age (Arkadeva, 1963a, 1963b; Schreiber & Green 1963; cited in Hamlen, Lukezic, & Bloom, 1972). These results outline the differences in exudation with plant species over time. It can be theorised that throughout plant development, exudation fluctuates which can potentially lead to a fluctuation of heavy metal and nutrient solubility correlating with plant age.

2.4.3.2 Soil Factors Influencing Root Exudation

Soil pH is a primary factor controlling root exudation. pH changes with the translocation of compounds by plants can occur and when plants uptake cations they release anions to balance the charge (Zeng, Chen, Miao, Wu, & Zhang, 2008). These changes in pH can alter the solubility and bioavailability of heavy metals and trace elements within bulk soil and the rhizosphere (Zeng et al., 2008).

In soils with elevated trace elements, exposure can trigger a plant defence mechanism (Javed, Stoltz, Lindberg, & Greger, 2013). Likewise, nutrient deficiency can cause exudation, which affects metal and nutrient solubility. Phytosiderophores are chelating substances which promote complexation in soils which are released from graminaceous species to acquire iron by making inorganic iron (III) soluble and mobile (Römheld & Marschner, 1986; Schenkeveld, Reichwein, Temminghoff, & van Riemsdijk, 2014; Violante et al., 2010).

In a broader sense, soil classification can directly influence exudations due to the different physiochemistry between soils, which in turn affects root growth and the subsequent release of exudates (Inderjit & Weston, 2003; Neumann et al., 2014). A recent study found exudation varied in three different soils, loess loam, alluvial loam and diluvial sand. Glucose concentrations were found to be high in loess loam, medium in diluvial sand and low in alluvial loam (Neumann et al., 2014). The findings were attributed to the different nutrient availabilities and microbial diversities (see section 2.4.3.4) of the varying soil types. Soil conditions may directly affect root exudations, which effects metal and nutrient solubility.

2.4.3.3 Environmental Factors Influencing Root Exudation

Photosynthates created by plants during photosynthesis can be rhizodeposited as metabolic products that are created by the plant and translocated to the root (Hale & Moore, 1979). Therefore, environmental factors that directly affect photosynthesis have an indirect influence on root exudation and in turn, metal and nutrient solubility in soils. The main limiting factors for photosynthesis are temperature, light intensity and carbon dioxide concentration, but nutrient availability and water availability can alter the rate of photosynthesis (Flexas et al., 2014). In terms of root exudation, temperature and light have a noticeable effect on exudations.

The different rates of photosynthesis, membrane permeability, translocation rates and different enzyme activity rates limit the understanding between temperature and photosynthesis-induced exudation. For instance, an elevated temperature is thought to increase exudation (Bokhari & Singh, 1974; Curl & Truelove, 2012; Shukla et al., 2011). Yet early root-exudation work shows how the influence of temperature on exudation can be plant-specific (Rovira, 1959, 1969; Rovira & Harris, 1961). Furthermore, an indirect link between plant foliage and exudation may exist because of changing soil temperatures owing to changes in soil albedo. A study by Smith (1972) found a link between defoliation and increased exudation quantity from sugar maple saplings. They found that out of fifteen exudates measured, six were affected by defoliation: mainly via an amino acid release. These studies suggest that seasonality may affect exudate release and in turn, affect trace element and nutrient solubility in soils.

Concerning the light intensity exposure, it is expected to follow a similar trend to that of temperature, generally increasing exudation with increased intensity (Curl & Truelove, 2012). Work by Cakmak, Erenoglu, Gülüt, Derici, and Römheld (1998) found that phytosiderophore release from wheat and barley under zinc and iron-deficient scenarios increased with increasing light intensities between 50 to 600 µmol m^{-2} s^{-1}. More recently, Cheng et al. (2014) found that citrate exudation by white lupin (*Lupinus albus L.*) showed an increase citrate exudation as light intensity increased from 200 µmol m^{-2} s^{-1} to 600 µmol m^{-2} s^{-1}.

These studies suggest that seasonality may affect exudate release and in turn, affect trace element and nutrient solubility in soils. From the studies reviewed, it can be summarised that different plants favour and release exudates at different temperatures while defoliation is thought to increase exudation quantity. If this is the case, different plants will evoke different metal and nutrient solubility in the surrounding soils and in areas of lesser vegetation exudation can be expected to increase, leading to varying dynamics between present metals, nutrients and exudates. Likewise, where there is lower vegetation coverage, and the light intensity received in soils is higher, exudation will be increased, and the same dynamics will be expected. It is evident that environmental factors play a direct role in exudate release and indirectly influence metal and nutrient solubility in soils.

2.4.3.4 Microbial Factors Influencing Root Exudation

To get the full picture of soil-plant interactions, one must consider the role of microbes. The rhizosphere is a ‘hot spot of microbial interactions’ where exudates and microorganisms interact with organic matter and act as a mediator between plants and soil (Brimecombe et al., 2007; Raaijmakers, Paulitz, Steinberg, Alabouvette, & Moënne-Loccoz, 2009). Root exudates are the most important factor that causes microbial changes within the rhizosphere and can cause stimulatory or inhibitory effects on soil-plant interactions, nutrient supply, growth and survival which are governed by microbial presence (Bais et al., 2006; Brimecombe et al., 2007; Martin, George, Price, Ryan, & Tibbett, 2014; Neumann et al., 2014). Three important factors which are altered with changes to microbe populations are (i) permeability of root cells, (ii) root metabolism and (iii) absorption and secretion of exudates by microorganisms (Rovira, 1969; Shukla et al., 2011). The consensus is that exudation increases with microbial populations (Brimecombe et al., 2007). When exudation occurs in large quantities, biological processes such as biodegradation can occur (Bertin et al., 2003; Inderjit & Weston, 2003). As described by Phillips, Greer, Farrell, and Germida, (2012), this is a synergistic process whereby roots secrete organic compounds that increase microbe activity and population, thus facilitating the degradation of organic compounds.

Table 2.2 Factors which may affect the release of LMWOAs from root systems

Factor	Type	Evidence	References
Plant	Species	Different amounts of exudation as different plants have different rates of (i) photosynthesis, (ii) enzyme rates, (iii) membrane permeability and (iv) translocation rates	Inderjit & Weston (2003), Shukla et al. (2011)
	Age	Exudation release changes with development stage of plant	Brimecombe et al. (2007), Inderjit & Weston (2003), Uren (2007)
Environment	Temperature	With increased temperature, exudation generally increases	Curl & Truelove, (2012)
	Light	As light intensity increases as does exudation	Curl & Truelove (2012) Rovira (1959)
Soil	pH	Soil pH affects exudation and also metal and nutrient solubility	Hinsinger et al, (2009); Zeng et al. (2008)
	Soil albedo	Greater albedo increases temperature and affects exudation rate	Smith (1972)
	Type	Different soils can affect exudation based of physiochemical properties of parent soil	Neumann et al., (2014)
	Nutrient availability	Plant release exudates to balance nutrient availability	Violante et al. (2010)
Microbial	Number of microbes	Root exudation increases with the number of microbes present in soil aiding biodegradation processes	Bertin et al. (2003), Inderjit & Weston, (2003) Phillips et al. (2012)
	Release of acids	Gluconic, oxalic and citric acids are released from microbes which give further energy to microbes creating a synergistic effect on metal and nutrient solubility	Rajkumar, Sandhya, Prasad, & Freitas. (2012)

As the scope of this research in constrained to inorganic compounds which are typically non-biodegradable, microbes can also influence inorganic nutrient and metal solubility. For example, microbes can affect metal solubility depending on environmental factors, rhizosphere physiochemistry and the type of microbe directly involved with the relating biogeochemical mechanisms (Gadd, 2004, 2010). Microbes, like root exudates, are also known to release low-molecular-weight compounds, which in turn affect the solubility of nutrients and metals in soils (Rajkumar, et al., 2012). Therefore, it can be speculated that if exudation increases microbial populations by the release of LMWOAs, which energise microbial populations, then an increased release of exudations will be found from microbes, creating an amplified synergistic effect on nutrient and metal solubility.

2.4.4 Low-molecular-weight organic acids

In the current work, LMWOAs are at the forefront of the research. LMWOAs are weak carboxylic acids which are commonly found in soils because of root exudation (Ash et al., 2016; Onireti & Lin, 2016) but also exist as secretions from microbes and the decomposition of animal and plants (Jiang, Jiang, Xu, & Li, 2012; Najafi & Jalali, 2015). In terms of root exudation, commonly found LMWOAs are acetic, citric, formic, fumaric, malic, malonic, oxalic, tartaric and succinic acids (Dinh, Li, Thu, Wang, & Liang, 2017). Figure 2.2 shows the chemical structure of three of the most commonly used model LMWOAs in the surveyed research, and those used in this thesis.

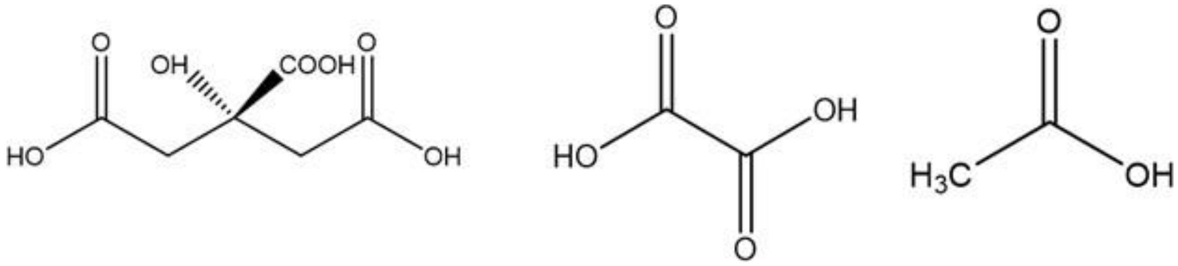

Figure 2.2 Chemical structures of three commonly found LMWOAs in soils: citric, oxalic and malic acid.

Previous studies have reported varying concentrations of LMWOAs ranging from 0.1 μm to 100 mmol kg^{-1} (Ash et al., 2016; Dinh et al., 2017). As shown in Table 2.2, LMWOA type and concentration are affected by factors such as soil pH, microbial activity and plant species. It is believed they are released from plant roots as a response to either anoxia, metal toxicity or nutrient deficiency (Agnello, Huguenot, Van Hullebusch, & Esposito, 2014). They are of importance as they can affect the solubility and therefore, bioavailability of metals within soils by the complexation and chelation with metal species.

The three primary ways in which LMWOAs can affect trace element solubilisation is via acidification, complexation and reductive dissolution (Jones, 1998; Jones, Dennis, Owen, & Van Hees, 2003; Nworie, Qin, & Lin, 2017; Onireti, Lin, & Qin, 2017; Schwab, Zhu, & Banks, 2008). Root-induced acidification can affect the fate and mobility of trace elements in soils by increasing their solubility (Houben & Sonnet, 2015; Najafi & Jalali, 2015). Reactions with the soil surface via root proton efflux may also change the variable charge of soil colloids affecting the natural attenuation of nutrients and trace elements to soil colloids (Guppy et al., 2005; Qin, Enya, & Lin, 2018).

Complexation is primarily affected by the functional groups of organic acids that can release protons into soil solution, creating a high complexation affinity to cationic metals (Potysz et al., 2017; Yang, Sheng, & Huang, 2006). As a result, divalent and trivalent organic acids such as citrate, malate and oxalate are often responsible for solubility of metals within the rhizosphere (Jones et al., 2003; Mench, Morel, Guckert, & Guillet, 1988). LMWOAs are also able to form complexes with trace elements and nutrients which generally increases their solubility (Guppy et al., 2005; Jones, 1998; Qin et al., 2018). The efficacy of LMWOAs to form such complexes is reliant on the properties of the organic acids, i.e. the dissociation properties, number of carboxylic groups and the amount of LMWOAs exuded from the root systems (Jones, 1998). The release of protons from the carboxyl groups increases the complexation affinity for cationic species, therefore, a more considerable amount of carboxyl groups generally relates to a higher affinity to form complexes with divalent and trivalent metals (Jones, 1998; Potysz et al., 2017). As such, complexation reactions between cations and divalent and trivalent organic acids such as citrate, malate and oxalate are often responsible for the speciation and solubility of trace elements and nutrients within the rhizosphere (Jones et al., 2003; Mench et al., 1988; Potysz et al., 2017). Concentrations of metal within soils and soil pH also affect the amount of metal complexation within soils (Najafi & Jalali, 2015). Several studies have revealed that LMWOAs can enhance organic acid-driven solubilisation of iron-arsenic complexes (Ash et al., 2016; Onireti & Lin, 2016; Onireti et al., 2017). Similarly, others have reported that organic acids can increase the solubility of chromium (Jean et al., 2008) and cadmium (Nigam, Srivastava, Prakash, & Srivastava, 2001).

Lastly, organic acid-induced dissolution of nutrients and trace elements is widely reported to affect metal solubility (Jones, Darah, & Kochian, 1996; Nworie et al., 2017; Onireti & Lin, 2016; Taxiarchou, Panias, Douni, Paspaliaris, & Kontopoulos, 1997). In calcareous soils, where the solubility of iron is restricted due to high pH, organic acids have been reported to mobilise ferric hydroxides (Jones, 1998). Like the efficacy of LMWOAs to form complexes depends on the number of functional groups, so does the efficacy for LMWOA-mediated dissolution (Huang et al., 2019). Oxalate ions can aid iron oxide dissolution via the formation of soluble iron oxalate at high oxalate concentrations (Onireti & Lin, 2016). In a study by Onireti and Lin (2016), the authors found that oxalate-induced reductive dissolution was a primary factor in mobilising soil-borne iron. Notably, citrate- and malate-induced reductive dissolution was also found but to a lesser extent. Overall, LMWOAs play an important role in governing trace element and nutrient solubility at the soil-plant interface. So, consideration for LMWOAs should be made in soil-related trace element and nutrient studies.

2.4.5 Plant uptake of trace elements

Plant uptake of trace elements and nutrients is an essential requirement for growth. Several factors such as plant species, soluble metal concentrations and soil conditions govern the uptake by plant tissues (Clemens, Palmgren, & Krämer, 2002). Based on the fractionation of trace elements within soils, only water-soluble and exchangeable fractions are available for plant uptake. As such, the first step in plant uptake mechanism involves the solubilisation of elements within the rhizosphere. Acidification of the rhizosphere by secreted LMWOAs leads to increased solubility, and the exudation of chelators promotes the formation of metal-chelates (Clemens et al., 2002). Following the solubilisation of trace elements, the fractions are sequestered in the vacuoles of root cells. Certain plant species may accumulate trace elements and nutrients within the root cells with limited transport to aerial foliage. Translocation of trace elements and nutrients to plant shoots is either via symplastic or apoplastic pathways (Clemens et al., 2002; Thakur et al., 2016). Predominate mechanisms are thought to be mostly symplastic with minerals entering the xylem via the root symplast before being transported to the plant shoots (Antoniadis et al., 2017; Clemens et al., 2002).

2.5 Soil remediation techniques

Various remediation methods can be used to treat contaminated land, which can be categorised as biological, chemical or physical methods (Khan et al., 2004) (Table 2.3). Biological methods of remediation or bioremediation can refer to the use of microbes or plants to target contaminants. Organic contaminants are often biodegradable, and studies have reported the use of microbes to either degrade or transform organic contaminants such as hydrocarbons, lowering the environmental risk (Chikere, Okpokwasili, & Chikere, 2011). In the case of metals and metalloids, the use of phytoremediation is common. Phytoremediation is an umbrella term referring to the use of plants for remediation. Phytotechnology is a relatively new concept (China, Das, & Maiti, 2014). There are various phytoremediation options available, depending on how plants and their rhizosphere interact with specific contaminants (Bolan, Park, Robinson, Naidu, & Huh, 2011). The most common choices are phytoextraction and phytostabilisation (Bolan et al., 2011; Cunningham, Berti, & Huang, 1995; Koptsik, 2014a, 2014b; Macnair, Tilstone, & Smith, 1999; Paz-Ferreiro, et al., 2014). Such biological methods usually require the caveat that target contaminants are suitable for such techniques and that soil conditions are healthy enough to sustain microbial activity and host plants. This should be considered before use, despite these methods being a greener and cheaper alternative to traditional methods. Furthermore, consideration regarding the disposal of metal-laden plant species should be given, as this is a possible further health hazard.

Table 2.3 The advantages and disadvantages of different soil remediation techniques

Technique	Type	Advantages	Disadvantages
Microbial interaction	Biological	Can use specific microbes for certain organic contaminants	Time consuming May be difficult to apply to multi-contaminated sites
Phytoaccumulation, stabilisation or extraction*	Biological	Green alternative Greater public approval	Time consuming Continued management required Waste disposal issues*
Manual excavation '*dig* and dump'	Physical	One-time removal of contaminated soil	Expensive, Invasive, Waste disposal issues
Soil dilution	Physical	Quick reduction in soil contamination	Expensive Invasive, Waste disposal issues
Soil washing	Physical and Chemical	May be able to reuse compounds washed from soils	Expensive Invasive, Waste disposal issues
Oxide application	Chemical	Oxides natural to soil environment	Largely effected by soil conditions and target contaminant
Amendment application	Chemical	Greener alternative Cheaper alternative	Release of compounds from amendments Long term management may be required

Physical methods of remediation typically refer to the removal of contamination using physical means (Yousfi & Bermond, 2000). This usually refers to a more ex-situ, hard engineering response where traditional 'dig and dump' techniques are used. Another method is soil dilution, which involves mixing contaminated soils with acceptable amounts of uncontaminated soil to dilute the total soil contamination (Lim, Shukor, & Wasoh, 2014). Despite the possible effectiveness of these techniques, they can be costly and time-consuming (Mulligan et al., 2001). Chemical methods of remediation can include the application of oxides to treat soils (Komárek, Vaněk, & Ettler, 2013) or use of amendments such as fly ash (Tsang, Yip, Olds, & Weber, 2014), green waste compost (Beesley, Moreno-Jiménez, & Gomez-Eyles, 2010) and manure composts (Liu, Chen, Cai, Liang, & Huang, 2009).

2.6 Biochar soil amendment

2.6.1 Biochar definitions and production

Biochar is a carbonaceous product typically created from the low oxygen pyrolysis of organic materials (Lehmann & Joseph, 2009). The thermochemical conversion of biomass to biochar is performed at temperatures ranging from 300 to 700 ^{o}C. Speed, retention time and heating temperatures during pyrolysis are critical determining factors for product yield. Fast pyrolysis typically refers to a quick process, lasting only seconds, at temperatures between 300 to 1000 ^{o}C. However, biochar yields when using fast pyrolysis processes are low because bio-oil is the main product (Fiore, Berruti, & Briens, 2018; Mohan, Pittman Jr, & Steele, 2006). As a result, slower pyrolysis techniques are favourable for biochar production for soil amendment. Intermediate pyrolysis is production over 10 to 20 seconds at around 500 ^{o}C and slow pyrolysis is the production between 100 to 1000 ^{o}C, over 2 to 4 hours with a slow heating rate (Kumarathilaka, Mayakaduwa, Herath, & Vithanage, 2015). Slower production rates have been found to produce higher biochar yields (around 20 to 35 per cent) (Ahmad et al., 2014; Brown, 2012) and as a result are more favourable when the ultimate goal is to produce biochar for environmental management practices, rather than for energy purposes.

The term biochar feedstock refers to the original biomass used to create the biochar by pyrolysis. Essentially, the processes of pyrolysis can be applied to any organic material to produce biochar, making biochar production an attractive option for waste management in addition to soil amendment. More common feedstock materials include waste materials such as hardwoods (Ippolito, Spokas, Novak, Lentz, & Cantrell, 2015; Ippolito, Stromberger, Lentz, & Dungan, 2014), softwoods (Heaney et al., 2018; Jiang et al., 2016), plant materials (Al-Wabel et al., 2015; Park, Cho et al., 2016; Park, Ok et al., 2016; Shen et al., 2018; Shen et al., 2017) and sewage sludge (Agrafioti, Kalderis, & Diamadopoulos, 2014; Hossain, Strezov, Chan, Ziolkowski, & Nelson, 2011). In recent literature, there has been a peaked interest in atypical feedstock such as microalgae (Amin & Chetpattananondh, 2019; Gan et al., 2018; Plácido, Bustamante-López, Meissner, Kelly, & Kelly, 2019) and peat (Kamran et al., 2018; Wang, Zhao, Shan, Wang, & Yuan, 2017). Different feedstock materials inherently have different chemical compositions of cellulose, hemicellulose and lignin. It is during the pyrolysis of the different feedstock materials, the rate of cellulose, hemicellulose and lignin degradation is varied (Aller, 2016). Therefore, by design, biochar materials produced from different feedstock at different pyrolysis temperatures are diverse in physicochemical characteristics.

2.6.2 Biochar physicochemical characteristics

Biochars' physicochemical characteristics are at the heart of our understanding of biochar application for soil amendment. A considerable amount of literature has been published on biochar characteristics (Aller, 2016; Brewer, Schmidt-Rohr, Satrio, & Brown, 2009; Cantrell, Hunt, Uchimiya, Novak, & Ro, 2012; Jindo, Mizumoto, Sawada, Sanchez-Monedero, & Sonoki, 2014; Keiluweit, Nico, Johnson, & Kleber, 2010; Mukome, Zhang, Silva, Six, & Parikh, 2013). It has been identified that the properties of biochar namely high surface area, carbon and nutrient content, functionality and porosity can improve soil fertility and sequester carbon (Woolf, Amonette, Street-Perrott, Lehmann, & Joseph, 2010). These properties are why biochar is also considered an excellent environmental sorbent (Ahmad et al., 2014; Aller, 2016). The heterogeneity of biochar materials, however, could lead to different sorbent performance in environmental matrices. Hence the importance of considering biochars physicochemical characteristics before soil application.

2.6.2.1 Physical characteristics

The physical structure of biochar is intrinsically like that of the original feedstock (Downie, Crosky, & Munroe, 2009). Organic feedstock or biomass contains cellulose, hemicellulose and lignin that degrade at different temperatures during pyrolysis. According to Aller (2016), hemicellulose degrades at 250 to 300 ^{o}C while lignin and cellulose degrade between 190 to 900 ^{o}C. In the same vein, Keiluweit et al. (2010) showed that as pyrolysis temperature increases, the original organic material becomes turbostratic crystallites with the breakdown of cellulose, hemicellulose and lignin content. Porosity is commonly reported as a significant characteristic that defines biochar concerning its ability to interact with soil-borne contaminants. According to Downie et al. (2009) the crystalline layers, referred to by Keiluweit et al. (2010), are linked, and such linkages form pores of various sizes among the biochar surface. During pyrolysis, the loss of oxygen content causes the formation of pores within the biochar, which increases surface area and the number of potential sorption sites (Xiao & Pignatello, 2015). At temperatures < 400 ^{o}C partial carbonisation limits surface area (Gai et al., 2014), whereas at > 450 °C, the formation of micropores (< 2 nm) and an increase in carbonisation, markedly increases surface area (Gai et al., 2014; Kookana, Sarmah, Van Zwieten, Krull, & Singh, 2011). However, by 700 ^{o}C micropores are reportedly destroyed (Kookana et al., 2011).

Although pore formation is related to the high surface area values commonly reported for biochars, biochar feedstock also significantly influences surface area. Ippolito et al. (2015) compiled the surface areas of several different biochar characterisation studies and demonstrated the different mean surface area depending on the original feedstock. Values reported ranged from 33.8 $m^2 g^{-1}$ for dairy manure-derived biochar compared to 194.2 $m^2 g^{-1}$ for softwood-derived biochar. The evidence discussed in this section suggests that surface area and porosity are functions of both pyrolysis temperature and feedstock choice.

Conventional methods employed to measure the structure, surface area and porosity of biochar include gas or mercury porosimetry (Brewer et al., 2014), microscopy and diffraction techniques. Brunauer-Emmett-Teller (BET) surface area analysis using nitrogen gas to measure pore size and the total surface area is standard throughout biochar related research. This is often used in conjunction with scanning electron microscopy (SEM) imagery and x-ray diffraction (XRD); however, use of imagery such as SEM may fail to measure the more discrete pore structures within the internal structure and lessen total pore size volume measurements.

2.6.2.2 Chemical characteristics

Measuring the pH value of biochar materials is a fundamental characterisation step before the application to soils because of the implications for soil pH and the effect on biogeochemistry. The pH of biochar materials is usually reported as above 7. However, some studies recognise that biochar pH can range from 4 to 10, and soil-aged biochar has been reported as acidic (Chan et al., 2007, cited in Ahmad et al., 2014). However, biochar pH was more affected by feedstock choice over pyrolysis temperature in a study by Enders, Hanley, Whitman, Joseph, and Lehmann (2012). They found that biochar materials could be easily grouped by feedstock type. Only wood-derived biochars had pH values < 7.5 whereas animal manure-derived chars and lignocellulosic-derived biochars had pH values > 7.5. In all instances, pH was strongly correlated with ash content and the total sum of biochar-borne potassium, calcium, magnesium and sodium.

Pyrolysis temperature does, however, have some implications on the final pH of the biochar material due to ash content and silicate and salt formation with increasing carbonisation. Case in point, Rehrah et al. (2014) found a significant positive correlation between biochar pH and pyrolysis temperature. Since the biochar pH is such a central characteristic, nearly all biochar-related papers examine the pH. This is typically done with a pH meter and probe. However, many papers use methods involving different biochar: solution ratios, contact times and background electrolytes. This practice can further complicate the generalisation of biochar pH values. In a recent publication, Singh, Dolk, Shen, and Camps-Arbestain (2017) used three different biochar: solution ratios to measure the pH in either deionised water or 0.01 M calcium chloride after either 1.5 h or 24 h contact time. The study recommended that a background electrolyte may not be necessary due to the soluble salts released from the biochar material and that a biochar: solution ratio of 1:10 or 1:20 was suitable for pH readings. An equilibrium time of 1.5 h was also found sufficient for pH readings. In brief, a correct procedure for biochar pH determination should be adopted, and if possible, a range of techniques should be applied and cross-examined given the implications of biochar pH when added to soils.

The alkalinity of biochar materials tends to lead to a negatively charged surface area and is a further characteristic that makes biochar an excellent adsorbent of cationic metals (Ahmad et al., 2014). An important characterisation technique is the determination of the point of zero charge. This refers to the point at which the surface of the biochar is neither positively nor negatively charged. When the pH is less than the point of zero charge, the surface of the biochar is positively charged, and when higher than the point of zero charge, the biochar is negatively charged. This has direct implications in the sorption affinities of biochar materials. For example, the well documented electrostatic attraction sorption mechanism between biochar and cationic metals assumes that the charge on the surface of the biochar is negative. However, in environmental mediums where the pH is less than the point of zero charge, any adsorption of cationic metals would have to be through other available sorption mechanisms given the positively charged surface. One example is in rhizospheric soils, where protonation of the biochar surface may occur as identified by (Alozie et al., 2018).

Seminal work by Fiol and Villaescusa (2009) explored three different methods of point of zero charge determination of absorbent materials: mass titration, potentiometric titration and the immersion technique. The study identified a small standard deviation of ± 0.1 to 0.2 between techniques, suggesting that either of the techniques could be used to determine the point of zero charge. Despite the variety of methods available few papers appear to characterise biochar for the point of zero charge. Perhaps this is due to an assumption the biochar will be positively charged at circumneutral pH. For instance, a paper by Essandoh, Kunwar, Pittman Jr, Mohan, and Mlsna (2015) using pinewood biochar produced at 698 ^{o}K had a reported point of zero charge of around 2. In contrast, a paper using a similar technique and pinewood biochar recorded a point of zero charge of 9.2 (Karunanayake et al., 2017). In summary, this shows the variance that production parameters have on biochar materials' point of zero charge and the importance of this parameter in biochar characterisation studies.

Biochar functionality refers to the oxygen-containing functional groups (e.g., carboxyl, hydroxyl and phenolic groups) that are present on the surface of biochar. Such functional groups increase the number of active sites for contaminant adsorption and can improve the cation exchange capacity of biochar-amended soils (Uchimiya, Chang, & Klasson, 2011). The presence of functional groups is central to the efficient biochar-contaminant interactions (for example, via complexation with metal species), and prior characterisation must be considered before application to soils. The most common method to examine biochar functionality found across biochar characterisation literature is the use of Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectroscopy can provide information on the chemical element arrangement of the biochar surface, and it has been found biochar materials often share mutual functional groups. Parikh, Goyne, Margenot, Mukome, and Calderón (2014) published a paper in which they compiled a useful list of common functional group assignments corresponding to biochar samples from six different literature sources. In an earlier paper, Mukome et al. (2013) identified that the biochar materials could be split explicitly into either wood or non-wood biochar materials, revealing the role of feedstock on biochar functionality. Indeed, several studies have found that there is a relationship between the functionality of biochar and the production parameters, may that be feedstock or pyrolysis parameters (Al-Wabel, Al-Omran, El-Naggar, Nadeem, & Usman, 2013; Jindo et al., 2014; Zhao, Cao, Mašek, & Zimmerman, 2013). Decreased functionality with increased production temperature has been widely documented as dehydration and deoxygenation occurs (Ahmad et al., 2014) and by 500 ^{o}C to 700 ^{o}C only C=C and C=O bonds remain (Chen & Chen, 2009). Biochar functionality tends to decrease with increased aromaticity, which is characterised with less defining peaks (Chen & Chen, 2009; Chen et al., 2016). Besides, the hemicellulose, cellulose and lignin content of the biochar feedstock and the degradation characteristics of the three components can directly affect the functionality (H. Yang et al., 2006). It is important that in trace element and nutrient studies involving biochar, that biochar functionality is determined.

Cation exchange capacity (CEC) refers to biochars ability to bind exchangeable cations in solution. The aforementioned large surface area of biochar materials with surface functionality leads to a high cation exchange capacity. The negatively charged surface of the biochar material under neutral and alkaline conditions allows for exchange with soil nutrients such as Ca^{2+}, K^{+}, and Mg^{2+}, improving biochars' efficacy to enhance soil quality (Kloss et al., 2012). Anion exchange capacity (AEC) is like that of CEC but refers to biochars ability to retain exchangeable anions such as NO_3^- and PO_4^{3-}. In contrast to CEC, AEC conditions are subject to conditions where pH is lesser than the point of zero net charge (Lawrinenko & Laird, 2015; Mukherjee, Zimmerman, & Harris, 2011).

As many of the biochar characteristics are, CEC is also a function of biochar production parameters (Ippolito, Lentz, & Novak, 2009). Mukherjee et al. (2011) found decreased CEC with low temperature produced biochar caused by the formation of more acidic biochar. Lignocellulosic biochar also had a slightly higher CEC compared to wood-based chars. These in part, reflect earlier findings by Singh, Singh and Cowie (2010), who found increased CEC with increased production temperature of different biochar materials and increased pH. A variation in CEC was reported with different biochar feedstock. The authors attributed the findings to water-soluble cations released from the studied materials.

2.6.3 Biochar for remediation

Biochar can immobilise labile metal ions in the soil, mainly as a function of its inherent properties developed either during production or from the original biochar feedstock itself (Aller, 2016). However, the associated sorption mechanisms between inorganic contaminants and biochar remain poorly understood, despite the importance of adsorption in controlling the solubility of contaminants in soils. Primary sorption mechanism can be grouped into physical or chemical sorption depending on the interaction with the biochar (Beesley, Moreno-Jimenez, Fellet, Carrijo, & Sizmur, 2015; Inyang et al., 2016). Regarding physical sorption, pore-filling has often been considered a primary mechanism for biochar-contaminant retention, characterised by a higher adsorption of adsorbates that are readily desorbed due to a lack of bonding (Cao et al., 2009; Inyang et al., 2016; Pignatello, Kwon, & Lu, 2006). Sorption of this type would, therefore, depend heavily on the initial concentration of the adsorbate, biochar application rate and the porosity of the chosen biochar material. Occupancy of biochar pores would decrease efficacy, and it believed soil organic matter can block pores further reducing effectiveness (Oleszczuk, Hale, Lehmann, & Cornelissen, 2012; Pignatello et al., 2006). The formation of precipitates on the biochar surface is a further physisorption mechanism that is generally a function of high pH chars. Precipitation can be on the surface of the biochar directly or via co-precipitation through interactions with biochar-borne minerals such as carbonate and phosphate. Studies have demonstrated the removal of soluble metals via the formations of metal precipitates such as lead-phosphate precipitates (Cao et al., 2009; Lu et al., 2012).

Sorption can also take place via interactions with the surface charge (electrostatic interactions), ion exchange and complexation. Many papers report that cations bind to the negatively charged surface of the biochar due to electrostatic attraction. This is dependent on the surface net charge of the biochar. By drawing on this, Chintala et al. (2013) were able to demonstrate increased nitrate adsorption by various biochar materials at decreasing pH. Other studies also found that electrostatic attraction is a primary sorption mechanism (Ding et al., 2016; Shen et al., 2018; Yang et al., 2018). In contrast, Zhang et al. (2015) presented an account of the sorption of arsenic III to sewage sludge-derived biochar. The authors accepted that at pH > point of zero charge, the biochar surface would be negatively charged favouring electrostatic attraction. However, they found comparable adsorption at pH > point of zero charge and pH < point of zero charge. Sorption of arsenic III at higher pH was attributed to ligand exchange between $H_2AsO_3^-$ and hydroxyl functional groups on the biochar surface. This demonstrates how ion chemistry can affect sorption behaviour, and therefore, solution pH can govern sorption behaviour. A second chemisorption mechanism is ion exchange. Ion exchange involves ions from groups 1 to 3 on the periodic table. These are ions that are selectively replaced or exchanged on the biochar surface functional groups with ions from the same groups based on their element characteristics (Inyang et al., 2016). For example, metals ion could be exchanged with biochar-borne Ca^{2+} or Mg^{2+}. Works by Uchimiya, Cantrell, Hunt, Novak, and Chang (2012) and Uchimiya, Klasson, Wartelle, and Lima (2011) have shown cation exchange to facilitate the adsorption of target metals in soils. Ion exchange mechanisms would depend on the cation or anion exchangeability of the biochar, the coexisting ions and the binding strength of target ions, i.e. weaker binding ions that can be readily displaced.

Finally, complexation can occur when complexes are formed with functional groups on the surface of the biochar, allowing specific adsorption to take place. Tong, Li, Yuan, & Xu (2011) found copper formed surface complexes with –COOH and phenolic hydroxyl groups found on various biochar (400 ^{o}C), identified with a shift in FTIR spectra. More recently, cadmium removal was reportedly caused by complexation with oxygen-containing functional groups such as –OH, –COOH, and –CH (H. Huang et al., 2018; Penido, Melo, Guilherme, & Bianchi, 2019). The functional group interactions would be limited by increasing pyrolysis temperature following decreased functionality (Jiang et al. 2012; Park et al., 2011; H. Huang et al., 2018). A summary of the mechanisms discussed is shown in Figure 2.3.

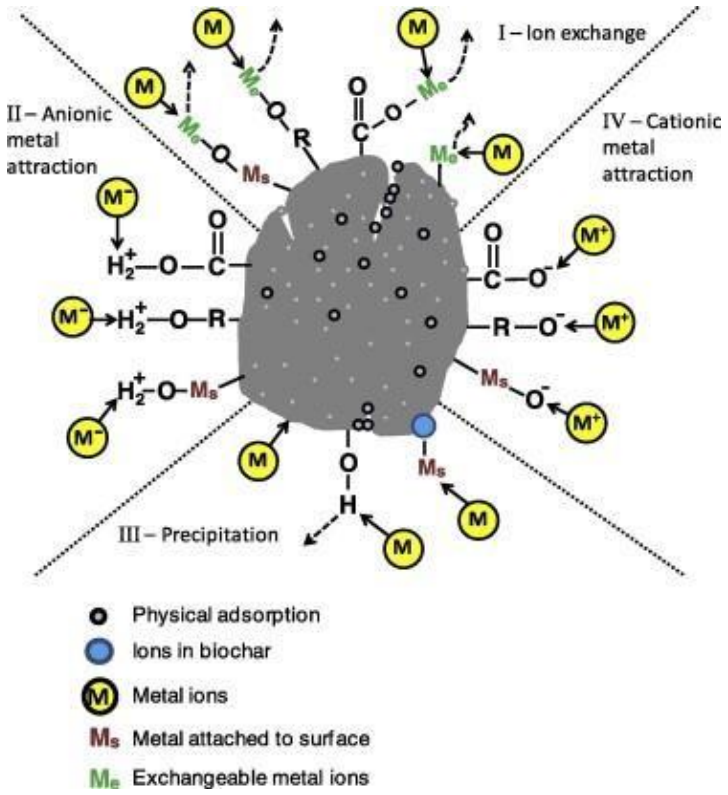

Figure 2.3 A schematic of the potential biochar-inorganic contaminant sorption mechanisms. Physical adsorption is indicated by grey circles on the biochar surface. I refers to ion exchange between target metals and exchangeable biochar-borne metals. II refers to the electrostatic attraction between anionic metals and the protonated surfaces of the biochar materials. III refers to the precipitation of soil-borne metals, and IV refers to electrostatic attraction of cationic metals to a negatively charged biochar surface. (Source: Ahmad et al., 2014).

2.6.4 Biochar affecting plant uptake

The large-scale variation in biochar physicochemistry and efficacy for contaminant retention has led to numerous works investigating the effect on plant uptake of trace elements and nutrients. Mostly, biochar application appears to limit uptake and improve soil nutrient status (Fellet, Marmiroli, & Marchiol, 2014; Kim et al., 2015; Park et al., 2011; Puga, Abreu, Melo, Paz-Ferreiro, & Beesley, 2015). This is due to the retention of soluble cations on the surface of the biochar limiting uptake and the release of water-soluble biochar-borne nutrients such as nitrogen and phosphorus.

Some studies have found results less determinate regarding biochar function in element uptake. Using a different biochar material (rice straw, 550 ^{o}C), Hu et al. (2014) found no effect on cadmium concentrations in Alfred stonecrop (*Sedum alfredii* Hance). Similarly, wood-based biochar has a variable species-specific effect on trace element uptake (Rees, Germain, Sterckeman, & Morel, 2015; Rees, Sterckeman, & Morel, 2016). In the first study, uptake was assessed in a non-hyper-accumulating species, ryegrass (*Lolium perenne* L.) and a hyper-accumulating species, alpine pennycress (*Noccaea caerulescens*). Concentrations in ryegrass plant tissues were limited following biochar application. Contrastingly, following a 5 per cent application of biochar the amount of cadmium and zinc in alpine pennycress tissues increased. It was postulated that increased biochar-driven immobilisation of nutrients within the rhizosphere lead to root-induced accumulation. Biochar further leads to increased root surfaces believed to increase metal uptake in roots for the case of maize (*Zea mays*) and shoots, in the case of alpine pennycress (Rees et al., 2016). Importantly, Rees et al. (2016) speculated that the changes to soil cation bioavailability following biochar application might not solely be because of increased retention. The authors hypothesised that application of biochar might affect the dissolution of metal-complexes increasing plant uptake.

These studies demonstrate the differences in results from a handful of biochar-related research concerning the uptake of trace elements and nutrients from soils. The variations are largely down to biochar-related chemical properties, species-specific propensities to uptake elements and soil characteristics that affect trace element mobility. Additionally, there is a lack of work focusing on vegetative species compared to phytoremediating species. Further work is required to enable generalisation with regards to biochar uptake efficacy in vegetable plants and biochar-contaminant dynamics in rhizospheric soils.

2.6.5 Studies using UKBRC standard biochar in the related field

Even with the amount of biochar-related publications available, few studies have utilised the UKBRC standard biochar materials to explore biochar-metal interactions in solutions and soils. Table 2.4 presents a summary of known papers that focus on UKBRC biochar and nutrient or heavy metal interactions in environmental mediums. The most prolific user of the UKBRC biochar is arguably Dr Zhengtao Shen, who has published numerous studies considering biochar-metal dynamics in solutions and soils (Table 2.4). Findings generally revealed that biochar such as wheat straw pellet biochar and miscanthus straw pellet biochar (produced at 550 ^{o}C and 700 ^{o}C), can retain heavy metals such as nickel and lead and sorption was more often thought to be via surface precipitation.

In terms of biochar-nutrient studies of a similar design, two studies have explored phosphorus sorption onto UKBRC biochar into solution and soil (Bornø, Müller-Stöver, & Liu, 2018; Melia, Busquets, Hooda, Cundy, & Sohi, 2019). Melia et al. (2019) studied all twelve of the UKBRC biochar materials. The author found the majority of the UKBRC set released water-soluble phosphorus apart from softwood-derived biochar. These biochar materials also could not retain soluble or exchangeable phosphorus. Rice husk biochars were also unable to retain phosphorus but released the most phosphorus into solution. Similar reduced efficacy for phosphorus retention was found for wheat straw pellet (produced at 700 ^{o}C) and was credited to soil behaviours (Bornø, Eduah, Müller-Stöver & Liu, 2018).

At the time of writing, no studies exploring nitrate sorption onto UKBRC biochar in solution were found. In a soil-based study, Heaney et al. (2018) explored the effect of softwood pellet biochar (produced at 550 ^{o}C and 700 ^{o}C) on trace element and nitrogen species. Specifically, regarding nitrogen retention, sandy soil amended with 0.5 g of softwood pellet biochar produced at either temperature and 1 g softwood pellet biochar produced at either temperature, retained 42, 46, 50 and 63 per cent of the added nitrogen species respectively. The authors found the softwood pellet biochar temporarily retained ammonia gas. In turn, a reduced rate of volatilisation was hypothesised, which increased the availability of ammonia for conversion into nitrate via nitrification. The findings are important in terms of UKBRC biochar-nutrient dynamics in soils. They imply that the addition of softwood pellet biochar may contribute to nitrate losses from fertilised alkaline sandy soils.

Adding to the lack of nutrient-UKBRC biochar dynamic literature available, fewer studies have examined the effect of LMWOAs on biochar-contaminant behaviour. Even less have utilised UKBRC standard biochar. Two key works from the University of Salford have used UKBRC materials and have included LMWOAs. The first strand examined the LMWOA-driven release of biochar-borne elements from SS550 and SS700 biochars (Vause et al., 2018). The authors found a differential release of biochar-borne elements such as zinc, nickel, lead and copper. No other work assessing the release of biochar-borne trace elements by LMWOAs could be found for UKBRC biochar. The second strand included an examination of the efficacy of biochar to retain trace elements in the presence of LMWOAs (Alozie et al., 2018). We found that softwood pellet biochar produced at 700 ^{o}C was unable to retain cationic species in the presence of LMWOAs but could retain anionic species attributed to the protonation of the biochar surface.

Concerning UKBRC biochar-plant dynamics, at the time of writing, only one author has been identified who has used UKBRC standard biochar using maize as a model plant in two published studies (Bornø, Eduah, et al., 2018; Bornø, et al., 2019).

The three recent studies examined firstly phosphorus uptake dynamics by maize plants, then later potassium, calcium, sulphur, magnesium, sodium, manganese and boron uptake in maize following phosphate-based fertiliser. Key findings included that rice husk and oil-seed rape biochar (produced at 550 ^{o}C) and wheat straw pellet biochar (produced at 700 ^{o}C) increased the total uptake of phosphorus by maize tissues. Furthermore, softwood pellet biochar (produced at 550 ^{o}C) was found to have limited effect on phosphorus availability in soils and did not affect uptake. This is in accord with the limited release of phosphorus from softwood pellet biochars found recently (Melia et al., 2019).

It is clear from this review that the UKBRC biochar materials have demonstrated efficacy to retain trace elements and nutrients to variable levels based primarily of their physicochemical characteristics. This corroborates with the well-documented works showing the potential of biochars for remediation in solutions and soils (Ahmad et al., 2016; Cao et al., 2009; Inyang et al., 2016; Lu et al., 2012).

Table 2.4 Summary of the studies using UKBRC standard biochar to explore biochar-contaminant interactions, the methods adopted and key findings. PTTEs refers to potentially toxic trace elements.

Author/s	**Biochar used**[1]	**Target PTTEs**[2]	**Medium**	**Method**	**Key findings** [1,2]
Alozie et al. (2018)	SWP700	As, Ba, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr & Zn.	Solution (Water, Citric acid, Malic acid, Oxalic acid, mixed organic acid solutions)	Batch incubations experiments	(1) LMWOAs protonate biochar surface favouring adsorption of anionic rather than cationic compounds
Bornø et al. (2019)	WSP700	K, Ca, S, Mg, Na, Mn & B.	Soil	Maize growth experiment	(1) Excess amounts of biochar-borne K released into soils leading to uptake the of Ca and Mg in maize foliage. (2) Differential effect on Mn uptake following biochar application. (3) Increased uptake of B regardless of increased pH following biochar application
Bornø, Eduah, et al. 2018)	SWP550 RH550 OSR550	P	Soil	Maize growth experiment	(1) Root exudations by maize altered with feedstock and P fertiliser application (2) RH and OSR increased total P uptake by maize plants (3) Feedstock influenced available P. SWP had no effect but RH and OSR increased available fraction hence the observed effect on uptake.
Bornø, Müller-Stöver et al. (2018)	WSP700	P	Soil	Batch sorption study Maize growth experiment	(1) Soil type strongly influenced the sorption of P by biochar materials (2) WSP700 had a small effect on P retention in low P containing soils due to the limited liming potential (3) WSP700 increased the total P content depending on soil type

Heaney et al. (2018)	SWP550 SWP700	As, Fe, Mn, Pb & Zn	Soil	Batch incubations experiments	(1) Dose-dependent ability to retain N-species in softwood biochar-amended sandy soil (2) SWP700 can successfully immobilise Zn under anaerobic conditions which improved with increased biochar amount
Melia et al. (2019)	MSP550 MSP700 OSR550 OSR700 RH550 RH700 SS550 SS700 SWP550 SWP700	P	Solution	Batch adsorption experiments	(1) All biochar except for SWP550 and SWP700 released water-soluble P (2) SWP-derived biochar did not retain any P (3) Rice husk-derived biochars were unable to retain P but released the most into solution. (4) Low temperature biochar with high Ca and Mg content was strongly correlated to P retention and was recommended for further use
Shen, Hou, et al. (2018)	WSP700	Cd, Cu & Pb	Soil washing residue	Batch adsorption experiments	(1) Positive but limited reduction in Cd, Pb and Cu from soil washing residue following 5 per cent application (2) Aging processes reduced biochar efficacy and increased leachable Cd, Cu and Pb from SWR.
Shen, Tian, et al. (2018)	WSP700	Pb	Solution (water)	Batch adsorption experiments	(1) WSP700 removed 95.17% of 1000 ppm from solution attributed to electrostatic attraction and precipitation mechanisms. (2) Coexistence with bioapatite improved removal efficacy
Shen, Zhang, Jin, Alessi et al. (2018)	MSP550 MSP700 SWP550 SWP700	Cu, Ni & Pb	Solution (water)	Batch adsorption experiments	(1) MSP improved efficacy for Ni^{2+} removal due to increased carbonisation and high amount of biochar- borne minerals compared to SWP. (2) Primary sorption mechanisms were surface precipitation and cation- π interactions because of biochar-induced alkalinity.

Shen, Zhang, Jin, McMillan, & Tabbaa (2017)	RH700 WSP700	Pb	Solution (water)	Sequential extractions following batch adsorption	(1) Low water-soluble fraction of adsorbed Pb indicating chemisorption rather than physisorption mechanisms. (2) Acidic soluble fraction of retained Pb is the largest fraction on studied biochars (75.61 %, WSP700 and 85.76 % for RH700) suggesting surface precipitation or cation-π interaction are primary sorption mechanisms.
Shen, Zhang, McMillan, et al. (2017)	RH550 RH700 WSP550 WSP700	Ni	Solution (water)	Batch adsorption experiments	(1) Immobilization to the order of WSP700 > WSP550 > RH700 > RH550 (2) Lower ability to retain Ni by rice husk-derived chars attributed to high ash content pore blocking. (3) Higher aromaticity, pH, CEC and K contents of wheat straw derived chars credited for improved retention compared to rice husk
Vause et al. (2018)	SS550, SS700	n/a	Water, Citric acid, Malic acid, Oxalic acid	Extraction	(1) Differential release of biochar-borne elements by citric, malic and oxalic acid. More released observed by oxalic acid. (2) Essential elements and those of potential toxicity released under LMWOAs presence.

[1] The twelve UKBRC biochar materials are referred to firstly by their feedstock acronym where MSP: Miscanthus straw pellet, OSR: Oil seed rape pellet, RH: Rice husk, SS: Sewage sludge pellet, SWP: Softwood pellet and WSP: Wheat straw pellet. Then the respective production temperature (i.e. 550 ºC and 700 ºC) is added as a suffix.

[2] As: Arsenic, B: Boron, Ba: Barium, Ca: Calcium; Cd: Cadmium, Co: Cobalt, Cr: Chromium, Cu: Copper, Fe: Iron, K: Potassium, Mg: Magnesium, Mn: Manganese, Na: Sodium, Ni: Nickel, P: Phosphorus, Pb: Lead, S: Sulphur, Sr: Strontium and Zn: Zinc.

2.7 Gaps in current knowledge, research progression and hypotheses

This research aims to examine the effect of biochar on trace element and nutrient solubility in the presence of LMWOAs. From the literature review, several knowledge gaps have been identified in which research hypotheses are derived. These form the framework for this research. In this section, each of the current shortcomings will be presented, and the associated research background and hypotheses that form the foundations of this thesis will be proposed. The hypotheses are indicated in bold.

2.7.1 Characterisation of the biochar materials

Biochar has demonstrated potential for a range of environmental applications such as carbon sequestration, soil fertility improvement and the remediation of contaminated wastewaters and soils (Ahmad et al., 2014; Beesley & Marmiroli, 2011; Jeffery, Verheijen, van der Velde, & Bastos, 2011; Tang, Zhu, Kookana, & Katayama, 2013). However, a key aspect of biochar-related research is the reported variations in physicochemical properties depending on the feedstock and production processes (Aller, 2016). Consequently, different biochar types could have varying efficacy for environmental remediation. For example, Tan et al. (2015) used four different biochar, created at the same temperature, to examine the effect of cadmium immobilisation in acidic soils. The results showed varied immobilisation efficacies based on feedstock type. Others have reported similar findings (Jiang & Xu, 2013; Park et al., 2013). Changes to biochar immobilisation efficacy based on pyrolysis temperature have also been identified (Ding et al., 2014; Shen et al., 2019). Given that the variations in biochar efficacy are related to the different fundamental production characteristics, full characterisation of biochar materials should be carried out before environmental application.

Nevertheless, considerable work has demonstrated that biochar can effectively retain cationic materials (see section 2.6.3 and 2.6.5). In contrast, there are varying reports of biochar's efficacy for anion removal (e.g. nitrate and phosphate) (Clough, Condron, Kammann, & Müller, 2013). Different observations that biochar can adsorb anions, cannot adsorb or even release anionic nutrients into the environment have been reported (Gai et al., 2014; Hollister, Bisogni, & Lehmann, 2013; Mishra & Patel, 2009). Despite the plethora of biochar-contaminant research, most of the work so far has used different biochar materials, making generalisability difficult.

To counteract this, the UK Biochar Research Centre (UKBRC) have produced a standard set of twelve biochar, produced from six feedstock materials at two temperatures (550 ^{o}C and 700 ^{o}C). The materials are readily available for research purposes from the UKBRC. The materials have been produced using a standardised procedure. Careful monitoring of pyrolysis temperature, retention time, feedstock supply and composition has allowed for easily reproducible and pre-characterised materials to be made (Mašek, Buss, & Sohi, 2018). By using a standard set of biochar reference materials, a generalisation of biochar efficacy for environmental applications can be carried out.

However, the combustion of biomass materials and the feedstock choice can both cause biochar materials to be high in trace elements of potential toxicity, presenting a negative side to biochar application for soil remediation (Hilber, Schmidt, & Bucheli, 2017; X. Yang et al., 2019). In response, several studies have studied the release of biochar-borne trace elements using different extractants. Commonly used extractants have included but are not limited to, distilled water (Gondek, Baran, & Kopeć, 2014; Gondek & Mierzwa-Hersztek, 2017; Kloss et al., 2012), dilute nitric acid, dilute sulphuric acid (de Figueredo, da Costa, Melo, Siebeneichlerd, & Tronto, 2017; Stefaniuk, Oleszczuk, & Bartmiński, 2016), pentetic acid or a pentetic acid, calcium chloride and triethanolamine mix (Liu, Liu, & Zhang, 2014; Meng et al., 2013). Liu et al. (2014) found evidence of copper, lead, zinc, cadmium and chromium in sewage sludge biochar but the bioavailable amount of the total metal content to be relatively low, to the order of zinc > chromium > lead > copper > cadmium. Similarly, Gondek and Mierzwa-Hersztek (2017) found cadmium, copper, lead and zinc present in similar biochar materials to the order of zinc > lead > cadmium > copper.

Of the work regarding the ecotoxicity of biochar materials, more focus is given on sewage sludge biochars that are typically higher in metal content compared to plant-derived biochar (Jeffery et al., 2011; Paz-Ferreiro et al., 2014). However, metal content is not limited to sewage sludge-derived biochar. Kloss et al. (2012) used three different lignocellulosic feedstock and found variable soluble boron, chromium, nickel, copper and molybdenum release from the different biochar materials. There is also emerging research exploring the potential of biochar created from metal rich-phytoremediation residue, which may contain more substantial quantities of metal content (H. Huang et al., 2018). This reiterates the heterogeneity of biochar characteristics and the importance of characterisation before application to soils.

Soil amendment is a widely reported application for biochar materials (Ahmad et al., 2014; El-Naggar et al., 2019; Panwar, Pawar, & Salvi, 2019). Within rhizospheric soils, LMWOAs are secreted from plant roots and exist due to the decomposition of organic materials (Jones & Darrah, 1994). Therefore, any application of biochar materials to soil means biochar materials are likely to encounter LMWOAs. This may lead to the release of biochar-borne elements via acidification, reduction or complexation (Alozie et al., 2018; Onireti & Lin, 2016). Previous studies have utilised extractants to explore the release of biochar-borne trace elements, for instance, Vause et al. (2018) found LMWOA-driven solubilisation of biochar-borne trace elements of potential toxicity which could have implications for the phytoavailability of biochar-borne trace elements. However, limited information exists about the chemical behaviour of biochar-borne elements in the presence of LMWOAs.

Recent studies have utilised the UKBRC standard biochar set, as reviewed in Chapter 2. However, an extensive review of all literature has found: (1) that few studies have characterised the functionality and surface morphology of all twelve biochar materials; (2) none of the existing studies mention the solubility of biochar-borne elements; and (3) few use extractants that are rhizospherically relevant (Vause et al., 2018). For this reason, the physicochemical characteristics of twelve UKBRC biochar will be analysed included a test to establish whether LMWOAs can extract biochar-borne trace elements of potential toxicity. The work will complement the existing characterisation of UKBRC standard biochar materials by providing further characterisation information and a novel insight into the LMWOA-released biochar-borne elements. The study will also provide insights into the biogeochemical mechanisms responsible for plant uptake of biochar-borne elements.

2.7.2 Research hypothesis 1

When the rhizospheric conditions are not in equilibrium, that is to say, the net number of anions and cations is unequal, plants are likely to respond via the efflux or influx of either protons (H^+) or bicarbonate (HCO_3^-) or hydroxyl ions (OH^-) to acidify or lime the rhizospheric pH (Hinsinger, 2001; Kahle, 1993). Plants may also respond to changes in rhizospheric pH by the release of LMWOAs in the anionic form such as citrate, malate and oxalate, which may contribute to root-induced acidification (Hinsinger, 2001; Qin et al., 2018). As such, in soils with excessive trace elements, it is likely that more LMWOAs are also secreted in response to stress conditions to alter trace element solubility (Jones, 1998).

Several earlier works have reported the effect of biochar on trace element solubility (Ahmad et al., 2014; Beesley et al., 2013; Park et al., 2011; Uchimiya, Wartelle, et al., 2011). One of the commonly cited features which makes biochar beneficial for cation immobilisation is the negatively charged surface. This is because of the amount of acidic surface functional groups on the biochar surface, which are likely negatively charged under normal soil pH conditions (Mukherjee et al., 2011). LMWOAs play an essential role in the maintenance of rhizospheric equilibrium. Namely, they have the potential to affect soil pH, which in turn affects trace element and nutrient solubility at the soil-plant interface (Hinsinger, Bengough, Vetterlein, & Young, 2009). For instance, when the number of cations in the rhizosphere is high, root-induced acidification can occur (Blossfeld, Perriguey, Sterckeman, Morel, & Lösch, 2010; Hinsinger, 2001; Houben, 2013). This can reduce the pH units compared to that of bulk soil (Hinsinger, 2000).

Such root-induced acidification may markedly affect biochar-contaminant dynamics at the soil-plant interface. However, few works have included LMWOAs despite the high likelihood of biochar encountering rhizospheric soils (Vause et al., 2018). In our earlier work, LMWOAs interfered with the biochar-contaminant dynamics and reduced the efficacy for cation retention (Alozie et al., 2018). It was found that upon contact with LMWOAs, the biochar surface was likely protonated. Such protonation would create unfavourable conditions for cationic metal adsorption (e.g. Cd^{2+}, Cu^{2+} and Pb^{2+}) while creating favourable conditions for anionic metal adsorption (e.g. AsO_4^{3-} and CrO_4^{2-}) via electrostatic interaction. Furthermore, the alkaline nature of biochar materials (see Chapter 3) may contribute an acid neutralisation effect which may affect the LMWOA-driven solubilisation of trace elements (Alozie et al., 2018; Bashir et al., 2018; Houben, 2013; Houben, Evrard, & Sonnet, 2013a, 2013b).

Information about the interactive effects of biochar and LMWOAs on trace element solubility is lacking, and a full understanding of how biochar application can affect trace element solubility in the presence of LMWOAs in aqueous and soil systems is required. Therefore, the related hypothesis to test is that **LMWOAs will inhibit the biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems.** The results will help the understanding of potentially toxic element solubility in the rhizosphere of biochar-amended soil systems.

2.7.3 Research hypothesis 2

Nitrogen and phosphorus are essential nutrients for plant growth (Hale et al., 2013). However, excessive application of nitrate and phosphate is a global concern (Huang, Xu, Ridoutt, Wang, & Ren, 2017). Whilst nitrate has a weak affinity to soil colloids the movement of phosphorus within soils depends on soil characteristics such as iron and aluminium content and organic matter (Barber, 1995; Dickinson & Murphy, 2008; Esteller, Martínez-Valdés, Garrido, & Uribe, 2009; Guppy et al., 2005). The leaching of inorganic fertilisers can, therefore, lead to increased levels of nutrients in receiving water environments, contributing to eutrophication in the affected aquatic ecosystems (Beeckman et al., 2018; Camargo & Alonso, 2006; Di & Cameron, 2002; Heaney et al., 2018).

Recent studies have found biochar could limit nitrate and phosphate leaching from soils (Chintala et al., 2013; Hagemann, Kammann, Schmidt, Kappler, & Behrens, 2017;

Kanthle, Lenka, Lenka, & Tedia, 2016; Libutti, Mucci, Francavilla, & Monteleone, 2016). However, there is also evidence to suggest biochar materials may have a negligible effect on soil nutrient losses or even leach nitrate and phosphate materials (Gai et al., 2014; Hollister et al., 2013; Yao et al., 2012). The varying results are likely accounted for by the differential properties of the biochar materials used. The use of a standardised biochar material would help generalise the efficacy of biochar to immobilise anionic nutrients.

However, limited studies using standardised biochar materials to elucidate phosphate-biochar interactions were found (Bornø, Müller-Stöver, et al., 2018; Melia et al., 2019) and at the time of writing, none examining nitrate-biochar in solution could be identified (section 2.6.5). Furthermore, no research examining biochar-nutrient interactions in the presence of LMWOAs was found. However, for the same reasons outlined in the previous section, LMWOA-driven protonation of the biochar surface as suggested by Alozie et al. (2018) would theoretically enhance the electrostatic attractions between biochar and anionic contaminants improving nutrient retention. If biochar can be activated by LMWOAs as suggested by Alozie et al., (2018) and by Liu et al. (2017), then findings relating to biochar anion immobilisation inefficacy may be misplaced. That is, in rhizospheric soil biochar may be activated by LMWOAs enhancing the anion retention capacity, which would increase nutrient residency time in the rhizosphere (Kameyama, Miyamoto, Shiono, & Shinogi, 2012). In turn, this would reduce the requirement for excessive application of nitrogen and phosphorus-based fertilisers and reduce the risk of eutrophication. For the reasons above, urgent work is needed to establish whether **LMWOAs facilitate the biochar-driven immobilisation of nitrates and phosphates in solution**. The results will help the evaluation of biochar’s role in rhizospheric nutrient dynamics and plant nutrient acquisition.

2.7.4 Research hypothesis 3

Soils present a more complex system to that of aqueous systems. Despite the recorded efficacy of biochar to reduce trace element and nutrient solubility (Beesley et al., 2010; Laird, Fleming, Wang, Horton, & Karlen, 2010; Park et al., 2011; Yakout, Salem, Mostafa, & Abdeltawab, 2019), their application to vegetated soils may be limited because of a failure to consider LMWOAs. In soils, biochar is likely to encounter LMWOAs which may affect biochars-contaminant dynamics. As shown in section 2.4.4 of this review, LMWOAs can affect trace element and nutrient solubility via acidification, complexation and reduction reactions (Jones, 1998; Jones et al., 2003; Nworie et al., 2017; Onireti et al., 2017; Schwab et al., 2008). Furthermore, LMWOAs may also alter the biochar surface characteristics (Alozie et al., 2018; Liu et al., 2017). To date, there is a lack of research that has reported on the behaviour of soil-borne trace elements and nutrients in biochar-amended soils in the presence of LMWOAs. Acid neutralisation via the biochar materials may counteract LMWOA-solubilisation, reducing LMWOA-driven solubilisation of trace elements and nutrients (Alozie et al., 2018). Likewise, if the biochar alkalinity is unable to neutralise the acidity of the LMWOAs, LMWOA-driven solubilisation of trace elements is likely. Hence why plant uptake of heavy metals reportedly decreases with increasing biochar application rates (Al-Wabel et al., 2015; Kim et al., 2015; Rees et al., 2015). This research will test if **biochar will reduce the LMWOA-driven solubilisation of trace elements and nutrients in soils**.

2.7.5 Research hypotheses 4a to 4d

The lack of standardisation in biochar production has led to different outcomes in terms of efficacy for environmental remediation (see sections 2.6.3 to 2.6.5). One common research theme is to evaluate the effect of varying biochar materials on plant growth performance. Parameters such as germination shoot heights and root lengths are frequently used as indicators for toxicity (Gascó, Cely, Paz-Ferreiro, Plaza, & Méndez, 2016). Species-specific plant growth requirements, paired with the variations in different biochar physiochemistry, have prevented a consensus on biochar efficacy to improve growth. Besides, many of the biochar used are ‘fresh’ rather than weathered or aged by soil biogeochemical processes. This may have caused an overestimation of the performance of biochars in agriculture.

Biochar activation or modification is a commonly adopted procedure to improve the physicochemical characteristics of either the feedstock or biochar material for targeted remediation applications (Sizmur, Fresno, Akgül, Frost, & Moreno-Jiménez, 2017). Several studies have utilised different activation techniques including but not limited to, metal oxides treatment (Micháleková- Richveisová et al., 2017), electrochemical treatment (F. Yang et al., 2019), steam activation (Lou, Rajapaksha, Ok, & Chang, 2016; Shim et al., 2015) and acid or base treatment (Feng & Zhu, 2018; Liu et al., 2017). Despite the reported improvements in biochar physicochemical characteristics, activation of biochar materials using chemicals may pose a secondary contamination risk to already sensitive environments (Qiu et al., 2019).

Few studies have attempted to modify biochar materials with more environmentally relevant substances such as LMWOAs, which biochar is likely to encounter in soils. In one study, eucalyptus sawdust biochar activated with LMWOAs was found to improve the surface functionality, aiding methylene blue adsorption (Sun et al., 2015). More recently, the enhanced cadmium and lead retention in soils amended with citric acid-activated chickpea biochar have been reported, attributed to the improved biochar functionality following activation (Nazari, Rahimi, & Khademi Jolgeh Nezhad, 2019). Other studies have also reported similar changes to biochar physicochemical characteristics (Liu et al., 2017; Zheng, Zhang, et al., 2019). These findings contrast those of Ren, Sun, Wang and Cao (2016) who found pig manure biochar that had been in contact with wheat root exudates had a lower porosity, surface area and ash content, suggesting that LMWOAs may reduce the efficacy of biochars to retain heavy metals via physisorption or intraparticle diffusion (Ren et al., 2016; Zhou et al., 2015). These studies exemplify how the variability of biochar properties may affect LMWOA-biochar interactions within the rhizosphere.

Compared to biochar-LMWOA dynamics, the effect of biochar on plant dynamics is frequently cited. For instance, biochar has been found to improve shoot and root lengths (Brennan, Jiménez, Alburquerque, Knapp, & Switzer, 2014; Prapagdee, Piyatiratitivorakul, Petsom, & Tawinteung, 2014) and provide a greater biomass (Nie et al., 2018; Rue, Rees, Simonnot, & Morel, 2019). The main mechanisms responsible for enhanced growth are improved soil texture, increased soil water holding capacity, improved microbial communities, increased nutrient availability and rhizospheric residency (Jeffery et al., 2011; Kameyama et al., 2012; Lehmann & Joseph, 2009). However, few studies have reported on the effect of LMWOA-activated biochar on plant growth. LMWOA-driven dissolution of trace elements and nutrients has been found in this research, and in previous studies (Liu et al., 2017; Vause et al., 2018). On the one hand, LMWOAs can liberate biochar-borne elements which are essential to plant growth such as nitrogen, phosphorus, potassium, iron, copper and zinc (Rawat, Saxena, & Sanwal, 2019; Vause et al., 2018). On the other hand, LMWOA-driven solubilisation of biochar-borne elements may increase the amount and availability of potentially toxic elements such as Al^{3+} (Vause et al., 2018). Research on this topic needs to be undertaken to allow for generalisations between LMWOA-activated biochar and plant performance to be made. **It was hypothesised that:**

1. **Biochar and LMWOA-activated biochar will:**
    - **a) Increase the germination percentage of different edible plant species,**
    - **b) Increase the fresh biomass of different edible plant species,**
    - **c) Increase the shoot height of different edible plant species, and**
    - **d) Increase the root length of different edible plant species.**

The findings will provide an understanding of the role of UKBRC standard biochar in soil-plant-biochar interactions and address the issues related to the possible rhizospheric modification of biochar by LMWOAs.

2.7.6 Research hypotheses 5a and 5b

Many studies exist examining how biochar soil amendment affects trace element uptake in various plant species by immobilising soluble trace elements within the soil (Fellet et al., 2014; Kim et al., 2015; Park et al., 2011; Puga et al., 2015). As shown in section 2.6.5, there has been little quantitative analysis of the effect of UKBRC standard biochar on trace element uptake by plants. Additionally, few works focus on edible species with more attention paid to phytoremediating species. Further studies, which take standardised biochar and edible plants into account, will need to be undertaken. It is hypothesised that **biochar will reduce trace element uptake by a selected plant species.**

Previous work has suggested LMWOAs can affect biochars' physicochemical characteristics (Alozie et al., 2018; Liu et al., 2017; Ren et al. 2016; Sun et al., 2016; Sun, Chen, Wan, & Yu, 2015; Zheng, Zhang, et al., 2019). Reported changes that would affect cationic trace element retention are changes to porosity, change to surface net charge and the dissolution of biochar-borne minerals (Alozie et al., 2018; Liu et al., 2017; Ren et al., 2016; Zheng, Zhang, et al., 2019). For instance, increased porosity would enhance the likelihood of intraparticle diffusion, which is frequently cited to be involved in trace element immobilisation (Ding, Dong, Ime, Gao, & Ma, 2014; Kołodyńska et al., 2012; Shen et al., 2019; Shim, Yoo, Ryu, Park, & Jung, 2015). Opposing this, Ren et al. (2016) reported root-aged biochar had a pore-blocking effect, reducing the adsorption efficacy of high-temperature produced biochars. LMWOAs may also impede the efficacy of biochars for trace element retention via protonation as found in our previous work (Alozie et al., 2018) or by LMWOA-driven dissolution of biochar-borne minerals which may block pores or compete with trace elements for surface sites (Liu et al., 2017; Sun et al., 2016; Vause et al., 2018).

Despite the research exploring the effect of biochar on trace element uptake by plants, no research has been found that investigated the effect of LMWOA-activated biochar materials on the uptake of trace elements in edible plant species. As biochar-trace element dynamics are unclear, a null hypothesis can be tested for LMWOA-activated biochar, that is **LMWOA-activated biochar will not affect trace element uptake by a selected plant species**. By using edible species and standard biochar materials, to examine the uptake of trace elements, the efficacy of biochar application to vegetated soils and the implications of UKBRC biochar use in an urban agricultural setting can be assessed. A further understanding of biochar-trace element dynamics in soils can be sought.

2.8 Conclusion

The aim, addressed in this chapter, was to establish a framework for the current research. LMWOAs play an essential role in governing trace element and nutrient solubility at the soil-plant interface (section 2.4.4). Furthermore, LMWOAs may modify the biochar surface, which may interfere with biochar-contaminant dynamics (section 2.6.5). Despite this, the majority of biochar-related work has studied trace element and nutrient solubility in the absence of LMWOAs (section 2.6.3 to section 2.6.5). The situation is further complicated by the different physicochemical characteristics of biochar materials produced at different production temperatures (section 2.6.2) which may behave differently under varying environmental conditions. Three critical research gaps have emerged from this review: (a) a lack of work on biochar-trace element dynamics in the presence of LMWOAs, (b) a lack of work on biochar-nutrient dynamics in the presence of LMWOAs and (c) trace element uptake by vegetable plants in biochar and LMWOA-activated biochar-amended soils. The work on biochar-contaminant dynamics should be examined using standardised biochar materials (section 2.6.5). In summary, there is an urgent need for research that examines the effect of biochar on trace element and nutrient solubility in the presence of LMWOAs which will be addressed by each of the research hypothesis outlined in section 2.7 and Chapter 1 of this thesis.

CHAPTER 3

Materials and methods

3.1 Overview

The former chapter identified the gaps in current knowledge and the various separate hypotheses (section 2.7) created to help meet the research aim, that is, to examine the effect of biochar on trace element and nutrient solubility in the presence of LMWOAs. Here, the materials and methods used to achieve the research aim are outlined. The chapter begins by explaining the philosophical orientation of the thesis (section 3.2), followed by the describing the biochar, soil and plants used in each experiment (section 3.3, section 3.4 and section 3.5, respectively). Each method adopted for the individual hypotheses is then reported (section 3.6 to section 3.8) before the statistical analysis process is outlined (section 3.9). Finally, a summary is presented (section 3.10)

3.2 Research philosophy

While it is often difficult to conceive the philosophical paradigms within the natural science domain (Heller, 2011; Bryman, 2016), all research is unpinned by a philosophical framework which is the building block of research (Grix, 2010; Heller, 2011). Ontology is the first consideration of the researcher and relates to the study of being, or what exists (Grix, 2010; Hathcoat, Meixner, & Nicholas, 2019; Marsh & Furlong, 2002). The ontological positions of the researcher can be distinguished as either ‘constructivism’ or ‘objectivism’. When phenomena and their significance are recurrently being realised by social actors, this pertains to ontological constructivism (Bryman, 2016). In brief, it is the belief that knowledge is a construct of the researcher owing to their own experiences and subjectivity of a concept (Jonassen, 1991). However, subjectivity has no role in the traditional sense of quantitative natural science research, but it does in the social sciences (Table 3.1). In contrast to constructivism, ontological objectivism adopts the paradigm that, 'social phenomena and their meanings have an existence' (Bryman, 2016). In other words, what exists, is external to the researcher's reality. Bryman continues that an objectivism paradigm is organised by a set of standardised procedures to study a phenomenon. Laboratory-based testing of soil samples is one example of a set of standardised procedures used to study a phenomenon.

Epistemology is also another fundamental building block of research that shapes methodologies (Grix, 2010). The term, epistemology, pertains to what is knowledge and the methods used to increase our knowledge (Grix, 2010; Hathcoat et al., 2019). Broadly, this can be categorised as either a 'positivism' or 'interpretivism' paradigm. Epistemological positivism is the paradigm that assumes relationships or phenomena can be understood through empirical methods and statistical analysis (Hathcoat et al., 2019). Grix (2010) explains that an important part of the positivism paradigm is the belief that theory can be used to generate hypotheses, which can be tested by observation, allowing for relationships to be proven. They also highlight that within the positivism paradigm, there is no difference between what reality is and what appears as reality, which removes subjectivity. It is, therefore, clear that a positivistic approach lends itself, by design, to more quantitative research methods such as those favoured in the natural sciences. In contrast, an interpretivism epistemology is the opposite of positivism, that is, the position that a subjective reality can be interpreted and as such, no phenomena or relationship can be proved with absolute certainty (Gummesson, 2003; Järvinen, 2016). This would not reflect a natural science approach (Table 3.1). However, in Gummesson's (2003) paper, a bold opening line read, 'Let's stop fooling ourselves: All research is interpretive!' Yet it is an important note that with the introduction of subjectivity, this *de facto* makes the interpretivism paradigm less favourable for the natural sciences, wherein facts are facts. Some of the ontological and epistemological philosophical paradigms that researchers may choose to adopt are summarised in Table 3.1.

Table 3.1 Fundamental differences between quantitative and qualitative research strategies adapted from Bryman (2016)

	Quantitative	**Qualitative**
Principal orientation to the role of theory in relation to research	Deductive; testing of theory	Inductive; generation of theory
Epistemological orientation	Natural science model, in particular positivism	Interpretivism
Ontological orientation	Objectivism	Constructivism/Constructionism

As the work herein is laboratory-based, this thesis followed the ideas that the existence of a phenomenon is separate to the researcher and can be studied empirically. So, the research philosophy adopted herein was that of ontological objectivism and an epistemological positivism approach.

3.3 Biochar used in the experiments

3.3.1 Biochar description

Twelve different biochar materials were purchased from the United Kingdom Biochar Research Centre (UKBRC), University of Edinburgh (Table 3.2 and Figure 3.1). Within these twelve biochar materials, there were six different feedstocks, which were produced at two different temperatures (550 and 700 ^{o}C) using slow pyrolysis in a continuous rotary kiln. The biochar feedstocks were:

- **Miscanthus straw pellet biochar (MSP)** which is produced from pelletised miscanthus grass;

- **Oilseed rape straw pellet biochar (OSR)** which is produced from pelletised oilseed rape or rapeseed (*Brassica napus*) straw;
- **Rice husk biochar (RH)** produced from rice husks or hulls which are the hard-outermost covering which protects rice grains;
- **Sewage sludge biochar (SS)** that is produced from pelletised sewage sludge;
- **Softwood pellet biochar (SWP)** which is produced from mixed softwood pellets and,
- **Wheat straw pellet biochar (WSP)** is produced from the pelletised wheat straw, an agricultural waste product of wheat harvesting.

Hereafter, the biochar materials are referred to using their acronym, for example, MSP, and their respective production temperature (i.e. MSP550 or MSP700). When presenting or discussing results refers to grouped feedstock (e.g. MSP550 and MSP700) the terminology miscanthus straw-derived or MSP-derived is applied, where applicable.

Before use in the experiments, the biochar was prepared by grinding using a mortar and pestle for 1 minute each. Samples were then passed through a 2 mm sieve. Sieved biochar was stored in sealed 250 mL glass Kilner jars as shown in Figure 3.2 until use to avoid exposure to liquid or gaseous contaminants as suggested by Hilber et al. (2017).

Table 3.2 The various biochar purchased from the UKBRC, with the corresponding pyrolysis temperature used for production and the acronyms used throughout this research

Feedstock	Acronym[1]	Temperatures purchased (°C)
Miscanthus straw pellet	MSP	550 and 700
Oil seed rape	OSR	550 and 700
Rice husk	RH	550 and 700
Sewage sludge	SS	550 and 700
Softwood pellet	SWP	550 and 700
Wheat straw pellet	WSP	550 and 700

[1] Acronyms assigned by the UK Biochar Research Centre

Figure 3.1 Feedstock and biochar samples as received from the manufacturer. A: Miscanthus straw pellet and biochar, B: Oil seed rape straw pellet and biochar, C: Rice husk and biochar, D: Sewage sludge and biochar, E: Softwood pellet and biochar and, F: Wheat straw pellet and biochar (Source: UK Biochar Research Centre, 2019).

Figure 3.2 The prepared biochar samples shown as stored in sealed and labelled 250 mL glass Kilner jars.

3.3.2 Biochar characterisation

3.3.2.1 Biochar pH, conductivity, redox potential and point of zero charge

Establishing the pH of biochar materials is a necessary part of the characterisation process. Different solid to solution ratios are often used. Briefly, 1 g of biochar was weighed out into 50 mL centrifuge tubes and 10 mL of deionised water was added before sealing (Cayuela et al., 2013; Lee et al., 2013). Samples were left to shake for 1 hour at 150 rpm on a mechanical shaker. Following shaking, samples were left to settle for 10 minutes before measurement. The electrical conductivity (EC) of biochar material relates to the release of biochar-borne salts into the solution phase, which affects the free exchange of ions in systems (Singh et al., 2017). Determination of biochar EC was completed at the same point as the pH measurement (Cayuela et al., 2013; Lee et al., 2013).

The redox potential of biochar materials is the capacity to either accept or donate electrons within different environmental media and is a recent addition to biochar characterisation (Graber, Tsechansky, Lew, & Cohen, 2014; Prévoteau, Ronsse, Cid, Boeckx, & Rabaey, 2016). Redox potentials of the twelve biochar materials were measured by adding 1 g of biochar into 50 mL centrifuge tubes. Then 10 mL of deionised water was added. Samples were shaken for 1 hour before allowing settling prior to measurement.

The point of zero charge refers to the pH value at which the net charge on the biochar surface is neither positive nor negative. As such, it is imperative to determine the point of zero charge for each biochar within the UKBRC standard set. An adapted method of the immersion technique (Fan et al., 2018; Fiol & Villaescusa, 2009; Trakal, Šigut, Šillerová, Faturíková, & Komárek, 2014) was used. Briefly, 0.5 g of biochar was added to 50 mL of 0.01 mol L^{-1} sodium chloride. The pH of the sodium chloride solution was adjusted to a range of pH values (2 to 12) using nitric acid and sodium hydroxide. Samples were shaken at 300 rpm for 24 hours until an equilibrium pH was achieved. The difference in pH from the initial pH was determined. Then the intercept between the pH change and initial pH determined the point of zero charge. Other techniques have been used such as mass titration and the potentiometric mass titration technique however the immersion technique is widely adopted due to its simplicity and has similar reported values to other methods (Fiol & Villaescusa, 2009).

For pH, redox potential and point of zero charge, the parameter determination was completed using a Hanna HI-2020 edge® Hybrid Multiparameter pH Meter and probe. Before use, the equipment was set up with a three-point calibration (pH 4, 7 and 10). In between readings, care was taken to rinse the probe with deionised water to avoid cross-contamination of samples and fouling of the probe. For EC determination, a MettlerToledo Five Go portable meter was used which was pre-calibrated with potassium chloride reference solution before use.

3.3.2.2 Biochar functional groups

Surface functionality of the biochar materials, that is to say, the identification of oxygen-containing functional groups on the biochar samples was determined using FTIR spectroscopy. FTIR spectroscopy passes infrared light through a sample and measures the amount absorbed and transmitted at different wavenumbers, typically in the range of 4000 cm^{-1} to 400 cm^{-1}. The latter is provided as the spectral output. The various peaks that are identified on the spectrum are associated with the biochar materials functional groups. Crushed biochar samples were placed onto the diamond plate of the spectrometer. An initial background scan was run before sample analysis to calibrate the machine. The spectral analysis was performed within a 4,000 cm^{-1} to 600 cm^{-1} scan range at a resolution of 4 cm^{-}

^{1}. A total of 100 scans were averaged, as this gives a better signal-to-noise ratio (Smith, 2011). Spectra were corrected for the presence of carbon dioxide and the results were plotted using Origin Lab 2019. All spectra were subjected to peak smoothing for better peak clarity. Peaks were assigned by comparison to wavelengths published in current literature (Keiluweit et al., 2010; Özçimen & Ersoy-Meriçboyu, 2010; Shen, Zhang, Jin, Alessi et al. 2018).

3.3.2.3 Biochar surface morphology

Surface morphology analysis of biochar materials using SEM is often used to view the pore structure, distribution and size on different biochar materials. SEM imagery was taken at Salford Analytical Services based at the University of Salford, UK. The procedure was carried out using a Philips XL30 SFEG, SEM with an accelerating voltage of 7 kV with a spot size of 3 and Secondary Electron detection (SE). Samples were prepared by coating with 5 nm of platinum/palladium using the Cressington 208 sputter coating unit. Different magnifications (5000x and 10000x) were used to collect a range of surface imagery to support the characterisation of biochar materials.

3.3.2.4 Total metal content

The total metal content of the twelve biochar materials used in the study was determined using x-ray fluorescence (XRF) spectrometry. XRF analysis involves the emission of primary x-ray radiation towards the sample of interest and then interacts with atoms within the sample itself. Secondary x-ray waves are then emitted and beamed towards a detector within the instrument that measures the energy and intensity, allowing a spectrum to be plotted. This is used for identification of elements within the sample.

Samples were dried for 48 hours at 100 °C and crushed using mortar and pestle. Then, samples were sieved to < 2 mm ready for analysis (Caporale et al., 2018). Firstly, a coning and quartering technique was used to allow sample homogenisation (see section 3.4.1). Approximately 3 g of a sample was added to soil pots and rings with 6 µm Mylar film to hold the sample in place. Samples then gently patted down with a glass rod to allow for full coverage next to the film. Before using the XRF (Thermo Scientific™ Niton™ XL2 XRF Analyser), the machine was calibrated using certified reference material (Buffalo river sediment, NIST® RM 8704). Following calibration, each sample was run in triplicate for 240 seconds and the results compared to the reference material.

While previous studies have utilised XRF for total metal content analysis of biochar materials (e.g. Fidel, Laird, Thompson, & Lawrinenko, 2017; Kim et al., 2013; Stefaniuk & Oleszczuk, 2015), several limitations exist with the methods used in the work presented in this thesis that must be acknowledged. Organic matter content is a major limiting factor to the success of element determination by XRF (Kern et al., 2019; Mcwhirt, Weindorf, & Zhu 2012; Longman, Veres, & Wennrich, 2019; Ravansari & Lemki, 2018). Firstly, the high levels of hydrogen, carbon, nitrogen and oxygen that are characteristic of organic matrices cannot be determined by XRF and cause a dilution effect that underestimates the concentrations of heavier elements (Kern et al., 2019; Longman et al., 2019). In response to the dilution effect, lighter elements such as magnesium, aluminium and silicon can also be overestimated (Kern et al., 2019). The current study used Buffalo river sediment NIST® RM 8704 to calibrate the machine, which is the certified reference material used at the University of Salford (Korai, 2012). However, river sediment is likely to have a lower organic content than biochar which inherently has a very high organic matter content (Downie, 2009). As such, the calibration of the XRF equipment would likely affect the accuracy of the analysis.

A second issue is that when comparing the total metal contents as reported in this study, with those values provided by the UKBRCs modified dry-ashing method followed by inductively coupled plasma optical emission spectrometry (ICP-OES) analysis (Appendix A3), there is a clear discrepancy between values. Namely, those supplied by the UKBRC typically report higher concentrations of elements of major concern such as arsenic, chromium and copper for several of the biochar materials. In the current study, ICP-OES analysis for biochar metal content was not completed due to the costs involved and the XRF determination for total element content can be affected by inter-lab preparative technique differences such as grinding technique, particle size, scanning time and film thickness (Kalnicky & Singhvi, 2001). Despite XRF being more cost and time effective, it is recognised as a less precise method compared to ICP-OES analysis but has good correlation with ICP-OES values overall (Kilbride, Poole & Hutchings, 2006; Radu & Diamond. 2009) (section 3.3.2.5). Therefore, it is plausible that a short scanning time and the use of larger particle biochar (< 2mm) may have affected the results in this work causing a discrepancy compared to the UKBRC reported total metal content values (Appendix A3).

These factors may have limited the accuracy of the results supplied in this work and hence considerations for future work should be made. Post-analysis element dependent corrections to compensate for the dilution effect of a majority organic matrix have been previously completed (Ravansari & Lemki, 2018). These should be applied where necessary if future work is to consider this technique. Next, efforts to extend scanning times and use of smaller biochar particles would greater increase the accuracy of element determination and should be considered (Hou, He, & Jones, 2004; Maruyama, Ogawa, Okada, & Kato, 2008). If this is not feasible, other methods such as laser-induced breakdown spectroscopy (LIBS) could be considered. LIBS reportedly boasts improved sensitivity, especially for light elements, some of which cannot be measured by XRF (Connors, Somers, & Day, 2016; Senesi, 2017). Lastly, before application to field scenarios, all biochar should undergo acid digestion and analysis via ICP-OES to better quantify total element concentrations because of the improved limits of detection compared to XRF methods.

3.3.2.5 Water and 0.01 mol L^{-1} calcium chloride-extractable biochar-borne elements

Water extractable biochar-borne elements are the soluble and available elements available for plant uptake. Quantification of the water-extractable biochar-borne elements is essential before application to contaminated environments. A series of extractions were carried out to determine the water-soluble anions and cations extracted from biochar materials. In triplicate, 1 g of the twelve biochar materials were weighed out into 50 mL polypropylene centrifuge tubes, then, 10 mL of deionised water was added to each before shaking for 1 h at 150 rpm on a rotary shaker. Samples were filtered using a nylon syringe filter (0.22 µm) and plastic syringe into a 15 mL centrifuge tube. Before storage, samples were acidified using one drop of 65 % nitric acid before the tubes were sealed and placed in the refrigerator (< 4 ^{o}C) until the day of analysis. Exchangeable trace elements were determined using the same method but with 0.01 mol L^{-1} calcium chloride as an extractant with a 2 h shaking time (Houba, Novozamsky, Huybregts, & Van der Lee, 1986; Pueyo, Lopez-Sanchez, & Rauret, 2004).

Determination of anions (fluoride, chloride, bromide, nitrate, phosphate and sulphate) in the deionised water extracts were carried out using ion-exchange chromatography (DIONEX ICS-1000) with the use of an autosampler (DIONEX AS40). Previously filtered samples were carefully placed into acid-washed 5 mL Polyvials. Each vial was then capped and placed into the auto-sampler cassettes. Standard calibration solutions were prepared for each run and were analysed before any samples and then after every forty samples to ensure machine calibration. For anion determination, an Ion Pac® AS14 anion analytical column (4 mm×250 mm), Ion Pac® AG14 guard column (4 mm × 50 mm) and ULTRA anion self-regenerating suppressor (4 mm) were used. A mixed 0.8 mmol L^{-1} sodium carbonate and 1 mmol L^{-1} sodium bicarbonate solution was used as the eluent mobile phase. For all measurements, the flow rate was set at 1.0 mL min^{-1} with a 20 µL injection volume. Qin, Lin, Cheruiyot, Mkpanam, and Duma (2017) previously used this set up with success.

Trace elements were measured using a Varian 720-ES ICP-OES set up with a four-point calibration curve. All standard solutions were made using analytical grade reagents and were made up either with water and acidified with nitric acid to ensure matrix matching. The wavelengths used for element determination are shown in Table 3.3.

Table 3.3 Primary and secondary wavelengths used for the determination of trace elements of interest using inductively coupled plasma optical emission spectroscopy (ICP-OES)

Element	Primary wavelengths (λ) used for element determination	Secondary wavelengths (λ) used for element determination
Aluminium	396.152	n/a[1]
Arsenic	193.636	n/a
Boron	241.678	208.959
Barium	455.403	n/a
Bismuth	306.772	n/a
Calcium	396.847	n/a
Cadmium	226.502	214.438
Cobalt	228.16	230.786
Chromium	284.325	205.552
Copper	324.754	n/a
Iron	259.940	n/a
Gallium	294.418	417.206
Indium	325.609	n/a
Potassium	766.490	n/a
Lithium	670.784	n/a
Magnesium	279.553	n/a
Manganese	257.610	259.373
Sodium	589.595	588.995
Nickel	221.647	n/a
Lead	217.000	220.353
Strontium	407.771	n/a
Titanium	377.572	n/a
Zinc	206.200	n/a

[1] n/a: not applicable

3.3.2.6 Organic acid-extractable major elements

LMWOA-extractable biochar-borne elements for the twelve biochar materials were assessed by carrying out a batch experiment with controls (water-extractable elements) and one treatment being set for each biochar material. For the controls, 2 g of each biochar was extracted using 20 mL of deionised water. For the treatments, 20 mL of 0.01 mol L^{-1} mixed citric, malic, and oxalic acid solution was used. Biochar was added to either the water or acid solutions in 50 mL polypropylene centrifuge tubes. Tubes were then shaken on a rotary shaker at 150 rpm for 1 hour. Following shaking, samples were immediately filtered into 15 mL polypropylene centrifuge tubes using a 0.22 µm nylon syringe filter and a 10 mL syringe. The pH of each extractant solution was measured. Samples were then analysed using ICP-OES as previously outlined (section 3.3.2.5). However, preliminary work identified a build-up of precipitates on the ICP-OES torch and nebuliser due to the high organic content and metal-organic acid complexes within samples when LMWOAs were introduced. This lowered the signal intensity, affecting the analysis reading. After numerous techniques were attempted, it was found that nitric acid run within sample batches could eliminate the issue. So, a solution of 3 mL concentrated nitric acid and 7 mL deionised water was run every twenty samples followed by a standard solution which was compared to the calibrated standards to ensure machine accuracy was maintained. Further maintenance included sonication of the nebuliser after each run to ensure parts were sufficiently clean.

The release rate of a biochar-borne element by each of the LMWOAs was then calculated using Equation 3.1, Where E_{LMWOA} stands for the concentration of an extracted element by the mixed LMWOA solution and E_{total} denotes the total concentration of that biochar-borne element measured by XRF (Vause et al., 2018).

$$\textbf{Release rate (\%)} = \frac{E_{LMWOA}}{E_{total}} \times 100$$

Equation 3.1 Determination of the release rate of biochar-borne elements by low-molecular-weight organic acids

3.4 Soil used in the experiments

3.4.1 Soil collection

Garden soil from a residential address in Holmfirth, West Yorkshire (SE 10021 10414) were taken for use in the experiments. Samples were stored in labelled and sealed polypropylene grip lock bags and transported to the University of Salford. In the laboratory, soils were transferred to paper bags and oven-dried at 40 ^{o}C until a constant weight was achieved. After drying, the soils were ground using a mortar and pestle then sieved through a 2 mm stainless steel sieve to achieve a consistent particle size. Debris > 2 mm were classified as non-soil constituents and discarded. At this stage, the soil samples were combined and homogenised using a coning and quartering technique to create one contaminated soil sample (Alozie et al., 2018) (Figure 4.1).

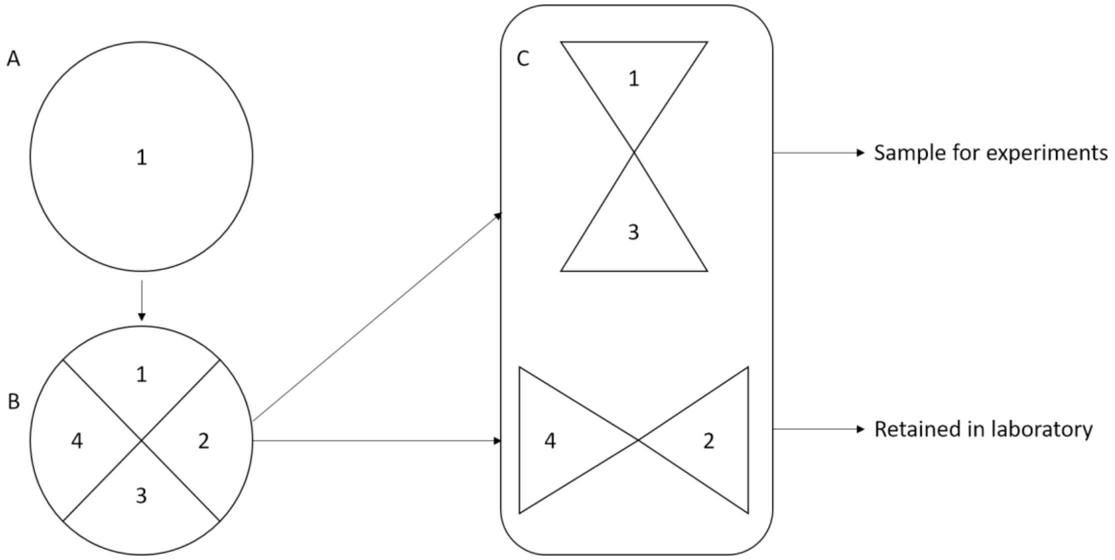

Figure 3.3 The coning and quartering technique used to homogenise and separate field-collected samples for use in the experiments completed within this thesis. A refers to the samples piled in a flattened cone shape. B indicates the quartering of the coned soil. C refers to the separation of subsamples where quarter 1 and quarter 3 are reserved for use in the experiments. Quarter 2 and quarter 4 are retained in the laboratory stores.

3.4.2 Soil characterisation

3.4.2.1 Soil pH and electrical conductivity

Once homogenised, the garden soil was characterised for a range of properties. First, pH and EC were measured by weighing 2 g of soil into a 100 mL glass beaker and adding 20 mL of ultrapure water. Samples were left to shake for 1 hour at 150 rpm on a mechanical shaker. Following shaking, samples were left to settle for 10 minutes before measurement using a pH and EC probe.

3.4.2.2 Soil particle size analysis

Soil particle size distribution analysis was carried out using a simplified hydrometer method (Kroetsch & Wang, 2008). Firstly, 50 g L^{-1} sodium hexametaphosphate was prepared as a dispersing agent. Into a 1 L glass beaker, 100 mL of sodium hexametaphosphate was added to make a blank solution. At the same time, 40 g of sieved and prepared soil sample (section 3.4.1) was added to a 1 L glass beaker before 100 mL of the sodium hexametaphosphate solution and 300 mL distilled water was added. Both the blank solution and samples were then placed on a magnetic stirrer with a stir bar and left to mix overnight. The following day, the blank and sample solutions were transferred to a 1 L plastic measuring cylinders. Care was taken to rinse each of the glass beakers with distilled water to make the volume of the cylinders to the 1 L mark. The cylinders were then sealed with parafilm before inverting each cylinder for 2 minutes each to ensure homogenisation and the time of each cylinder inversion was recorded. At this point, a hydrometer was used to take a reading of the blank solution (R_L). After 40 seconds, a calibrated hydrometer was placed into each cylinder and a reading was taken ($R_{40\text{ s}}$). The cylinders were all left to stand and after 7 hours, a second reading was taken ($R_{7\text{ h}}$). The amount of sand (%), clay (%) and silt (%) were then calculated as in Equation 3.2, Equation 3.3 and Equation 3.4.

$$\boldsymbol{Sand}\ (\%) = \mathbf{100} - (\boldsymbol{R_{40\,s}} - \boldsymbol{R_L})\boldsymbol{x}\ \frac{\mathbf{100}}{\boldsymbol{Oven\ dried\ soil}\ (\boldsymbol{g})}$$

Equation 3.2 Determination of the sand percentage per soil sample according to the simplified hydrometer method

$$\boldsymbol{Clay}\ (\%) = \mathbf{100} - (\boldsymbol{R_{7\,h}} - \boldsymbol{R_L})\boldsymbol{x}\ \frac{\mathbf{100}}{\boldsymbol{Oven\ dried\ soil}\ (\boldsymbol{g})}$$

Equation 3.3 Determination of the clay percentage per soil sample according to the simplified hydrometer method

$$\boldsymbol{Silt}\ (\%) = \mathbf{100} - (\boldsymbol{sand}\ \% + \boldsymbol{clay}\ \%)$$

Equation 3.4 Determination of the silt percentage per soil sample according to the simplified hydrometer method

3.4.2.3 Total metal content

The total metal content of the soil samples used in the study was determined using XRF spectrometry. Previously prepared soil samples (section 3.4.1) were used for analysis. The preparation of the soil pots and rings, calibration technique and run time was carried out as previously described for the biochar characterisation in section 3.3.2.4.

3.4.2.4 Calcium chloride-extractable elements

Lastly, a series of calcium chloride and LMWOA-extractions took place to determine the exchangeable and plant available trace elements. The method used was as previously described for the biochar characterisation in section 3.3.2.5, respectively. Element concentrations were determined using a Varian 720-ES ICP-OES which was calibrated using a four-point calibration curve (0, 0.01, 1 and 10 mg kg^{-1}) to the methods discussed in section 3.3.2.5 and 3.3.2.6. All standard solutions were made using analytical grade reagents and were made up either with deionised water or LMWOAs. The wavelengths used for element determination are the same as described in Table 3.3. The garden soil baseline characteristics are shown in Table 3.4. The soil had a pH of 6.63 and an EC of 0.154 mS cm^{-1}. The particle size analysis revealed 32 % of the soil was < 0.002 mm, 43 % was between 0.002 to 0.05 mm and 25 % was > 0.5 mm to 2 mm, classifying the soil as clay loam. These preliminary soil quality investigations revealed below advisable levels for heavy metals and therefore represent a ‘clean soil’.

Table 3.4 Baseline characteristics of the garden soil that was used in the experiments before the spiking procedure

Parameter	Garden soil (before spiking)	Method
pH	6.63	pH meter (1:10 w/v)
Electrical conductivity (mS cm^{-1})	0.154	EC meter (1:10 w/v)
Organic matter (%)	3.25	Loss-on-ignition
Soil particle fraction < 0.002 mm (%) (clay)	32	Hydrometer method
Soil particle fraction 0.002 – 0.05 (%) (silt)	43	
Soil particle fraction 0.05 - 2 mm (%) (sand)	25	
Total arsenic (mg kg^{-1})	21.88	XRF
Total cadmium (mg kg^{-1})	< LOD	
Total copper (mg kg^{-1})	41.48	
Total iron (mg kg^{-1})	20040.65	
Total manganese (mg kg^{-1})	300.71	
Total lead (mg kg^{-1})	273.25	
Total zinc (mg kg^{-1})	187.12	
CaCl$_2$[1] extractable arsenic (mg kg^{-1})	< LOD	ICP-OES
CaCl$_2$ extractable cadmium (mg kg^{-1})	< LOD	
CaCl$_2$ extractable copper (mg kg^{-1})	< LOD	
CaCl$_2$ extractable iron (mg kg^{-1})	< LOD	
CaCl$_2$ extractable manganese (mg kg^{-1})	< LOD	
CaCl$_2$ extractable lead (mg kg^{-1})	< LOD	
CaCl$_2$ extractable zinc (mg kg^{-1})	< LOD	

[1] CaCl$_2$; 0.01 mol L^{-1} Calcium chloride

3.4.3 Soil spiking procedure

To represent a contaminated soil, the garden soil was spiked according to a modified procedure from Khalkhaliani, Mesdaghinia, Mahvi, Nouri, and Vaezi (2006). First, to make stock solutions of 25 mmol L^{-1}, 7.7123 g of cadmium nitrate tetrahydrate ($Cd(NO_3)_2.4H_2O$) was weighed out and then 7.4373 g of zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and made up to 1 L volumetric flask using ultrapure water. Then in 125 mL acid-rinsed bottles, 10 g of soil was mixed with 50 mL of the metal-containing solutions. The sealed bottles were shaken at 150 rpm for 1 h on a rotary shaker to allow for the complete wetting of the soil particles. Afterwards, bottles were removed and placed in the dark to stand for seven days. No further drying of the soil took place as experiments would be carried out under inundated conditions.

The procedure for the creation of nutrient spiked soils was similar however rather than metal solutions, two stock solutions of 0.1 mol L^{-1}, 8.4995 g of sodium nitrate

($NaNO_3$) was weighed out and then 11.9977 g of sodium dihydrogen phosphate (H_2NaO_4P) and made up to 1 L volumetric flask using ultrapure water. In 125 mL acid-rinsed bottles, 10 g of the garden soil was placed with 50 mL of the nutrient-containing solution. Storage and handling were as previously described for the metal spiking procedure.

3.4.4 Soil for growth assays and plant metal uptake experiments

For the growth assays and plant metal uptake experiments, contaminated soil and garden soil were used as a composite. Contaminated soil was collected from two sites within a closed landfill site at Moston Brook in Greater Manchester. These sites were the Football Ground (SD 89811 02171) and the White Hills (SD 89661 02059). Numerous studies have used these soils for research confirming metal contamination (Heaney et al., 2018; Mukwaturi & Lin, 2015; Nworie et al., 2017; Nworie, Qin, & Lin, 2019; Onireti & Lin, 2016; Onireti et al., 2017; Qin et al., 2018; Qin, Nworie, & Lin, 2016). Samples from both sites were taken to include a wide range of metals and metalloids. At both Moston Brook and the garden sites, the surface vegetation was removed, and a surface soil sample (0 to 10 cm) was taken using a stainless-steel hand trowel (Mukwaturi & Lin, 2015). Samples were stored, transported and prepared for use in the same manner as described in section 3.4.1. Soils were then combined using the coning and quartering method described in section 3.4.1 and Figure 3.3 to make a composite soil consisting of 50 % mix of garden and contaminated soil were used herein known as ‘Moston Brook Composite soil’. The reason for this was that preliminary trials showed how 100 % contaminated soil from this site either with or without biochar could not sustain the growth of lettuce (*Lactuca sativa*), Indian mustard (*Brassica juncea*) or cress (*Lepidium sativum*). That is, obvious and signs of necrosis and stunted growth were found (Appendix A4). Therefore, dilution with garden soil was deemed a necessary step to promote plant growth. The Moston Brook Composite soil was characterised as described in section 3.2.2 and the basic properties are shown in Table 3.5. The soil had a pH of 7.92 and an EC of 0.037 mS cm^{-1}. The particle size analysis revealed 38 % of the soil was < 0.002 mm, 35 % was between 0.002 to 0.05 mm and 27 % was > 0.5 mm to 2 mm, classifying the soil as clay loam.

Table 3.5 Baseline characteristics of the Moston Brook Composite soil that was used in the experiments

Parameter	Moston Brook Composite	Method
pH	7.92	pH meter (1:10 w/v)
Electrical conductivity (mS cm^{-1})	0.037	EC meter (1:10 w/v)
Soil particle fraction < 0.002 mm (%) (clay)	38	Hydrometer method
Soil particle fraction 0.002 – 0.05 (%) (silt)	35	
Soil particle fraction 0.05 - 2 mm (%) (sand)	27	
Total aluminium(mg kg^{-1})	76521.03	ICP-OES
Total arsenic (mg kg^{-1})	596.37	
Total cadmium (mg kg^{-1})	3.38	
Total cobalt (mg kg^{-1})	200.51	
Total copper (mg kg^{-1})	1416.69	
Total chromium (mg kg^{-1})	295.2167	
Total iron (mg kg^{-1})	95403.70	
Total manganese (mg kg^{-1})	4127.67	
Total nickel (mg kg^{-1})	1768.86	
Total lead (mg kg^{-1})	1422.55	
Total zinc (mg kg^{-1})	504.31	
CaCl$_2$[1] extractable arsenic (mg kg^{-1})	2.25	ICP-OES
CaCl$_2$ extractable cadmium (mg kg^{-1})	< LOD	
CaCl$_2$ extractable copper (mg kg^{-1})	74.12	
CaCl$_2$ extractable iron (mg kg^{-1})	< LOD	
CaCl$_2$ extractable manganese (mg kg^{-1})	< LOD	
CaCl$_2$ extractable lead (mg kg^{-1})	1.74	
CaCl$_2$ extractable zinc (mg kg^{-1})	24.32	

[1] CaCl$_2$; 0.01 mol L^{-1} Calcium chloride

3.5 Plants used in the experiments

Two of the research hypotheses (4 and 5) use plants in the experiments. Five different plant species were selected for use in the growth experiments. Firstly, lettuce (*Lactuca sativa*) is a leafy salad crop from the Asteraceae family. The second plant is white mustard (*Sinapis alba*) from the Brassicaceae family that is grown for salad garnish and seed production. Thirdly, pea (*Pisum sativum*) is a leguminous crop from the Fabaceae family that is grown for its seeds (peas) or pea shoots. The fourth plant selected was radish (*Raphanus sativus*) a root vegetable used in salads from the Brassicaceae family. Lastly, tomato (*Solanum* *lycopersicum*) is a fruit from the Solanaceae family used in salads and as the base of many dishes. The different seed types were bought from Wilko Retail Ltd, United Kingdom. All of the plant species were selected for their ability to be grown in a greenhouse setting from early spring. Lettuce, pea and tomato were also identified as commonly consumed vegetables within the UK (Frankowska, Jeswani, & Azapagic, 2019).

3.6 Methods for hypotheses 1 and 2

3.6.1 Biochar selection

Rice husk biochar (RH700) was selected as a model biochar for all further work in this thesis. There are three reasons for this decision. Firstly, the results obtained from the method detailed in section 3.3.2 demonstrated that rice husk biochar had several favourable characteristics for adsorption such as a porous surface, a high pH (11.17), a mid-ranged point of zero charge (8.59), reasonably low levels of water-extractable trace elements, nutrients and LMWOA-extractable trace elements, compared to chars such as sewage sludge. A porous surface may aid the retention of trace elements and nutrients, whereas a high pH and mid-ranged point of zero charge would be beneficial for use in acidic solutions. The comparably low levels of extractable trace elements and nutrients would also limit any competitive sorption effects that may occur (Park et al., 2016; Xu et al., 2013). The UKBRC data also revealed RH700 biochar to have the highest ash content (47.93 %) (Appendix A3). There is a debate that ash content can be an indicator for adsorption (Zhu, Wang, & Ok, 2019). However, previous studies have reported maximum metal adsorption to positively correlate with ash content (Abbas et al., 2018; Hass & Lima, 2018). Furthermore, Glover (2009) wrote how high-ash biochars from less homogenous biomass sources such as agricultural wastes would be ideal for biochar production owing to the extra fertiliser effect from the ash content. Therefore, with the characteristics shown, it was decided RH700 may be an interesting biochar of choice to examine trace element and nutrient dynamics in the presence of LMWOAs.

Secondly, rice is a staple food around the globe. The production of rice crops for consumption leads to rice husk and kernel by-products. Roughly 120 Mt yr^{-1} of rice husks is agricultural waste material (Giddel & Jivan, 2007; Shackley et al., 2012). The easy availability of rice husk wastes presents an excellent opportunity for biochar production justifying the inclusion of rice husk-derived biochar materials as one of the six-feedstock materials utilised by the UKBRC. Lastly, several papers discussed in Chapter 2 have successfully demonstrated potential for biochar driven-immobilisation of contaminants using rice husk biochar from the UKBRC standard biochar set (Shen, Zhang, Jin et al., 2017; Melia, Busquets, Hooda, Cundy, and Sohi, 2019). This further supports the choice of RH700.

3.6.2 Experimental design

A series of batch sorption experiments were performed to test if LMWOAs will inhibit the biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems (Table 3.6). For hypothesis 1, solutions of 0.01 mol L^{-1} cadmium, lead and zinc were prepared by weighing 3.0849 g cadmium nitrate tetrahydrate ($Cd(NO_3)_2.4H_2O$), 3.3123 g lead nitrate ($Pb(NO_3)_2$) and 2.9749 g zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) into separate 1 L volumetric flasks. Then, different solutions of 0.02 mol L^{-1} citric, malic and oxalic acid were made by weighing, 4.2030 g, 2.6818 g and 2.5208 g respectively, into separate 1 L volumetric flasks. All solutions were made up with ultrapure water. These acids were selected due to their prevalence within the rhizosphere (Jones, 1998; Sun et al., 2016). The concentration of LMWOAs was selected to reflect the upper limit of acids within the rhizosphere to exacerbate any effects observed between biochar and the LMWOAs (Vause et al., 2018).

Table 3.6 Details of the experimental design used for hypothesis one, to test if LMWOAs will inhibit the biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems

Treatment	**Biochar ($g\ L^{-1}$)**	**Metal solution (mL)**[1]	**Water (mL)**	**Citric acid (mL)**[2]	**Malic acid (mL)**[2]	**Oxalic acid (mL)**[2]
Biochar only	1	0	100	0	0	0
Biochar and citric acid	1	0	0	100	0	0
Biochar and malic acid	1	0	0	0	100	0
Biochar and oxalic acid	1	0	0	0	0	100
Metal only	0	10	90	0	0	0
Metal and citric acid	0	10	0	10	0	0
Metal and malic acid	0	10	0	0	10	0
Metal and oxalic acid	0	10	0	0	0	10
Biochar and metal	1	10	90	0	0	0
Biochar, citric acid and metal	1	10	0	10	0	0
Biochar, malic acid and metal	1	10	0	0	10	0
Biochar, oxalic acid and metal	1	10	0	0	0	10

[1] Metal solution (10 mmol L^{-1}), [2] Organic acid solutions (20 mmol L^{-1})

Acid-washed Nalgene bottles (125 mL) were rinsed with deionised water then left to air dry before use. Bottles without metal were filled with 100 mL of water or one of the three LMWOA solutions. Treatment bottles were filled with 90 mL of water or the different LMWOAs and 10 mL of either cadmium, lead or zinc stock solution. This was to achieve an initial concentration of 1 mmol L^{-1}. Next, pre-weighed biochar (1 g) was added where required. Bottles were then sealed and placed on a rotary shaker for 1 h at 150 rpm in the dark.

At 1 h, the pH and EC were determined before taking a 10 mL aliquot using a 10 mL polypropylene syringe. The aliquot was immediately filtered into individual 15 mL polypropylene centrifuge tubes through a 0.22 µm nylon syringe filter. Samples were stored at < 4 °C before analysis. Bottles were then returned to the shaker, and the sampling procedure was repeated at 24, 72 and 120 h to examine the temporal changes to cadmium, lead and zinc concentrations.

For nutrient studies (hypothesis 2), a similar batch sorption approach was adopted to that used for hypothesis 1. Rather than metals, solutions of 0.01 mol L^{-1} nitrate and phosphate were prepared by weighing 0.8500 g sodium nitrate ($NaNO_3$) and 1.1998 g sodium dihydrogen phosphate (H_2NaO_4P) into separate 1 L volumetric flasks. Then the bottles containing the previously prepared citric, malic and oxalic acid or water solutions were spiked to make a final concentration of 1 mmol L^{-1} of nitrate or phosphate-containing solution before the biochar was added. An experimental design is provided in Table 3.7.

Table 3.7 Details of the experimental design used for hypothesis two, to test if LMWOAs will facilitate the biochar-driven immobilisation of nitrate and phosphate in aqueous systems

Treatment	**Biochar ($g L^{-1}$)**	**Nutrient solution (mL)**[1]	**Water (mL)**	**Citric acid (mL)**[2]	**Malic acid (mL)**[2]	**Oxalic acid (mL)**[2]
Biochar only	1	0	100	0	0	0
Biochar and citric acid	1	0	0	100	0	0
Biochar and malic acid	1	0	0	0	100	0
Biochar and oxalic acid	1	0	0	0	0	100
Nutrient only	0	10	90	0	0	0
Nutrient and citric acid	0	10	0	10	0	0
Nutrient and malic acid	0	10	0	0	10	0
Nutrient and oxalic acid	0	10	0	0	0	10
Biochar and nutrient	1	10	90	0	0	0
Biochar, citric acid and nutrient	1	10	0	10	0	0
Biochar, malic acid and nutrient	1	10	0	0	10	0
Biochar, oxalic acid and nutrient	1	10	0	0	0	10

[1]Nutrient solution (10 mmol L^{-1}), [2]Organic acid solutions (20 mmol L^{-1})

Parameter determination (pH and EC) and aliquot removal were carried out at 1 h, 24 h, 72 h and 120 h in the same manner as described for the metal batch sorption experiments (hypothesis 1). Samples were frozen before analysis with ion-exchange. All samples were slowly defrosted at room temperature on the day of analysis.

3.6.3 Model fitting

Three models, the pseudo-first-order, pseudo-second-order and intraparticle diffusion model were chosen to help elucidate sorption kinetics for metals and nutrients onto the biochar material. Linearised pseudo-first-order kinetics were applied following work by Hyder, Begum and Egiebor (2015). The linearised pseudo-first-order kinetics is shown Equation 3.5 (where Qe (mmol g^{-1}) is sorption capacity at equilibrium, Qt (mmol g^{-1}) is the sorption capacity at time t (h), and k_1 (hr^{-1}) is the first-order rate constant). The linearised pseudo-second-order model applied also followed work by Hyder et al. (2015) and is shown in Equation 3.6 (where Qe (mmol g^{-1}) is sorption capacity at equilibrium, Qt (mmol g^{-1}) is the sorption capacity at time t (h), and k_2 (mmol g^{-1} h^{-1}) is the second-order sorption rate constant):

$$\mathbf{Log}(\boldsymbol{Q_e - Q_t}) = -\frac{\boldsymbol{k_1}}{\mathbf{2.303}}\boldsymbol{t} + \boldsymbol{LogQ_e}$$

Equation 3.5 Linearised pseudo-first-order kinetic model

$$\frac{\boldsymbol{t}}{\boldsymbol{Q_t}} = \frac{\mathbf{1}}{\boldsymbol{qQ_e}}\boldsymbol{t} + \frac{\mathbf{1}}{\boldsymbol{k_2 \cdot Q_e}^{\mathbf{2}}}$$

Equation 3.6 Linearised pseudo-second-order kinetic model

Weber and Morris (1962, cited in (1962, cited in Aichour, Zaghouane-Boudiaf, Iborra, & Polo, 2018) suggested adsorbate transport towards solids is by intraparticle diffusion and could be expressed by Equation 3.7 (where Qt (mmol g^{-1}) is adsorbed metals at amount at time t (h), k_i is the intraparticle diffusion model rate constant (mmol g^{-1} $h^{0.5}$), C is the adsorption constant (mmol g^{-1})):

$$\boldsymbol{Q_t = k_i\sqrt{t} + C}$$

Equation 3.7 Intraparticle diffusion model

Where release of nutrients into solution was observed, the nutrient release percentage (%) was determined using a modified equation from Chintala et al. (2013) as shown in Equation 3.8, where % R was the percentage of nutrient (nitrate or phosphate) released into solution at each time interval, *C released* was the amount of nutrient released into solution (mg L^{-1}) and *C initial* is the initial concentration of nutrient at the experiment start point.

$$\%\boldsymbol{R} = \frac{\boldsymbol{Creleased}}{\boldsymbol{Cinitial}}\ \boldsymbol{x\ 100}$$

Equation 3.8 The nutrient release percentage (%)

3.6.4 Analytical methods

The pH and EC parameters in all the solution samples were measured using a Hanna HI-2020 edge® Hybrid Multiparameter pH Meter and probe and a Mettler Toledo EC meter, respectively. The meters were calibrated as described in section 3.2.2.1. For cadmium, lead and zinc analysis, all samples were analysed on a Varian 720ES ICP-OES. The procedure for machine set up has been described in section 3.3.2.5 and 3.3.2.6, respectively.

For nitrate and phosphate concentrations, all samples were analysed using ion-exchange chromatography (DIONEX ICS-1000) with the use of an autosampler (DIONEX AS40). Calibration of the machine using nitrate and phosphate-containing standards were carried out (0, 0.01, 0.1 and 1 mmol L^{-1}). All samples were placed into 5 mL Polyvials and sealed before loading onto the sampler. The machine set up for anion determination has been described in section 3.3.2.5. No changes were made in sample preparation, eluent mix or column selection.

3.6.5 Methodological limitations

Several caveats need to be noted regarding the present study. The batch sorption method adopted throughout this research has various advantages and limitations. This method is both simple and quick allowing for easy determination of solute concentrations (Limousin et al., 2007). However, there are various disadvantages to batch sorption methods. Generally, shaking over a long time can destroy adsorbent particles which may alter the accuracy of the results (Limousin et al., 2007). However, it is the aliquot removal and filtration that is perhaps the most limiting factor to the employed method. Removal of 10 mL aliquots as adopted in this research was done to reflect those of past research, both inclusive (Alozie et al., 2018; Heaney et al., 2018) and exclusive (Mukwaturi & Lin, 2015; Onireti & Lin, 2016; Onireti et al., 2017) of biochar, where 10 mL or greater was removed. However, removal of 10 mL over time is likely to affect the solid/solution ratio within the batch reactor. The solid/solution ratio can be defined as the mass of biochar (g) divided by the volume of liquid within the batch reactor (L) (Phillippi et al., 2007). In some instances, a replenishment of liquid to maintain a solid/solution ratio is made (Shepherd, Sohi, & Heal, 2016). This, however, was not carried out in the current work. Similar batch sorption studies have followed a non-replenishment approach (i.e. Alozie et al., 2018; Heaney et al., 2018; Mukwaturi & Lin, 2015; Onireti & Lin, 2016; Onireti et al., 2017) to which this thesis followed.

Theoretically, if the solution was to decrease over the six intervals measured but the solid was to remain constant, there would be 60 per cent change in solid/solution ratio. In the current study it was inferred that given the hydrophobic nature of the biochar material which floated once shaking ceased, filtration of the materials would remove small amounts of adsorbent at each interval to limit solid/solution effects. Indeed, the removal of small amounts of adsorbent with sampling is a reported disadvantage to the batch method (Patel, 2019). However, as no quantification of the removed biochar was made at each interval and no liquid was replenished over time, it is important to state that solid/solution ratio effects may limit the validity of these results to some extent, leading to overestimations of adsorbed amounts as solid/solution ratios decreased.

The pH of the organic acids used in the batch experiments is also a methodological limitation worthy of mention. As outlined in Chapter 2, the pH of the rhizosphere is typically 2 to 3 units less than bulk soil (Nason, Miller, Karthikeyan, & Pedersen, 2018; Tagliavini, Masia, & Maurizio, 1995; Youssef & Chino, 1989). However, the concentrations of the LMWOAs used herein are rarely encountered in soils and severely decreased systems to an unrealistic pH for soils. For instance, Häussling, Leisen, Marschner and Römheld (1985) found rhizosphere pH values of Norway Spruce to be between 3.9 and 5.4 when grown in acid mineral soil (pH 4.5). In the current text, this concentration of LMWOAs was chosen to represent a stressed rhizospheric environment. However, the high concentration used and subsequent low pH would be rarely encountered. This limitation means that study findings need to be interpreted cautiously.

Finally, the current study only infers sorption mechanisms from kinetic modelling. Further post-sorption testing to elucidate sorption mechanisms would have been useful. For instance, various studies have utilised post-sorption SEM imagery, XRD or FTIR spectroscopy techniques to characterise the sorbed metal-complexes (Inyang et al., 2012; Yao et al., 2011). Batch desorption modelling would have also strengthened the methods employed, as carried out by others (Hale et al., 2013; Kołodyńska, Krukowska, & Thomas, 2017). Given the issues aforementioned with the batch sorption design, the kinetic modelling findings might not be transferable to systems following a different approach.

3.7 Methods for hypothesis 3

3.7.1 Experimental design

To examine hypotheses three, a series of batch experiments were implemented to test if biochar will reduce the LMWOA-driven solubilisation of cadmium and zinc in soils. Each of the spiked soils in the 125 mL bottles (section 3.4.3) had either 50 mL water or 0.02 mmol L^{-1} citric, malic or oxalic acid added. Where required, 1 g of biochar was added (Table 3.8). Bottles were then shaken in the dark at 150 rpm for 1 h.

Table 3.8 Details of the experimental design used for hypothesis three, to test whether biochar will reduce the LMWOA-driven solubilisation of cadmium and lead in soil.

Treatment	**Soil (g)**	**Water (mL)**	**Bio-char (g)**	**0.02 mol L^{-1} Citric acid (mL)**	**0.02 mol L^{-1} Malic acid (mL)**	**0.02 mol L^{-1} Oxalic acid (mL)**	**25 mmol L^{-1} kg^{-1} metal solution[1]**
Soil only	10	50	0	0	0	0	No
Soil and biochar	10	50	1	0	0	0	No
Soil and citric acid	10	0	0	50	0	0	Yes
Soil and malic acid	10	0	0	0	50	0	Yes
Soil and oxalic acid	10	0	0	0	0	50	Yes
Soil, biochar and citric acid	10	0	1	50	0	0	Yes
Soil, biochar and malic acid	10	0	1	0	50	0	Yes
Soil, biochar and oxalic acid	10	0	1	0	0	50	Yes

[1] Either cadmium, lead or zinc-containing solution

After 1 h, pH and EC were measured before a 10 mL aliquot was removed using a 10 mL polypropylene syringe. Bottles were resealed and left to stand in the dark. Meanwhile, the aliquot was transferred to a 25 mL polypropylene centrifuge tube and centrifuged at 5000 rpm for 10 minutes. The supernatant was filtered using a 10 mL polypropylene syringe and a 0.22 µm nylon syringe filter into a 15 mL polypropylene centrifuge tube. Samples were stored at < 4 ^{o}C before analysis. The sampling procedure was repeated at 24 h, 72 h and 120 h.

For the nutrient batch sorption studies, similar batch sorption experiments were carried out reflective of those for cadmium and zinc, however, soils were spiked with 0.1

mol L^{-1} of either nitrate or phosphate-containing solution and then biochar was added. An experimental design is provided in Table 3.9. Parameter determination (pH and EC), centrifugation and aliquot removal were carried out at 1 h, 24 h, 72 h and 120 h in the same manner as done for the metal experiments. Then samples were frozen before analysis with ion-exchange. All samples were slowly defrosted at room temperature on the day of analysis.

Table 3.9 Details of the experimental design used for hypothesis three, to test whether biochar will reduce the LMWOA-driven solubilisation of nitrate and phosphate in soil

Treatment	**Soil (g)**	**Water (mL)**	**Biochar (g)**	**0.02 mol L^{-1} Citric acid (mL)**	**0.02 mol L^{-1} Malic acid (mL)**	**0.02 mol L^{-1} Oxalic acid (mL)**	**0.1 mol L^{-1} kg^{-1} nutrient solution**[1]
Soil only	10	50	0	0	0	0	No
Soil and biochar	10	50	1	0	0	0	No
Soil and citric acid	10	0	0	50	0	0	Yes
Soil and malic acid	10	0	0	0	50	0	Yes
Soil and oxalic acid	10	0	0	0	0	50	Yes
Soil, biochar and citric acid	10	0	1	50	0	0	Yes
Soil, biochar and malic acid	10	0	1	0	50	0	Yes
Soil, biochar and oxalic acid	10	0	1	0	0	50	Yes

[1] Either nitrate or phosphate-containing solution

3.7.2 Methodological limitations

As previously described in section 3.6.5, there are various limitations to the batch method employed in this thesis. Explicitly, the aliquot removal of 10 mL, the acidity of the systems, and the high concentration of the LMWOAs used also apply to the methods applied to test hypothesis 3. The anaerobic state of the batch system is also likely to have affected the system to some extent. Under a partially closed system, where the batch reactors were only opened for sample removal, it is likely a reduction in dissolved oxygen caused reducing conditions which could lead to the reductive dissolution of iron and manganese oxides and their associated compounds (Ajmone-Marsan et al., 2019; Heaney et al., 2018; Mukwaturi & Lin, 2015). It is plausible that the liberation of metals and metalloids within the garden soil used in the experiments may compete with the cadmium, zinc, nitrate and phosphate ions, introduced via the spiking procedure, for sorption sites on the biochar surface (Park et al., 2015). As such reductive conditions likely increased the co-existing trace elements, and the biochar-trace element dynamics are potentially affected. Ideally, redox potential readings and iron, manganese and other trace elements concentrations should have monitored alongside the proposed method or comparison with aerobic systems should be made in future work.

3.7.3 Analytical methods

The equipment for pH and EC measurement follows that described in section 3.3.2.1. Equally, the analytical determination of metal and nutrient content was done using ICP-OES and ion chromatography respectively according to the methods outlined in section 3.3.2.5 and 3.3.2.6.

3.8 Methods for hypotheses 4 and 5

3.8.1 Creation of LMWOA-activated biochar

For hypotheses 4 and 5b, LMWOA-activated biochar was used in the experiments. LMWOA-activation of the biochar materials was applied to simulate biochar that had been aged in rhizospheric soils. Briefly, 100 g of RH700 biochar was mixed with an equimolar solution of 0.01 mol L^{-1} solution containing citric, malic or oxalic acid in a 1:10 w/v mixture. The slurry was mixed in a 1 L glass beaker with a magnetic stir bar, on full power for 2 h and then extracted under vacuum using a Buchner funnel and Whatman 54 filter paper. The resulting biochar was then oven-dried for overnight at 100 ^{o}C. Following drying, the biochar was stored in a 250 mL glass Kilner jar until use.

3.8.2 Experimental designs

3.8.2.1 Germination and growth parameter assay

For hypothesis 4, germination and plant growth assays were carried out. These took place in the greenhouse in Newton Building, University of Salford, United Kingdom, during February 2019. Plastic seedling trays (L 37 cm x W 24 cm x D 5.5 cm) containing fifteen cells were used to plant in. Each cell had one Whatman 1 filter paper placed at the bottom to stop the substrate from escaping. In a laboratory setting, each cell was filled with 100 g of composite soil (see section 3.4.4) that had either no biochar, 1% w/w biochar (unactivated) or 1 % w/w activated biochar. An experimental design is provided in Table 3.10 and a schematic of the cell set up is shown in Figure 3.4.

Table 3.10 Details of the experimental design used for the hypotheses four and five, to test if biochar and LMWOA-activated biochar will improve the chosen growth parameters and affect trace element uptake by pea plants

Treatment	**Weight (g)**	**Biochar (g)**	**Activated biochar (g)**
Contaminated soil	100	0	0
Contaminated soil with 1% biochar	100	1	0
Contaminated soil 1% activated biochar	100	0	1

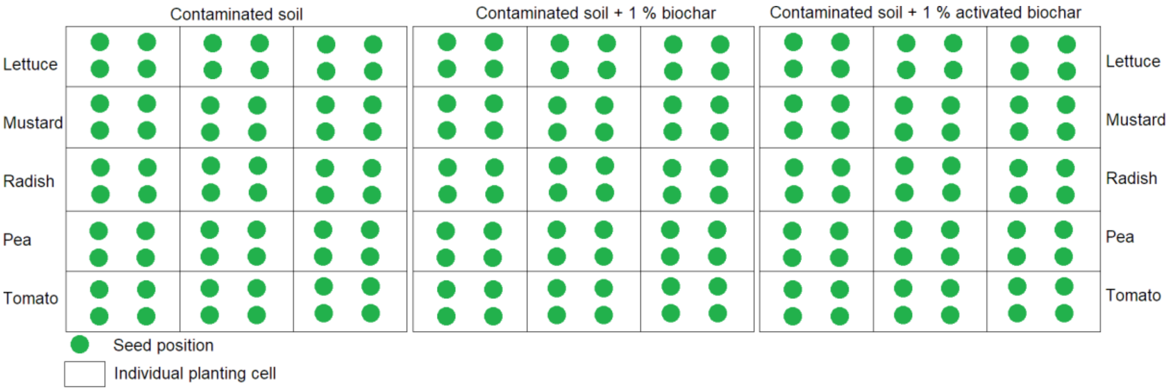

Figure 3.4 A schematic diagram of the germination and early growth traits study showing the seed placement of the five different plant species. Each planting tray is denoted by a rectangle made up of 15 cells. The seed position is shown as a circle.

Trays were then transferred to the greenhouse where the average temperature was approximately 15 ^{o}C with a daily photoperiod of 10 h. Trays were sprayed with water and then left for three days. After the settling period, four seeds of each plant species were planted 0.5 cm deep and then lightly covered with soil. Cells were then watered and left. Watering took place every day in the first week and every other day in the second week. After seven days, a germination assay was carried out visually counting the emerging seedlings. Seeds were reported as germinated when there was visible radicle protrusion through the seed coat (Luo et al., 2018). The number of germinated seeds was expressed as the percentage germination rate, calculated, as shown in Equation 3.9.

$$\boldsymbol{Germination\ rate}\ (\%) = \frac{\boldsymbol{Seeds\ germinated}}{\boldsymbol{Total\ number\ of\ seeds\ planted}}\ \boldsymbol{x\ 100}$$

Equation 3.9 Determination of the seed germination rate (%)

After a further seven days, the plants were harvested, and the plant parameters were recorded. Fresh biomass, plant root length and plant shoot height were measured. Fresh weight included the plant root and shoot in its entirety. Although measurements of the root and shoot would be a better indicator of toxicity because of the different plant species used and the lack of root length in some species, the decision to report whole weight was taken. Plant root measurements were taken from the root-shoot junction to the tip of the longest primary plant root (Liu, Zhang, Shan, & Zhu, 2005). The shoot measurements were from the base of the plant shoot to the uppermost leaf tip. All length and height measurements were recorded in millimetre using a Magnusson digital Vernier calliper (150 mm) that was calibrated after each cell to ensure accuracy. Each plant was washed using tap water and patted dry with a paper towel before measurement to ensure no soil was remaining on the plant.

3.8.2.2 Trace element uptake experiment

3.8.2.2.1 Growth parameter measurements

As the pea plants showed better development during the earlier growth assay compared to the other plant species, they were selected for use in further study. For hypothesis 5, a greenhouse pot trial was carried out to analyse the effect of biochar and LMWOA-activated biochar on trace element content in pea plants. The study was undertaken at the greenhouse in Newton Building, University of Salford, during spring 2019. Using the composite soil described in section 3.4.4, the cells were filled with soil as described in section 3.8.2.1. Then, cells each had one seed added, each totalling fifteen seeds in each tray, as shown in Figure 3.5. The different treatments and controls are shown in Table 3.10.

During the experiment, the cells were watered to maintain soil moisture to a level close to field capacity. After one month, plants were harvested. Plants were then carefully removed from each cell and washed in tap water to remove soil and separated into the root and shoot portions using stainless steel scissors. After blotting dry with a paper towel, each plant portion was weighed to determine fresh biomass. Weight, length and height measurements were taken as previously described (section 3.8.2.1). Plant tissues were then dried to a constant weight at 65 ^{o}C for around 72 hours (Nworie et al., 2019). Samples were then removed and placed in a desiccator to allow for cooling then were weighed to determine dry biomass (g).

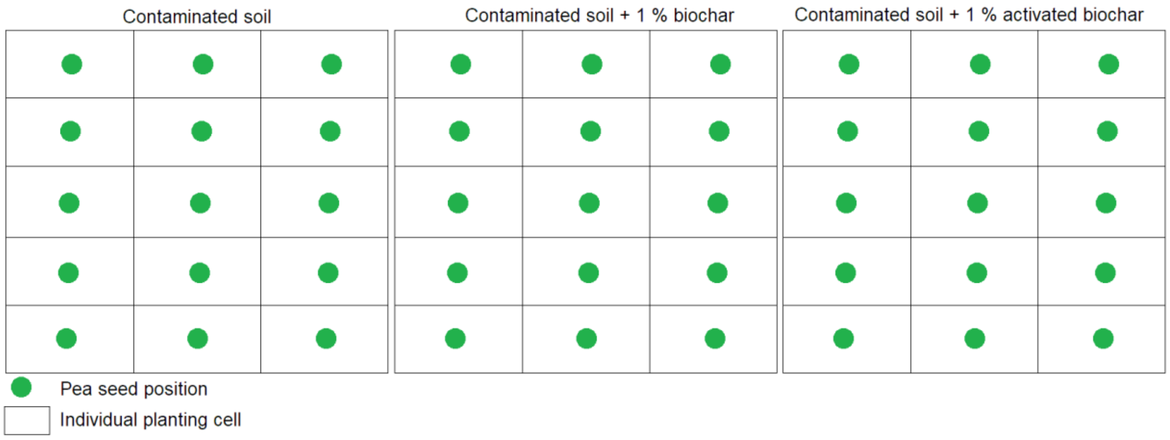

Figure 3.5 Schematic diagram of the pea growth and trace element uptake study showing the placement of each pea seed. Each planting tray is denoted by a rectangle made up of 15 cells. The pea seed position is shown as a circle.

3.8.2.2.2 Chlorophyll content in pea leaves

Chlorophyll content in the plant leaves grown in different treatments was determined according to the method by Lichtenthaler (1987). Following the plant harvest, 0.2 g of fresh pea leaves were accurately weighed out into a 25 mL centrifuge tube and mixed with 15 mL of 80 % acetone. The tubes were placed in a fridge (< 4 ^{o}C) overnight. Next, the samples were centrifuged at 5000 rpm for 15 minutes. The supernatants were decanted into polystyrene cuvettes in preparation for spectrophotometric measurement. The cuvettes had an absorbance range of 285 to 740 nm and a holding capacity of 4.5 mL. The ultraviolet-visible spectrophotometer was calibrated using an 80 % acetone blank. Measurements for chlorophyll a (Equation 3.10) and chlorophyll b (Equation 3.11) were taken at 645 nm and 663 nm, respectively. Total chlorophyll was obtained by the addition of both values (Equation 3.12). In Equations 3.10 and 3.11, *A* refers to the wavelength used for determination.

$$\boldsymbol{Chlorophyll\ a = 12.25\ (A_{663}) - 2.79(A_{645})}$$

Equation 3.10 Calculation for chlorophyll a content in the pea leaves

$$\boldsymbol{Chlorophyll\ b = 21.50\ (A_{645}) - 5.1(A_{663})}$$

Equation 3.11 Calculation for chlorophyll b content in the pea leaves

$$\boldsymbol{Total\ chlorophyll\ content = Chlorophyll\ a + \ Chlorophyll\ b}$$

Equation 3.12 Calculation for total chlorophyll content in the pea leaves

3.8.2.2.3 Plant tissue trace element determination

Plant tissues were dried to a constant weight at 65 °C for around 72 hours (Nworie et al., 2019). Samples were then removed and placed in a desiccator to allow for cooling then were weighed to determine dry biomass (g). Following the drying procedure, the plant material was ground into a fine powder using a mortar and pestle. For the plant digestion procedure, 0.2 g of plant powder was weighed into 55 mL MARSXpress Teflon microwave digestion vessels. Then 7 mL 65 % nitric acid and 2 mL hydrogen peroxide were added to mineralise the sample. All tubes were capped using a Teflon plug, sealed with a vessel cap and placed into the CEM MARSXpress microwave digester. The digestion procedure was carried out in four stages. The first stage hated the samples from 25 °C to 90 °C over 4 minutes, followed by a maintenance of 90 °C over 2 minutes. Then a further increase to 180 °C over 2 minutes until a final digestion temperature of 180 °C was reached for 10 minutes (Nworie et al., 2019). After samples were left to cool and the digested sample was filtered using a 0.22 µm nylon syringe filter into a 50 mL volumetric flask. Samples were then made up to the final volume using ultrapure water. All filtered samples were stored in the fridge at < 4 °C before trace element analysis using ICP-OES.

3.8.2.2.4 Post-experiment soil analysis

After the plants were harvested, soil samples were taken to ascertain the effect of biochar amendment on trace element content in vegetated soils. A stainless-steel spatula was used to remove 1 g of material from each cell. Every five cells were combined as a composite. Samples were subjected to tests for total metal content and 0.01 mol L^{-1} calcium chloride extractions. For total metal content, a nitric acid digestion was used. Briefly, 0.5 g of soil was weighed into 55 mL MARSXpress Teflon microwave digestion vessels. Next, 10 mL of 65% nitric acid was added before samples were digested in a CEM MARSXpress microwave digester. After digestion, samples were filtered through a 0.22 µm syringe filter and diluted for analysis via ICP-OES. After analysis, values were attained following the application of the dilution factor calculations. Bioavailable soil-borne trace elements were assessed via a 0.01 mol L^{-1} calcium chloride extraction like that described in section 3.3.2.5. Samples were stored overnight in a fridge (< 4 ^{o}C) before analysis by ICP-OES the following day.

3.8.3 Analytical methods

Trace element analysis in the plant tissues and post-experiment soils using ICP-OES followed that as described in section 3.3.2.5.

3.8.4 Methodological limitations

The greenhouse studies were limited by several factors. The reader should bear in mind that the trace element uptake findings were gathered for only one plant type (*Pisum sativum*) and one biochar feedstock (rice husk). Therefore, the applicability of these results to other plant species or soils amended with different biochar feedstock is limited because of the heterogeneous nature of different biochar and the complex soil-plant-biochar interactions. Secondly, due to practical constraints, this thesis cannot provide a comprehensive review of growth parameters and trace element uptake in the studied plant species due to a limited growth period (2 week and 1 month, respectively) due to time constraints. It is likely that with a longer growth period, a more exacerbated effect may be seen with regards to biochar efficacy to reduce plant trace element uptake and provide more significant changes in growth parameters. Next, no LMWOAs were measured in the soils for the greenhouse study. Previous studies have measured the changes in LMWOAs in greenhouse studies (Fu, Yu, Li, & Zhang, 2019; Zhan, Li, Zhang, Yu, & Zhao, 2018). This may affect the number of trace elements mobilised within soils and therefore help elucidate the behaviour between biochar and LMWOA in soil systems. Lastly, it must be noted that when comparing the findings of hypotheses 4 and 5 to those of hypothesis 3, care must be taken. The soils used for these experiments were from a different site as those used in for hypothesis 3. It is likely the organic matter contents were different and as such the biochar-trace elements dynamics may differ.

3.9 QA/QC and statistical analysis

For quality assurance and control, all experiments used chemical reagents of analytical grade and deionised water (18.2 MΩ/cm). All analysis was performed in triplicates and results are presented as mean values ± standard error of the mean, unless stated otherwise. One-way analysis of variance (ANOVA) was used to test if a statistically significant difference existed between the mean values. For all the characterisation of biochar materials, biochar type was the independent factor. For the batch experiments used to examine hypotheses 1, 2 and 3, in each experiment, two separate ANOVA tests were performed for the different dependent variables (pH, EC, metal concentration or nutrient concentration). Firstly, an analysis was run with treatment as the independent factor for each of the time intervals. Then a second analysis was run with time (1 h, 24 h, 72 h and 120 h) for each variable. Further to the one-way ANOVAs, separate Pearson's correlation coefficient analysis (bivariate correlation) was run to examine correlations between solution pH and the different metal and nutrient concentrations in solution. However, as the solution pH was not buffered and is extremely low (see section 3.6.5), the results of the correlation coefficient analysis should be interpreted with caution. For hypotheses 4 and 5, one-way ANOVAs were performed for each plant type. The dependent variables were the growth parameters and the individual trace element concentrations. Treatment was always the independent factor.

For every ANOVA completed, Duncan's multiple range tests were used to determine the statistical significance ($p < 0.05$) between the different factors. Statistical significance was denoted as the difference between letters. All graphs were plotted using Origin Lab 2019 Student edition and all statistical analysis was done using IBM SPSS version 24.

3.10 Summary

In this chapter, the research philosophy and methods adopted to meet the aim of this thesis have been outlined. In summary, the research presented in this thesis has adopted ontological objectivism and epistemological positivism research philosophy to test each hypothesis (section 3.2). Before testing the hypotheses outlined in Chapter 2, all the UKBRC biochar materials were characterised (section 3.3), the soil was characterised (section 3.4) and the plants used in the research were described (section 3.5). To see whether LMWOAs will inhibit the biochar-driven immobilisation of cadmium, lead and zinc but facilitate the biochar-driven immobilisation of nitrate and phosphate in aqueous systems, a combination of batch sorption tests were conducted (section 3.6). Secondly, to test if biochar will reduce the LMWOA-driven solubilisation of cadmium, zinc, nitrate and phosphate in soils, further batch sorption tests were applied (section 3.7). Next, a series of growth assays were outlined to examine whether biochar and LMWOA-activated biochar will affect certain growth parameters of different plant species (section 3.8). Finally, a greenhouse study was conducted to examine whether biochar will reduce trace element uptake by a selected plant species and whether LMWOA-activated biochar will have no effect on trace element uptake by a selected plant species (section 3.8). The following chapter will present the results of the biochar characterisation and the results for each hypothesis.

CHAPTER 4

Results

4.1 Overview

In the previous chapter, the ontological and epistemological approach (section 3.2) and the various experimental methods adopted to test each hypothesis were outlined (section 3.3 to section 3.9). In the current chapter, the research results are presented. Firstly, the physical and chemical properties of the different UKBRC biochar are reported (section 4.2). Then, the results of each experiment used to test each hypothesis are reported (section 4.3 to section 4.7). The chapter is summarised by highlighting key findings (section 4.8).

4.2 Biochar characterisation

4.2.1 Biochar pH and electrical conductivity

Table 4.1 presents the biochar pH results obtained from the 1:10 w/v extraction. It is apparent from the data that the pH values for all twelve biochar materials were alkaline ($pH > 7$) ranging from 7.58 to 11.17. The wheat straw derived biochar (WSP550 and WSP700), oil seed rape straw-derived biochar (OSR550 and OSR700) and the miscanthus straw biochar materials (MSP550 and MSP700) all showed a decreased pH with increased production temperature. However, these were not statistically significant ($p > 0.05$). For rice husk-derived biochar (RH550 and RH700), softwood pellet-derived biochar (SWP550 and SWP700) and sewage sludge biochar (SS550 and SS700) an increase in pH was observed with increased production temperature. Although, statistical significance was only observed for SWP and SS materials, respectively ($p < 0.05$). Notably, the pH for softwood pellet and sewage sludge-derived chars was much lower than the other biochar materials (< 10).

Table 4.1 pH values and electrical conductivity values (mS cm^{-1}) (1:10 w/v %) for the twelve UKBRC standard biochar materials after 1 hour contact time

Biochar	**pH**	**Electrical conductivity (mS cm^{-1})**
MSP550	10.94±0.02ab	0.97±0.05c
MSP700	10.48±0.06bc	2.82±0.38c
OSR550	10.71±0.04ac	3.66±0.12c
OSR700	10.25±0.54c	4.56±1.14c
RH550	10.97±0.06ab	0.55±0.06c
RH700	11.17±0.10a	0.58±0.06c
SS550	7.58±0.22f	35.60±5.42a
SS700	9.53±0.01d	26.40±4.62b
SWP550	8.44±0.05e	0.73±0.39c
SWP700	9.30±0.05d	1.80±1.01c
WSP550	11.14±0.06a	1.39±0.52c
WSP700	10.89±0.10ab	3.96±0.31c

All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The EC values for the biochar materials ranged from 0.55 to 35.60 mS cm^{-1} (Table 4.1). EC showed large variation between plant, wood and sewage sludge-derived biochar. However, in all instances, a difference with pyrolysis temperature was observed. For SS550 and SS700, EC significantly decreased with pyrolysis temperature ($p < 0.05$). In contrast, the inverse was true for the remaining biochar, which had much smaller EC values. However, the difference observed for the other biochar materials was not significant at the $p < 0.05$ level.

4.2.2 Redox potential

Redox potential results for all biochar samples are presented in Figure 4.1. All the biochar extracts had very low redox potentials ranging from -251.2 to 115.93 mV, showing that the biochar materials have a strong reduction potential. For the miscanthus straw-derived biochar (MSP550 and MSP700), oil seed rape straw-derived biochar (OSR550 and OSR700) and the softwood derived-biochar (SWP550 and SWP700), the redox potential significantly increased with production temperature ($p < 0.05$). For rice husk-derived biochar (RH550 and RH700) and wheat straw-derived biochar (WSP500 and WSP700) redox potential significantly decreased as production temperature increased ($p < 0.05$). There was no difference observed between values reported for sewage sludge-derived biochar (SS550 and SS700) ($p > 0.05$).

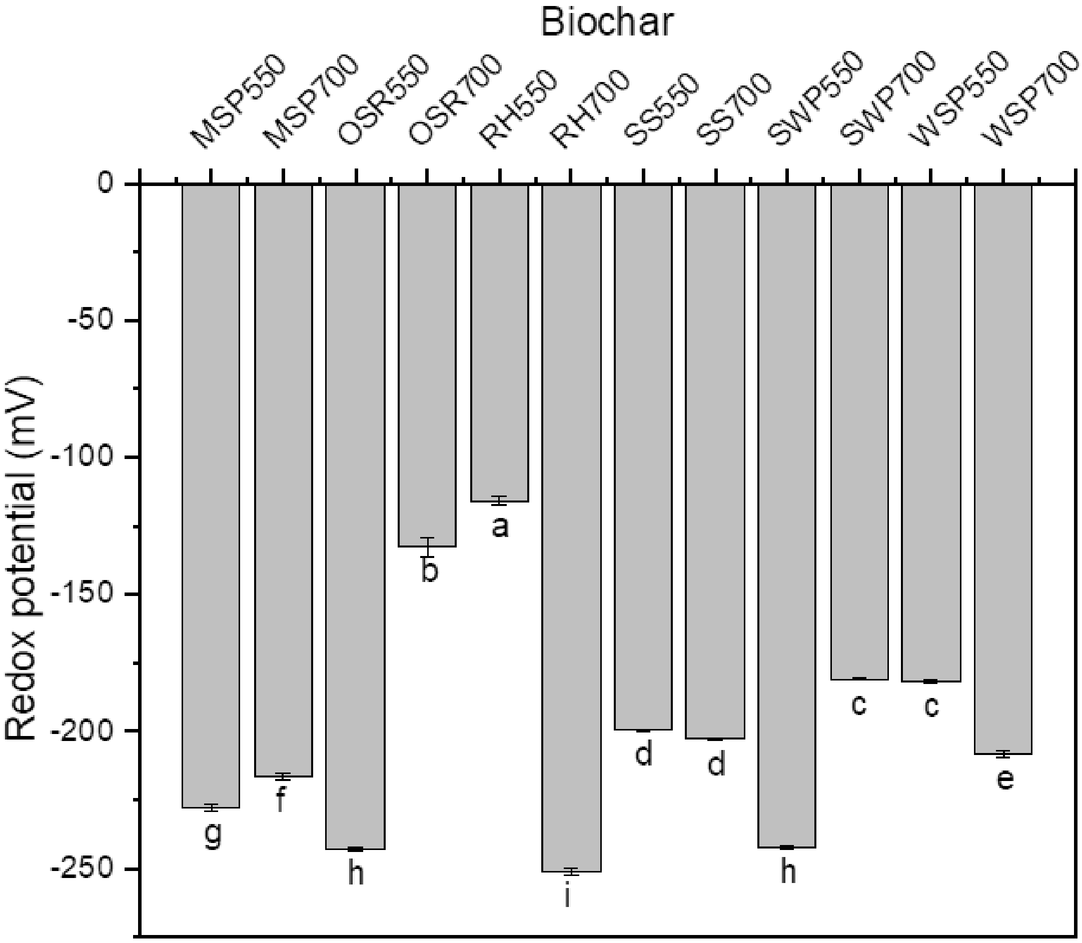

Figure 4.1 Redox potential values (mV) (1:10 w/v %) for the twelve UKBRC standard biochar materials determined after 1 hour of contact time. All values are presented as the mean ± standard error ($n = 3$). Statistical significance is denoted by different lowercase letters below each error bar, according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

4.2.3 Point of zero charge values

The results for the point of zero charge characterisation are shown in Table 4.2 and values range from 7.76 to 11.33. The point of zero charge increased with production temperature.

The data suggests that when all the twelve biochar materials are in environmental mediums with a pH > 7.76, the surface net charge of the materials is negative and when pH < 7.76 the surface net charge is positive.

Table 4.2 Point of zero charge values for all twelve UKBRC standard biochar materials

Biochar	**Point of zero charge**
MSP550	8.63
MSP700	10.01
OSR550	10.79
OSR700	11.09
RH550	7.96
RH700	8.59
SS550	7.79
SS700	8.44
SWP550	7.76
SWP700	8.32
WSP550	9.20
WSP700	11.33

4.2.4 Biochar surface functionality

Functional group analysis of the twelve biochar was carried using FTIR spectroscopy. A comparison of the assigned functional groups for each biochar is offered in Table 4.3, and the spectra are presented in Figure 4.2. Mainly, the biochar materials created at 550 °C displayed greater functionality than those created at 700 °C. The peaks between 1500 to 1580 cm^{-1} corresponded to aromatic C=C stretching and were identified for MSP500, OSR550, RH550 and SWP550 biochar. The peak from 1035 to 1080 cm^{-1} was assigned to C-O-C stretching vibrations of hemicellulose and cellulose. This was observed for all biochar except, softwood and sewage sludge derived-biochar materials. The broad peak identified at 1000 to 1020 cm^{-1} corresponded to aliphatic ether C-O and was assigned to sewage sludge-derived biochar. Lastly, small vibrations at 700 to 900 cm^{-1} were observed for MSP550, MSP700, OSR550, RH550 and SWP550 biochar. These are likely ascribed to aromatic C-H bonding.

Table 4.3 A summary table of the functional group assignments identified for the twelve UKBRC standard biochar materials

Associated Wavelength (cm^{-1})	**Functional group assignment**	**MSP550**	**MSP700**	**OSR550**	**OSR700**	**RH550**	**RH700**	**SS550**	**SS700**	**SWP550**	**SWP700**	**WSP550**	**WSP700**
700-900	C-H [ab]	√	√	√	-	√	-	-	-	√	-	-	-
1000-1020	C-O [c]	-	-	-	-	-	-	√	√	-	-	-	-
1035-1080	C-O-C [ab]	√	√	√	√	√	√	-	-	-	-	√	√
1500-1580	C=C [b]	√	-	√		√	-	-	-	√	-		

[a] Shen, Zhang, Jin, Alessi et al. (2018); [b] Keiluweit et al. (2010); [c] Özçimen and Ersoy-Meriçboyu (2010).

√ : denotes corresponding functional group was identified for this biochar material; -: denotes an unidentifiable peak.

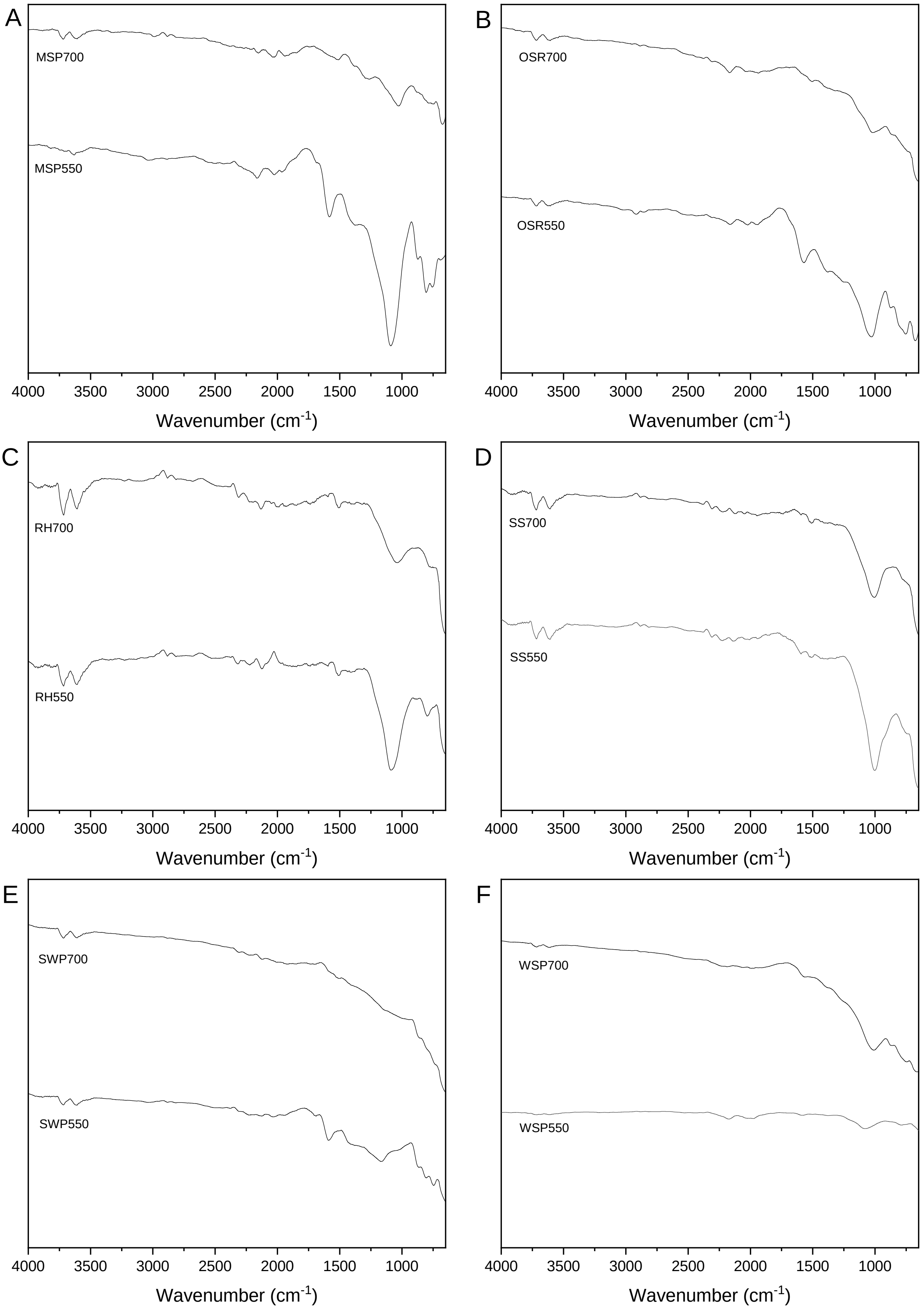

Figure 4.2 FTIR spectra for (A) MSP-derived biochar, (B) OSR-derived biochar, (C) RH-derived biochar, (D) SS-derived biochar, (E) SWP-derived biochar and (F) WSP-derived biochar.

4.2.5 Biochar surface morphology

SEM imagery of the UKBRC standard biochar materials was taken to observe the surface morphology (Figure 4.3 and Figure 4.4). The surface morphology of miscanthus straw-derived biochar revealed a macroporous and mesoporous structure for MSP550 and more so for MSP700, with a visible development of micropore networks within the mesopores (Figure 4.3). This supported the BET surface area values supplied by the UKBRC that show MSP550 to have a surface area of 33.6 m^2 g^{-1} compared to a slightly larger surface area for MSP700 of 37.2 m^2 g^{-1} (Appendix A3). The porous surface as previously described for the miscanthus straw-derived biochar materials is not as abundant in the oil seed rape-derived biochar materials (Figure 4.3) and these materials have a lower surface area in comparison, 7.3 m^2 g^{-1} (for OSR550) and 25.2 m^2 g^{-1} (for OSR700) (Appendix A3). However, there is a discernible difference between OSR550 and OSR700 SEM imagery (Figure 4.3). With increased temperature, OSR700 appears to have increased surface lamination and thinning of the pore walls compared to the relatively thick pore walls shown for OSR550. Both rice husk-derived biochar have a flaky surface with microparticles present and a clear pore structure (Figure 4.3). Notably, for RH700, the presence of cylinder-like tube structures are visible to the left of the image and the pore network is more diverse in size compared to that pictured for RH550. The increased pore distribution seen in RH700 compared to RH550 is supported by the increased BET surface area from 20.1 m^2 g^{-1} for RH550 to 42.0 m^2 g^{-1} for RH700 (Appendix A3).

Sewage sludge-derived biochar also had a flaky structure like that of RH550 and RH700, with numerous microparticles present (Figure 4.4). No precise pore distribution could be seen. For SS550, a small area of pores exists central in the image, and for SS700, a lateral band of pores is present. There were no significant observable morphological differences between the two images and the manufacturer provided no BET surface area information. In sharp contrast to sewage sludge-derived biochar, the surface morphological changes between SWP550 and SWP700 are visible. Regions of a regular honeycomb-like structure were clear for SWP700 compared to a more irregular folded structure for SWP550 (Figure 4.4). For the softwood-derived biochar, higher magnifications showed thinning of cell walls as pyrolysis temperature increased and the formation of meso- and micropores within the macropores relating to the observed BET surface area increase from 26.4 m^2 g^{-1} for SWP550 to 123.6 m^2 g^{-1} for SWP700. SWP700 also showed a glossier and laminated surface compared to SWP550. Finally, WSP550 appears to have a well-defined porous structure with thin pore walls and few microparticles (Figure 4.4). On some of the pore walls pictured, smaller pores are visibly forming. For WSP700, the pore network is irregular and the surface looks rough with evidence of folding (Figure 4.4.). Unlike the other biochar materials examined, these changes appear to be in reverse in response to the increased production temperature. These were also supported by the BET surface area measurements for WSP550 (26.4 m^2 g^{-1}) which were higher than WSP700 (23.2 m^2 g^{-1}) (Appendix A3).

In summary, the SEM imagery confirms the heterogeneous nature of different biochar materials. No two images were identical. Most of the biochar images showed pore networks that were more defined at a higher temperature. Furthermore, the presence of microparticles and cylinder structures were frequent amongst the studied materials. However, the SEM imagery only observes one point on the biochar surface. Furthermore, the observations are subjective so can only provide a qualitative approach to biochar characterisation and must be compared with the other characterisation methods applied in this chapter.

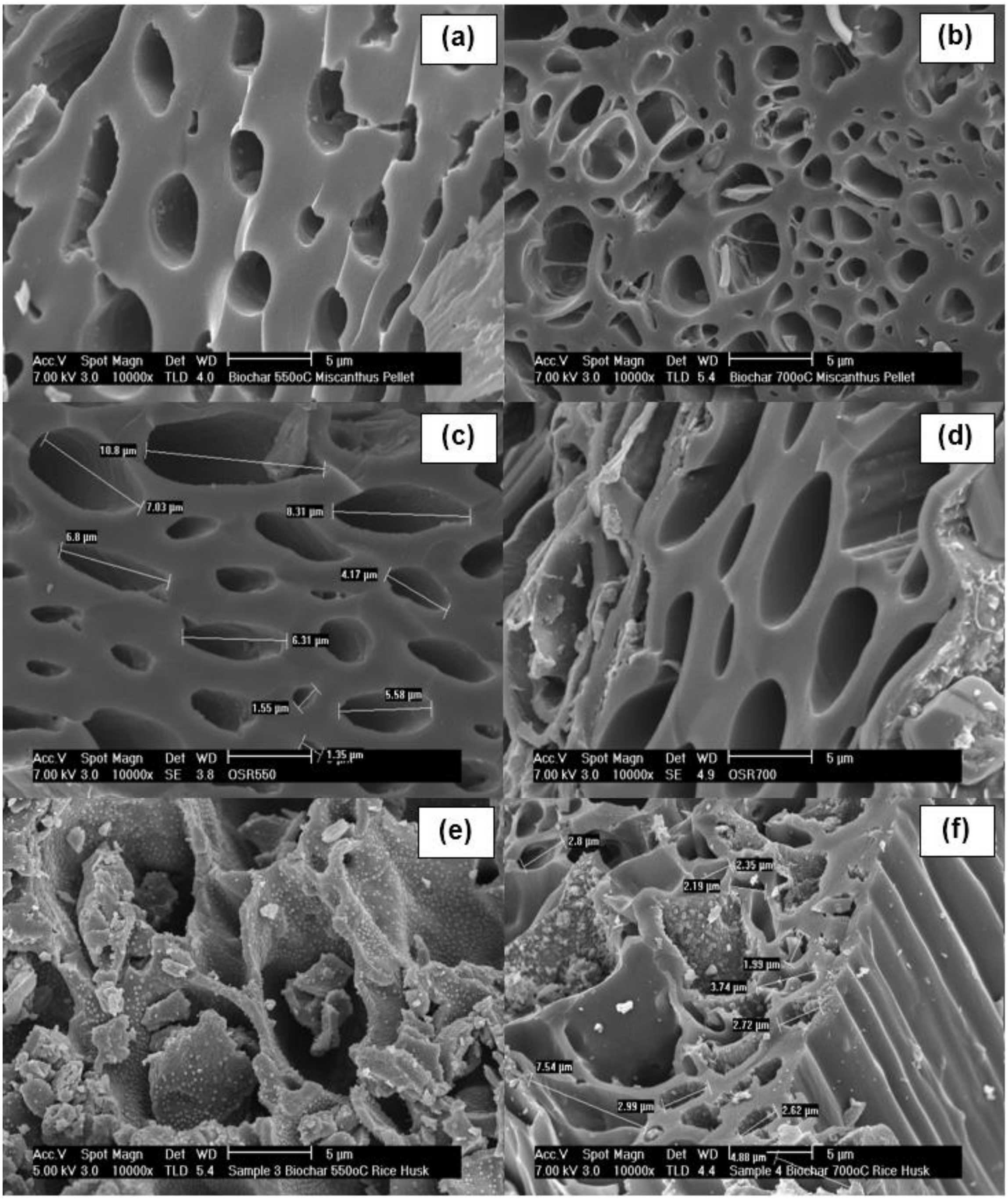

Figure 4.3 Scanning electron microscopy results for (a) MSP550, (b) MSP700, (c) OSR550, (d) OSR700, (e) RH550 and (f) RH700 observed at 10,000-x magnification.

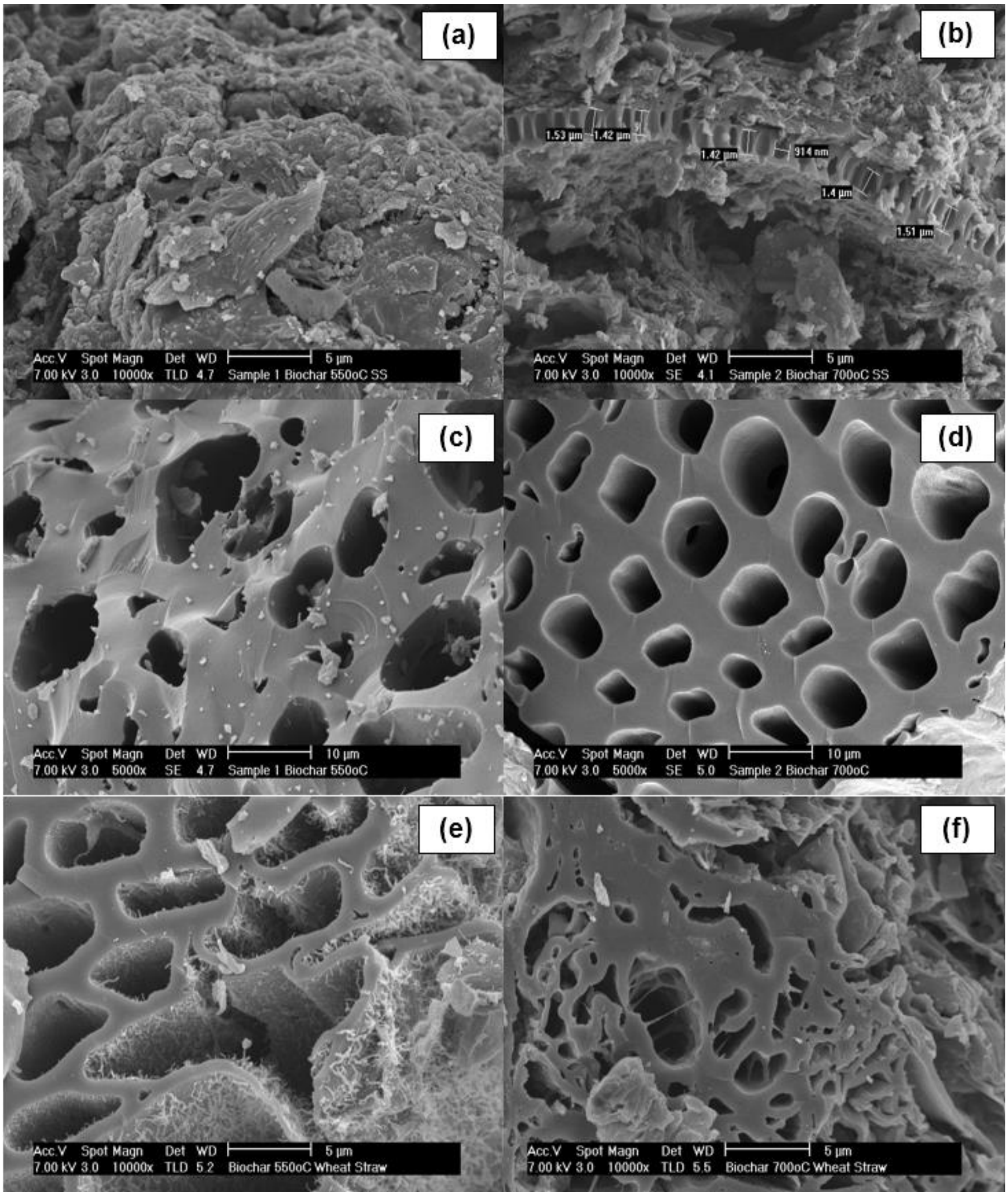

Figure 4.4 Scanning electron microscopy results for (a) SS550, (b) SS700, (c) SWP550, (d) SWP700, (e) WSP550 and (f) WSP700. The biochar were observed at either 10,000-x magnification or 5000-x magnification for SWP-derived materials.

4.2.6 Total metal content

Table 4.4 compares the mean total metal content for the UKBRC standard biochar materials, obtained by XRF analysis. Total cadmium and mercury values were below the limit of detection in all the biochar materials. Mean total arsenic concentrations were below the limit of detection for all biochar materials except for SS550 and SS700. Although total arsenic concentration was slightly elevated for SS500 compared to SS700, the result was not statistically significant ($p > 0.05$). Similarly, to arsenic, chromium concentrations were not detected in any biochar material except for SS550 and SS700. Concentrations were 471.54 mg kg^{-1} and 477.05 mg kg^{-1}, respectively. There was no significant difference between the chromium concentrations in both biochar materials ($p > 0.05$). Copper was detected in all biochar materials except for RH700. Values ranged from 5.41 mg kg^{-1} to 343.94 mg kg^{-1}. SS550 and SS700 contained the highest amounts of detected copper, which were significantly different from the other biochar materials ($p < 0.05$). For MSP550, MSP700, OSR550, OSR700, RH550, RH700, WSP550 and WSP700, copper concentrations tended to decrease with increased production temperature. However, for SS550, SS700, SWP550 and SWP700, the inverse was true.

Mean total lead concentrations were only detected for MSP500, RH700, SS550 and SS700, ranging from 1.38 mg kg^{-1} to 242.26 mg kg^{-1}. Once again, SS550 and SS700 were highest in concentration, which increased with pyrolysis temperature. Total molybdenum concentrations ranged from 4.30 mg kg^{-1} to 9.99 mg kg^{-1}. Interestingly, WSP700 had the highest total molybdenum, which was significantly higher than WSP550 (5.56 mg kg^{-1}) ($p < 0.05$). SS550 and SS700 also had high molybdenum concentrations but no significant difference was observed with increased production ($p > 0.05$). For the remaining biochar materials, no statistical difference in copper concentrations were observed with increased production temperature. For total nickel and selenium, values were below the limits of detection for all biochar except SS550 and SS700. Total nickel and selenium both appeared to decrease with production temperature; however, statistical significance was only observed for selenium ($p < 0.05$). Total zinc was detected in all biochar materials ranging from 31.70 mg kg^{-1} to 1151.05 mg kg^{-1} and SS550 and SS700 contained the most substantial amount of total zinc. MSP550 also had a relatively high amount of total zinc (204.04 mg kg^{-1}), which was significantly higher than that found for MSP700 ($p < 0.05$). For the remaining biochar materials, no statistically significant change in concentration was observed with increased production temperatures ($p > 0.05$).

In brief, the biochar materials appeared to contain varying metal contents with both feedstock material and pyrolysis temperature. Total zinc, molybdenum and Cu were most prevalent in all biochar materials. In contrast, the biochar materials did unanimously contain no detectable cadmium or mercury. Interestingly only SS550 and SS700 contained detectable concentrations of arsenic, chromium, nickel and selenium, compared to the other ten biochar studied.

Table 4.4 Mean total arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) content (mg kg^{-1}) for the twelve UKBRC standard biochar analysed by XRF.

Biochar	**Total metal content (mg kg^{-1})**									
	As	**Cd**	**Cr**	**Cu**	**Pb**	**Hg**	**Mo**	**Ni**	**Se**	**Zn**
MSP550	<LOD[1]	<LOD	<LOD	79.22±36.32b	2.22±1.22b	<LOD	6.70±0.17cd	<LOD	<LOD	204.04±19.74b
MSP700	<LOD	<LOD	<LOD	12.12± 1.52b	<LOD	<LOD	6.28±0.11de	<LOD	<LOD	88.31± 0.12c
OSR550	<LOD	<LOD	<LOD	12.86± 0.46b	<LOD	<LOD	4.59±0.50f	<LOD	<LOD	25.85± 0.40d
OSR700	<LOD	<LOD	<LOD	9.70± 0.33b	<LOD	<LOD	4.45±0.36f	<LOD	<LOD	26.60± 0.78d
RH550	<LOD	<LOD	<LOD	20.06± 1.51b	<LOD	<LOD	4.30±0.35f	<LOD	<LOD	68.09±4.78cd
RH700	<LOD	<LOD	<LOD	<LOD	1.38±0.69c	<LOD	4.61±0.29f	<LOD	<LOD	66.63±2.01cd
SS550	19.67±0.99a	<LOD	471.35±13.50a	339.57± 8.79a	234.90±5.75a	<LOD	8.52±0.11b	88.18±8.29a	1.56±1.56a	1110.89±34.20a
SS700	17.96±2.31a	<LOD	477.05±5.82a	343.94± 9.20a	242.26±9.68a	<LOD	8.57±0.52b	84.03±7.01a	1.06±1.06a	1151.05±46.02a
SWP550	<LOD	<LOD	<LOD	11.67± 1.18b	<LOD	<LOD	6.65±0.18cd	<LOD	<LOD	51.16±1.57cd
SWP700	<LOD	<LOD	<LOD	15.82± 0.29b	<LOD	<LOD	7.26±0.20c	<LOD	<LOD	38.33±0.54cd
WSP550	<LOD	<LOD	<LOD	7.80±0.77b	<LOD	<LOD	5.56±0.15e	<LOD	<LOD	31.70±0.71cd
WSP700	<LOD	<LOD	<LOD	5.41±2.77b	<LOD	<LOD	9.99±0.14a	<LOD	<LOD	74.34±0.86cd

[1] <LOD denotes a concentration less than the limit of detection by XRF analysis.

All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

4.2.7 Calcium chloride-extractable biochar-borne metals

The 0.01 mol L^{-1} calcium chloride-extractable biochar-borne metal results are presented in Table 4.5. Calcium chloride-extractable boron ranged from 0.23 mg kg^{-1} to 3.76 mg kg^{-1}. For MSP550, MSP700, OSR550, OSR700, SS550 and SS700, calcium chloride-extractable boron significantly increased with pyrolysis temperature ($p < 0.05$). However, for RH550, RH700, SWP550 and SWP700, calcium chloride-extractable boron significantly decreased with pyrolysis temperature ($p < 0.05$). No difference was observed between the extractable boron released from WSP550 and WSP700 ($p > 0.05$). Calcium chloride-extractable magnesium ranged from 1.85 mg kg^{-1} to 125.32 mg kg^{-1}. For MSP550, MSP700, RH550, RH700, SS550, SS700, WSP550 and WSP700, a statistically significant increase in extractable magnesium was observed with increased pyrolysis temperature, most notably for SS550 and SS700 (19.37 mg kg^{-1} compared to 125.32 mg kg^{-1}) ($p < 0.05$). For OSR550 and OSR700, extractable magnesium significantly decreased with production temperature increase ($p < 0.05$) and no noticeable difference in concentration was found between SWP550 and SWP700 ($p > 0.05$). Only SS550, SWP550 and SWP700 released manganese into solution and all values were similar (3.55 mg kg^{-1}, 3.40 mg kg^{-1} and 3.23 mg kg^{-1}, respectively). Although the values were similar for SWP550 and SWP700, a statistically significant decrease in extractable manganese was observed ($p < 0.05$). Unlike manganese, extractable sodium was detected for all twelve biochar, ranging from 79.27 mg kg^{-1} to 258.03 mg kg^{-1}. For OSR550, OSR700, SWP550, SWP700, WSP550 and WSP700, an increase in extractable sodium was observed with production temperature ($p < 0.05$). Extractable sodium also increased between MSP550 and MSP700, and RH550 and RH700; however, the post-hoc analysis did not reveal any significant difference ($p > 0.05$). For SS550 and SS700, a decrease in extractable sodium was observed with increased production temperature. However, this was not statistically significant ($p > 0.05$).

Table 4.5 Mean 0.01 mmol L^{-1} calcium chloride-extractable arsenic (As), boron (B), magnesium (Mg), manganese (Mn), sodium (Na), lead (Pb) and zinc (Zn) (mg kg^{-1}) for the twelve UKBRC standard biochar.

Biochar	**0.01 mmol L^{-1} calcium chloride-extractable elements (mg kg^{-1})**						
	As	**B**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
MSP550	0.40±0.02ab	0.39±0.01e	33.04±0.50c	<LOD	79.27±1.39f	1.25±1.04ab	<LOD
MSP700	0.43±0.07a	1.45±0.06c	41.62±0.97b	<LOD	153.70±2.56f	1.11±1.11ab	<LOD
OSR550	<LOD[1]	0.56±0.00d	11.06±0.14f	<LOD	152.11±1.55b	<LOD	0.70±0.44ab
OSR700	0.10±0.02cde	1.39±0.02c	6.76±0.08g	<LOD	154.34±1.43a	<LOD	0.43±0.31ab
RH550	0.30±0.09abcd	1.51±0.03c	12.80±0.40f	<LOD	164.57±4.58ef	<LOD	1.17±0.39ab
RH700	0.01±0.01e	0.70±0.02d	15.84±0.27e	<LOD	243.00±2.10ef	<LOD	1.35±0.42ab
SS550	0.17±0.10abcde	1.68±0.06b	19.37±0.62d	3.55±0.16a	223.61±6.05e	3.66±1.84a	1.44±0.26ab
SS700	0.34±0.08abc	3.76±0.10a	125.32±3.12a	<LOD	147.21±3.15e	0.94±0.55b	1.16±0.65ab
SWP550	0.02±0.02e	0.62±0.02d	1.85±0.04h	3.40±0.04b	102.11±1.05h	<LOD	0.15±0.17ab
SWP700	0.17±0.17bcde	0.23±0.00f	3.63±0.08h	3.23±0.02c	209.54±3.06g	<LOD	1.18±0.40ab
WSP550	0.18±0.12abcde	0.64±0.01d	12.39±0.26f	<LOD	163.72±2.09d	<LOD	1.13±0.65ab
WSP700	0.06±0.06de	0.65±0.05d	22.03±0.81d	<LOD	258.03±7.59c	2.08±1.58ab	1.21±0.45a

[1] <LOD denotes a concentration less than the limit of detection by ICP-OES analysis.
All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different, according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

In terms of calcium chloride-extractable biochar-borne heavy metals, extractable arsenic values ranged from 0.01 mg kg^{-1} to 0.43 mg kg^{-1}. MSP550 and MSP700 cumulatively had more extractable arsenic than other feedstock materials. A statistically significant difference in extractable arsenic was observed between RH550 and RH700 which increased with production temperature ($p < 0.05$). Although the similar trend was seen for other feedstock; MSP, OSR, SS and SWP, no statistical difference was revealed with post-hoc analysis ($p > 0.05$). Extractable lead was only released from MSP550, MSP700, SS550, SS700 and WSP700 with values ranging from 0.94 mg kg^{-1} to 2.08 mg kg^{-1}. Notably, the value significantly increased with production temperature for SS550 and SS700. Finally, all biochar materials released zinc except for those derived from miscanthus straw. Values were ranging from 0.15 mg kg^{-1} to 1.44 mg kg^{-1}. No trends appeared at a higher temperature.

In summary, a release of calcium chloride-extractable metals was observed between different biochar feedstock materials. In most instances, SS550 and SS700 showed elevated element release compared to other feedstock. For some elements, a trend could be observed between increased production temperature and either decreased, or increased element release. However, the data show that biochar-borne bioavailable metals exhibit differential release due to feedstock and production temperatures.

4.2.8 Water-extractable biochar-borne ions

Results for the water-extractable biochar-borne ions are shown in Figure 4.5. Water-extractable fluoride was extracted from all biochar except OSR700. Interestingly, at a lower production temperature, OSR550 showed a much higher fluoride content (798.17 mg kg^{-1}) than all other biochar (4.63 to 57.97 mg kg^{-1}). All biochar exhibited decreased fluoride content with increased production temperature ($p < 0.05$), except for SWP550 and SWP700. Water-extractable chloride was detected for all biochar, notably from MSP700 (1680.77 mg kg^{-1}) and WSP700 (1103.90 mg kg^{-1}) but no broad trend relating to production temperature could be identified. Apart from SWP550 and SWP700, the biochar materials showed a statistically significant change in chloride concentration with changing production temperature ($p < 0.05$). Water-extractable bromide was only extracted from SS550, SS700, OSR550, OSR700 and WSP700 biochar (0.53 to 8.13 mg kg^{-1}). However, a statistically significant difference in concentration between production temperatures was only observed for WSP700 compared to WSP550, for which no bromide was detected ($p < 0.05$).

Water-extractable nitrate was not detected from SWP550, SWP700, MSP550, OSR550 and OSR700 but was detected for the remaining biochar materials. Generally, increased production temperature caused increase nitrate extraction, however, this was only statistically significant for MSP550 and MSP700 ($p < 0.05$). Water-extractable phosphate was detected for all biochar (8.60 to 281.43 mg kg^{-1}). For MSP550, MSP700, RH550 and RH700, phosphate concentrations increased with production temperature ($p < 0.05$). Interestingly, for OSR550, OSR700, SS550, SS700, WSP550 and WSP700, the opposite trend was observed; extracted phosphate decreased with production temperature, yet none of these differences were statistically significant. Finally, water-extractable sulphate was detected for all biochar (5.30 to 981.57 mg kg^{-1}). Mean sulphate concentrations were found to decrease with production temperature for all biochar materials, except for WSP550 and WSP700 ($p < 0.05$). For WSP550 and WSP700, mean sulphate increase with production temperature ($p < 0.05$).

In summary, the water extraction of biochar-borne anions confirmed the differential release of anions from the different biochar materials. Although it was observed that in some instances this was influenced by production temperature, a generalisation of the trends observed for all chars cannot be made. What is clear is that the UKBRC standard biochar materials may have the potential to increase the water-soluble anions in soils, mainly regarding chloride, phosphate and sulphate. This may have implications for application to soils.

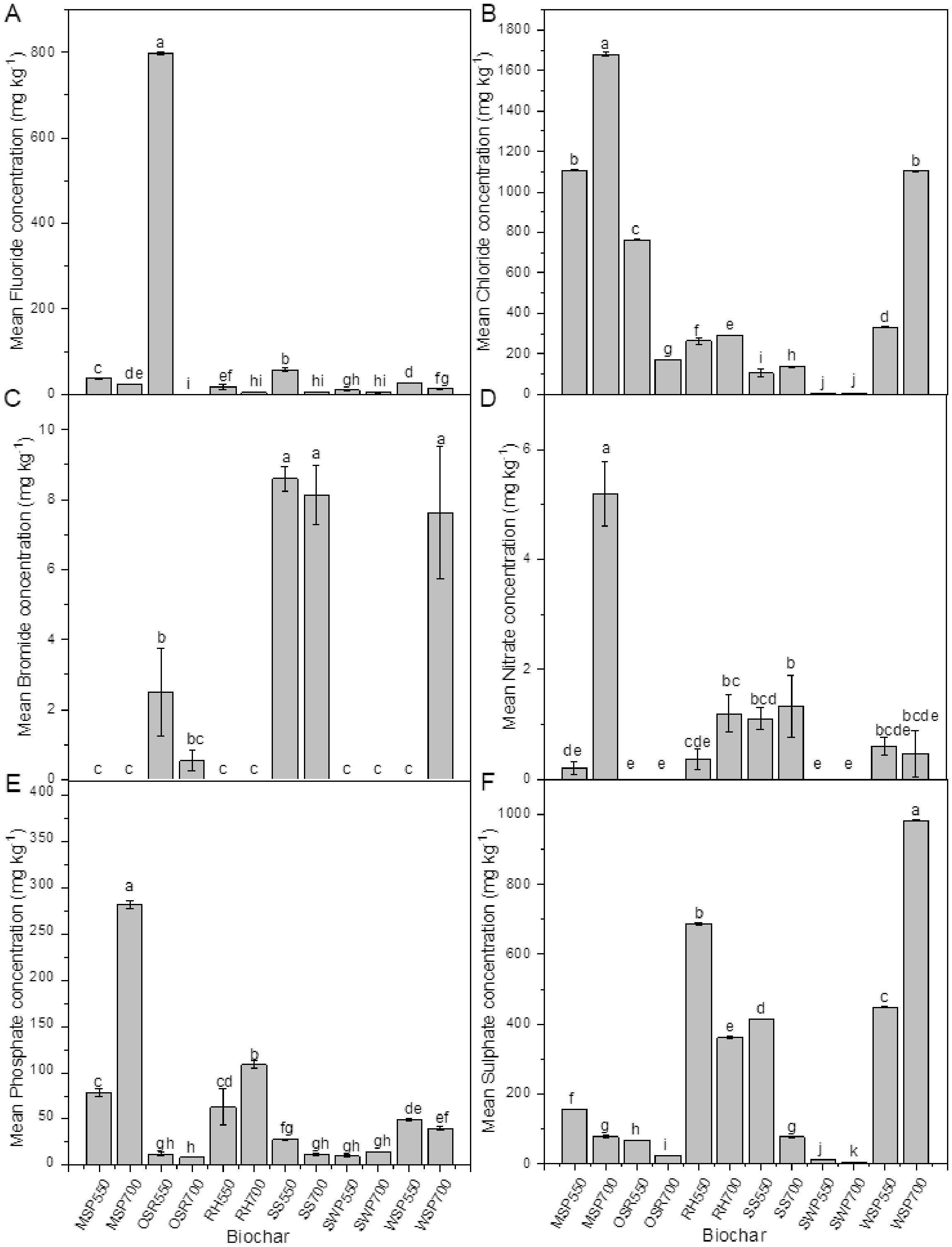

Figure 4.5 Mean water-extractable biochar-borne (A) Fluoride, (B) Chloride, (C) Bromide, (D) Nitrate, (E) Phosphate and (F) Sulphate concentrations (mg kg^{-1}). Values are presented as mean concentration ± standard error (n = 3). Statistical significance is denoted by different lowercase letters above each error bar, according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

4.2.9 LMWOA-extractable biochar-borne elements

4.2.9.1 pH of the water and LWMOA-extracts

The pH results for the water extracts and the LMWOA-extracts are presented in Table 4.6. The pH of the water-containing solutions was much higher than the LMWOA solutions as expected ($p < 0.05$). For the controls, pH ranged from 8.78 to 10.33. In comparison, all LMWOA-containing treatments ranged from 2.27 to 4.98.

Table 4.6 pH of the water-containing and LMWOA-containing solutions

Biochar	**Extractant**	**pH**
MSP550	Water	10.33±0.03c
	Organic acids	2.29±0.03o
MSP700	Water	8.78±0.08g
	Organic acids	3.63±0.13j
OSR550	Water	9.98±0.01e
	Organic acids	3.27±0.03k
OSR700	Water	9.41±0.02f
	Organic acids	4.98±0.04h
RH550	Water	10.30±0.00c
	Organic acids	2.78±0.10m
RH700	Water	8.69±0.04g
	Organic acids	2.49±0.06n
SS550	Water	10.10±0.00de
	Organic acids	2.47±0.04n
SS700	Water	9.52±0.04f
	Organic acids	2.48±0.03n
SWP550	Water	10.60±0.06b
	Organic acids	2.03±0.02p
SWP700	Water	10.90±0.00a
	Organic acids	2.27±0.05o
WSP550	Water	10.77±0.03a
	Organic acids	3.04±0.04l
WSP700	Water	10.20±0.00cd
	Organic acids	4.55±0.03i

All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different, according to one-way ANOVA and Duncan’s post-hoc test ($p < 0.05$).

4.2.9.2 Water-extractable and LMWOA-extractable elements

The results for water-extractable and LMWOA-extractable aluminium, calcium, iron, potassium and magnesium are shown in Table 4.7. No aluminium was detected in the water extracts, except for OSR700 (13.75 mg kg^{-1}). LMWOA-extractable aluminium was released by all biochar, ranging from 1.02 to 330.03 mg kg^{-1}. SS550 and SS700 had considerably more LMWOA-extractable aluminium than the other materials (330.03 mg kg^{-1} and 170.83 mg kg^{-1}). A statistically significant decrease in LMWOA-extractable aluminium occurred with increased pyrolysis temperature for RH550, RH700, SS550 and SS700 biochar ($p < 0.05$). No other observed trends for aluminium were statistically significant. No water-extractable calcium was detected for SS700, SWP550, SWP700 and WSP700. However, the water-extractable calcium for the remaining biochar ranged from 45.24 mg kg^{-1} to 606.49 mg kg^{-1}. LMWOAs extracted more calcium for RH550, RH700, SS550, SS700, SWP550, SWP700 and WSP700 compared to water-extractable calcium, but the trend was only significant for SS550, SWP550 and WSP700, respectively ($p < 0.05$). Whereas for MSP700, OSR550 and OSR700 significantly less calcium was extracted by LMWOA compared to water ($p < 0.05$). Generally, extractable calcium was observed to decrease with pyrolysis temperature, but this was only significant for SS550 and SS700 ($p < 0.05$). No water-extractable iron was detected for any of the biochar. However, LMWOA-extractable iron ranged from 3.12 mg kg^{-1} to 958.01 mg kg^{-1}. Notably, more LMWOA-extractable iron was found in SS550 and SS700 compared to other feedstock. In terms of changes to LMWOA-extractable iron with pyrolysis temperature, the amount for SWP550, SWP700, WSP550 and WSP700 increased, but no significance was found ($p > 0.05$). Whereas for RH550, RH700, SS550 and SS700, LMWOA-extractable iron significantly decreased with increased pyrolysis temperature ($p < 0.05$). LMWOAs released more potassium compared to water and this was statistically significant for MSP550, MSP700, OSR550, OSR700, WSP550 and WSP700 ($p < 0.05$).

Table 4.7 Mean water and 0.01 mmol L^{-1} equimolar LMWOA-extractable aluminium (Al), calcium (Ca), iron (Fe), potassium (K) and magnesium (Mg) (mg kg^{-1}) from the twelve UKBRC standard biochar.

Biochar	**Extractant**	**Mean extractable element (mg kg^{-1})**				
		Al	**Ca**	**Fe**	**K**	**Mg**
MSP550	Water	<LOD[1]	65.89±0.16fhi	<LOD	2475.37±17.08fg	29.40±0.21hj
MSP700	Water	<LOD	606.49±66.68b	<LOD	64.30±5.26g	34.29±7.26hj
OSR550	Water	<LOD	82.12±0.40fg	<LOD	4379.13±9.48ef	50.79±1.19ghj
OSR700	Water	13.75±0.18df	232.43±0.79d	<LOD	22.18±0.78g	82.23±0.39fh
RH550	Water	<LOD	72.00±1.51fh	<LOD	4535.73±1.86ef	56.35±1.73ghj
RH700	Water	<LOD	62.07±1.00fhij	<LOD	95.93±1.59g	<LOD
SS550	Water	<LOD	168.44±8.97e	<LOD	4477.84±1.82ef	91.71±1.85fh
SS700	Water	<LOD	<LOD	<LOD	13.00±1.15g	<LOD
SWP550	Water	<LOD	<LOD	<LOD	681.61±121.73g	40.71±12.31ghj
SWP700	Water	<LOD	<LOD	<LOD	147.23±0.12g	<LOD
WSP550	Water	<LOD	45.25±1.67fhk	<LOD	582.52±3.89g	50.48±0.53ghj
WSP700	Water	<LOD	<LOD	<LOD	0.66±0.66g	<LOD
MSP550	Organic acids	4.26±2.52df	36.02±19.63ghk	7.92±4.53ef	2664.58±556.05fg	91.49±52.01fh
MSP700	Organic acids	3.84±1.92df	7.29±3.26jk	5.40±2.70ef	9376.26±466.00d	112.03±55.94fg
OSR550	Organic acids	37.38±0.53d	14.55±1.52hk	31.68±0.27de	15784.10±127.00c	323.09±12.58bc
OSR700	Organic acids	23.99±2.46df	9.07±4.06ik	21.63±0.61df	29956.10±667.01a	264.60±3.57cd
RH550	Organic acids	143.45±3.26b	99.51±8.96f	82.63±2.40c	6262.11±283.69de	365.86±16.49b
RH700	Organic acids	89.55±50.10c	64.73±34.29fhij	43.46±24.96d	2331.43±121.00fg	137.06±75.59ef
SS550	Organic acids	330.03±12.27a	939.46±30.89a	958.01±33.44a	198.41±8.90g	201.58±3.70de
SS700	Organic acids	170.83±14.39b	430.50±31.13c	531.45±29.77b	171.53±14.14g	546.01±36.12a
SWP550	Organic acids	1.02±0.14ef	25.45±1.37ghk	3.12±0.14ef	71.72±6.44g	5.81±0.64ij
SWP700	Organic acids	7.76±0.57df	58.70±0.95fhij	9.49±0.61ef	1011.10±52.64g	20.57±0.89hj
WSP550	Organic acids	30.83±3.07de	14.54±1.85hk	17.74±1.68df	9096.44±465.32d	79.93±4.32fhi
WSP700	Organic acids	28.64±0.60df	21.80±3.25hk	22.34±0.22df	22312.00±718.05b	284.45±7.72c

[1] <LOD denotes a concentration less than the limit of detection by ICP-OES analysis.

All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different, according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The variability of potassium release from water to LMWOAs was pronounced, for instance, only 22.18 mg kg^{-1} water-extractable potassium was released from OSR700 compared to 29956.10 mg kg^{-1} LMWOA-extractable. These values were the lowest and highest amount of potassium released for each extraction solution, respectively. However, for SS550, significantly more potassium was extracted by water compared to LMWOAs ($p < 0.05$). Three out of six feedstock had significantly more LMWOA-extractable potassium as pyrolysis temperature increased (MSP, OSR and WSP) ($p < 0.05$). No further observed trends for potassium were statistically significant. No water-extractable magnesium was detected for RH700, SS700, SWP700 or WSP700. For the other biochar, water-extractable magnesium ranged from 5.81 mg kg^{-1} to 546.01 mg kg^{-1}. LMWOA-extractable magnesium was greater than water-extractable magnesium for all biochar ($p < 0.05$), except MSP550, SWP550 and WSP500 ($p > 0.05$).

The results for water-extractable and LMWOA-extractable manganese, sodium, arsenic, chromium and copper are shown in Table 4.8. No-water extractable manganese was detected for any biochar. In contrast, LMWOA-extractable manganese ranged from 1.06 mg kg^{-1} to 124.92 mg kg^{-1}. Notably, RH550, RH700, SS550 and SS700 had significantly less LMWOA-extractable manganese with increasing pyrolysis temperature ($p < 0.05$). No water-extractable sodium was detected for RH700, SS700, SWP550, or WSP700. The remaining biochar materials released between 6.54 mg kg^{-1} to 69.61 mg kg^{-1} of sodium in water. More sodium tended to be extracted by the LMWOAs, ranging from 1.83 mg kg^{-1} to 408.83 mg kg^{-1}. This was significant for MSP700, OSR700, RH550 and SS550 ($p < 0.05$). Concerning changes with pyrolysis temperature, MSP550, MSP700, WSP550 and WSP700 showed increased LMWOA-extractable sodium with increased temperature ($p < 0.05$), whereas less sodium was extracted from SS700 compared to SS550 ($p < 0.05$).

Table 4.8 Mean water and 0.01 mmol L^{-1} equimolar LMWOA-extractable manganese (Mn), sodium (Na), arsenic (As), chromium (Cr) and copper (Cu) (mg kg^{-1}) from the twelve UKBRC standard biochar.

Biochar	**Extractant**	**Mean extractable element (mg kg^{-1})**				
		Mn	**Na**	**As**	**Cr**	**Cu**
MSP550	Water	<LOD[1]	33.91±1.66fg	<LOD	<LOD	<LOD
MSP700	Water	<LOD	51.74±8.84df	<LOD	<LOD	<LOD
OSR550	Water	<LOD	69.61±2.73cdg	<LOD	<LOD	<LOD
OSR700	Water	<LOD	7.79±0.11fg	<LOD	<LOD	<LOD
RH550	Water	<LOD	6.54±0.28fg	<LOD	<LOD	<LOD
RH700	Water	<LOD	<LOD	<LOD	<LOD	<LOD
SS550	Water	<LOD	20.62±0.24fg	<LOD	<LOD	<LOD
SS700	Water	<LOD	<LOD	<LOD	<LOD	<LOD
SWP550	Water	<LOD	27.54±6.34fg	<LOD	<LOD	<LOD
SWP700	Water	<LOD	<LOD	0.13±0.12e	<LOD	<LOD
WSP550	Water	<LOD	32.50±1.66fg	<LOD	<LOD	<LOD
WSP700	Water	<LOD	<LOD	0.50±0.29a	<LOD	<LOD
MSP550	Organic acids	7.13±3.97cd	8.48±8.48fg	<LOD	0.05±0.02cgh	0.52±0.21bc
MSP700	Organic acids	4.28±2.13d	173.56±87.53b	<LOD	0.01±0.01fgh	0.38±0.13bd
OSR550	Organic acids	1.51±0.02d	<LOD	<LOD	0.05±0.01cdf	0.41±0.05bd
OSR700	Organic acids	1.06±0.02d	124.61±20.04bd	<LOD	0.03±0.01dgh	0.36±0.03bd
RH550	Organic acids	124.92±6.85a	133.68±15.60bc	<LOD	0.07±0.01cd	0.47±0.02bd
RH700	Organic acids	37.77±20.93b	110.72±69.26bde	<LOD	0.05±0.03cg	0.28±0.07d
SS550	Organic acids	32.86±0.78b	123.91±4.82bd	<LOD	1.33±0.07a	0.54±0.02b
SS700	Organic acids	19.01±1.05c	1.83±0.96fg	<LOD	0.49±0.03b	1.37±0.17a
SWP550	Organic acids	2.48±0.27d	<LOD	<LOD	0.02±0.01efgh	0.37±0.05bd
SWP700	Organic acids	9.46±0.47cd	40.81±12.26efg	<LOD	0.08±0.00c	0.49±0.04bc
WSP550	Organic acids	5.80±0.39cd	52.03±11.33df	<LOD	0.04±0.00cgh	0.32±0.03cd
WSP700	Organic acids	6.56±0.10cd	408.83±19.67a	<LOD	0.07±0.01cde	0.40±0.01bd

[1] <LOD denotes a concentration less than the limit of detection by ICP-OES analysis.

All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different, according to one-way ANOVA and Duncan’s post-hoc test ($p < 0.05$).

Water-extractable arsenic was only released from SWP700 (0.13 mg kg^{-1}) and WSP700 (0.50 mg kg^{-1}) (Table 4.8). No LMWOA-extractable arsenic was detected for any biochar. No water-extractable chromium was released from any biochar. LMWOA-extractable chromium ranged from 0.01 mg kg^{-1} to 1.33 mg kg^{-1}. Notably, SS550 and SS700 contained the highest amounts of LMWOA-extractable chromium (1.33 mg kg^{-1}and 0.49 mg kg^{-1}), which was significantly higher when produced at a lower temperature ($p < 0.05$). Like chromium, no water-extractable copper was released but LMWOA-extractable copper for all the biochar ranged from 0.28 mg kg^{-1} to 1.37 mg kg^{-1}. The amount of LMWOA-extractable copper was significantly higher in SS700 biochar compared to SS550 ($p < 0.05$). No other observable trend was statistically significant.

The results for water-extractable and LMWOA-extractable nickel, lead, zinc, boron and barium are shown in Table 4.9. Nickel was only extracted from SS550 (0.11 mg kg^{-1}) and SS700 (0.48 mg kg^{-1}) using LMWOAs. Interestingly, the amount extracted increased significantly with pyrolysis temperature ($p < 0.05$). Minimal amounts of extractable lead were detected. Of the twelve biochar, only OSR550, RH550, SS550 and SWP550 released lead in LMWOAs, and SS550 released significantly more lead compared to the other biochar materials ($p < 0.05$). No water-extractable zinc was detected for any of the biochar. LMWOA-extractable zinc was detected for all materials ranging from 0.69 mg kg^{-1} to 34.21 mg kg^{-1}. The amount of LMWOA-extractable zinc notably decreased with increased pyrolysis temperature for MSP550, MSP700, RH550, RH700, SS550 and SS700 ($p < 0.05$).

Water-extractable boron was not detected for WSP700 but was released by all biochar materials. The amount released for SS700 was negligible. Values for other biochar ranged from 0.09 mg kg^{-1} to 4.62 mg kg^{-1}. Water-extractable boron tended to significantly decrease with increased pyrolysis temperature ($p < 0.05$), except for SS550 and SS700.

Table 4.9 Mean water and 0.01 mmol L^{-1} equimolar LMWOA-extractable nickel (Ni), lead (Pb), zinc (Zn), boron (B) and barium (Ba) (mg kg^{-1}) from the twelve UKBRC standard biochar.

Biochar	**Extractant**	**Mean extractable element (mg kg^{-1})**				
		Ni	**Pb**	**Zn**	**B**	**Ba**
MSP550	Water	< LOD[1]	< LOD	< LOD	4.62±1.21a	< LOD
MSP700	Water	< LOD	< LOD	< LOD	1.00±0.09cg	< LOD
OSR550	Water	< LOD	< LOD	< LOD	2.28±0.18b	< LOD
OSR700	Water	< LOD	< LOD	< LOD	1.28±0.14cd	< LOD
RH550	Water	< LOD	< LOD	< LOD	1.42±0.22c	< LOD
RH700	Water	< LOD	< LOD	< LOD	0.35±0.18efgh	< LOD
SS550	Water	< LOD	< LOD	< LOD	1.19±0.11cde	< LOD
SS700	Water	< LOD	< LOD	< LOD	0.08±0.08h	< LOD
SWP550	Water	< LOD	< LOD	< LOD	0.42±0.24dgh	< LOD
SWP700	Water	< LOD	< LOD	< LOD	0.09±0.07h	< LOD
WSP550	Water	< LOD	< LOD	< LOD	1.11±0.08cdf	< LOD
WSP700	Water	< LOD	< LOD	< LOD	< LOD	< LOD
MSP550	Organic acids	< LOD	< LOD	8.04±4.42cd	0.51±0.18dgh	6.92±3.75fh
MSP700	Organic acids	< LOD	< LOD	4.32±2.13ef	0.45±0.23dgh	3.90±1.94gh
OSR550	Organic acids	< LOD	0.04±0.04bce	2.51±0.06eg	0.53±0.02dgh	16.97±0.49cde
OSR700	Organic acids	< LOD	<LOD	1.55±0.08eg	0.24±0.02gh	12.91±0.13df
RH550	Organic acids	< LOD	0.04±0.04ce	10.93±0.42c	0.97±0.05cg	40.53±1.72b
RH700	Organic acids	< LOD	<LOD	5.21±2.87de	0.46±0.24dgh	21.36±11.66c
SS550	Organic acids	0.11±0.02b	0.50±0.35a	34.21±0.63a	0.16±0.08gh	48.65±1.84a
SS700	Organic acids	0.48±0.03a	< LOD	27.29±1.49b	2.67±0.21b	24.39±1.16c
SWP550	Organic acids	< LOD	0.04±0.04e	0.69±0.04fg	0.21±0.02gh	2.69±0.23gh
SWP700	Organic acids	< LOD	< LOD	3.39±0.13eg	0.24±0.02fgh	6.55±0.38fh
WSP550	Organic acids	< LOD	< LOD	1.89±0.10eg	0.70±0.06cgh	10.35±0.59efg
WSP700	Organic acids	< LOD	< LOD	4.83±0.06de	0.76±0.01cgh	18.55±0.34cd

[1] <LOD denotes a concentration less than the limit of detection by ICP-OES analysis.

All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different, according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

For MSP550, MSP700, OSR550, OSR700 and SS550, more boron was released in water compared to LMWOAs ($p < 0.05$). However, for SS700, significantly more boron was released in LMWOAs compared to water ($p < 0.05$). Considering all biochar, the amount of LMWOA-extractable boron ranged from 0.16 mg kg^{-1} to 2.67 mg kg^{-1}. Only SS550 and SS700 showed increased LMWOA-extractable boron with increased pyrolysis temperature. Lastly, barium was only extracted in LMWOAs in values ranging from 2.69 mg kg^{-1} to 48.53 mg kg^{-1}. For four out of the six biochar, the observed trend was decreased barium release with increased pyrolysis temperature. This was statistically significant for RH550, RH700, SS550 and SS700 ($p < 0.05$), but not significant for MSP550, MSP700, OSR550 or OSR700 ($p > 0.05$). For SWP550, SWP700, WSP550 and WSP700, the reverse was true; however, a statistical difference was only obtained between WSP550 and WSP700 ($p < 0.05$).

In terms of LMWOA-extractable biochar-borne element release rates (Table 4.10) only SS550 and SS700 could be calculated for chromium, where the biochar released 0.28 and 0.10 % of the total chromium. For copper, all biochar released small amounts of the total amount of copper, ranging from 0.06 % for MSP550 to 1.29 % for WSP700. Only SS550 and SS700 released calculable amounts of nickel compared to the total nickel amount, 0.12 % and 0.57 % for SS550 and SS700, respectively. Only SS700 had a calculable lead release (0.21 %). For zinc, the release rate ranged from 1.3 % for SWP550 to 16.1 % for RH550. Overall, relatively small proportions of metals and metalloids were released in comparison to the total metal content measured in this study.

Table 4.10 A comparison of the biochar-borne element release rates (%) calculated from UKBRC standard biochar materials

Biochar	**Comparison of biochar-borne element release rate (%)[1,2]**				
	Chromium	**Copper**	**Nickel**	**Lead**	**Zinc**
MSP550	uc[3]	0.06	uc	uc	3.9
MSP700	uc	0.08	uc	uc	4.9
OSR550	uc	0.39	uc	uc	9.7
OSR700	uc	0.31	uc	uc	5.8
RH550	uc	0.35	uc	uc	16.1
RH700	uc	uc	uc	uc	7.8
SS550	0.28	0.39	0.12	0.21	3.1
SS700	0.10	0.14	0.57	uc	2.4
SWP550	uc	0.17	uc	uc	1.3
SWP700	uc	0.51	uc	uc	8.8
WSP550	uc	0.51	uc	uc	6.0
WSP700	uc	1.29	uc	uc	6.5

[1]Extracted via a 0.01 mmol L^{-1} equimolar mixed solution of citric, malic and oxalic acid. [2]Values where total metal content was above the limit of detection and had LMWOA-extractable trace element release are displayed. [3]uc; unable to calculate.

4.3 Results for hypothesis 1

4.3.1 Systems with biochar and no-added metals

The pH and EC results for systems with biochar and no-added metals are shown in Table 4.11. In the water, the pH ranged from 9.89 to 10.40. After 1 h, there was a significant increase in pH of around 0.46 units ($p < 0.05$). After 24 h, no temporal variations in pH were significant ($p > 0.05$). Expectedly, the pH in the LMWOA-containing solutions was acidic compared to water ($p < 0.05$) (< 2.89) to the order of malic acid > citric acid > oxalic acid. All the LMWOA-containing solutions were significantly different from each other ($p < 0.05$). The biochar citric acid treatment was only significantly higher in pH after 120 h ($p < 0.05$). However, the pH of the malic acid systems increased between 1 h and 24 h then again between 72 and 120 h ($p < 0.05$). For oxalic acid systems, the pH was the same at the first two intervals before increasing at 72 h ($p < 0.05$). Finally, a decrease in pH occurred between 72 h and 120 h, from 2.31 to 2.13 ($p < 0.05$).

Table 4.11 pH and electrical conductivity (EC) results (mS cm^{-1}) at various time intervals (h) in either water, citric, malic or oxalic acid-containing solutions with the addition of 1 g dL^{-1} RH700 biochar and no added metals

Parameter	**Solution**	**Sampling interval**			
		1 h	**24 h**	**72 h**	**120 h**
pH	Water	9.89±0.06Ba	10.35±0.09Aa	10.34±0.08Aa	10.40±0.04Aa
	Citric	2.56±0.01Bc	2.63±0.01ABc	2.62±0.04ABc	2.68±0.02Ac
	Malic	2.75±0.00Cb	2.83±0.01Bb	2.85±0.01Bb	2.89±0.02Ab
	Oxalic	1.98±0.02Cd	1.97±0.01Cd	2.31±0.05Ad	2.13±0.02Bd
EC (mS cm^{-1})	Water	0.50±0.01Cd	1.37±0.06Bb	1.56±0.05Ab	1.70±0.05Ab
	Citric	1.33±0.06Ab	1.23±0.04Ac	1.25±0.01Ac	1.21±0.02Ac
	Malic	0.94±0.01Ac	0.87±0.01Cd	0.90±0.01Bd	0.87±0.01BCd
	Oxalic	3.81±0.04Aa	3.63±0.03Ba	3.87±0.02Aa	3.38±0.04Ca

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The EC of the water systems increased with contact time from 0.50 mS cm^{-1} to 1.70 mS cm^{-1}. The increases were significant until 72 h, after which the increase was insignificant ($p > 0.05$). The EC of the LMWOA-containing systems followed the order of oxalic acid > citric acid > malic acid. The EC in the citric acid system decreased over time from 1.33 to 1.21 mS cm^{-1}. However, this was not significant ($p < 0.05$). Malic acid systems also decreased from 1 h to 24 h ($p < 0.05$). Then an increase of around 0.03 mS cm^{-1} occurred by 72 h ($p < 0.05$). No further temporal variations were significant ($p > 0.05$). The EC of the oxalic acid system was significantly higher than all other systems ($p < 0.05$). Overall, the EC decreased with time ($p < 0.05$), but there was no difference between the EC at 1 h and 72 h, respectively ($p > 0.05$).

4.3.2 Cadmium-containing systems

4.3.2.1 pH and electrical conductivity of the cadmium-containing solutions

The pH and EC results can be seen in Table 4.12. In the water control, the pH increased over time, but significance was only achieved at 72 h ($p < 0.05$). No further significant change in pH was observed. The addition of biochar significantly increased the pH at all intervals ($p < 0.05$). Expectedly, the addition of LMWOAs decreased the solution pH in both control and biochar-treated solutions, following a decreasing order of water > malic acid > citric acid > oxalic acid-containing systems.

Table 4.12 pH and electrical conductivity (EC) (mS cm^{-1}) results at various time intervals (h) in cadmium-containing solutions with either water, citric, malic or oxalic acid and the addition of 0 or 1 g dL^{-1} RH700 biochar

Parameter	**Solution**	**Biochar (g dL^{-1})**	**Sampling interval**			
			1 h	**24 h**	**72 h**	**120 h**
pH	Water	0	4.96±0.04Bb	5.91±0.30Bb	5.95±0.01Ab	6.42±0.01Ab
	Citric	0	2.36±0.00Bf	2.36±0.01Bd	2.41±0.00Af	2.42±0.02Af
	Malic	0	2.45±0.03Be	2.49±0.01ABd	2.50±0.01ABe	2.53±0.01Be
	Oxalic	0	1.83±0.01Ag	1.82±0.01ABe	1.75±0.02Bh	1.87±0.03Ah
	Water	1	5.88±0.07Ca	7.14±0.05Ba	7.29±0.04Ba	7.41±0.01Aa
	Citric	1	2.57±0.01Bd	2.69±0.01Acd	2.74±0.02Ad	2.75±0.04Ad
	Malic	1	2.72±0.02Cc	2.90±0.02Bc	2.90±0.04Bc	3.13±0.01Ac
	Oxalic	1	1.92±0.02BCg	1.90±0.01Ce	1.96±0.02Bg	2.08±0.02Ag
EC (mS cm^{-1})	Water	0	0.24±0.00Af	0.26±0.00Ae	0.27±0.00Af	0.29±0.00Af
	Citric	0	1.55±0.00Ac	1.62±0.00Ac	1.57±0.00Ac	1.59±0.01Ac
	Malic	0	1.20±0.01Ade	1.23±0.02Acd	1.20±0.01Ad	1.21±0.01Ad
	Oxalic	0	6.02±0.04Aa	6.03±0.37Aa	6.08±0.04Aa	6.09±0.03Aa
	Water	1	0.23±0.00Af	0.23±0.00Ae	0.23±0.00Af	0.23±0.00Af
	Citric	1	1.32±0.03Ad	0.91±0.01Cd	1.10±0.04Bd	1.06±0.04Bd
	Malic	1	1.02±0.02Be	1.16±0.02Ad	0.85±0.02Ce	0.77±0.01Ce
	Oxalic	1	5.23±0.16Ab	4.92±0.00Bb	4.15±0.17Bb	3.76±0.14Cb

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The pH in both the control and treated citric acid solutions increased over time, achieving significance at 72 h and 24 h, correspondingly ($p < 0.05$). No further observable increase was significant ($p > 0.05$). The addition of biochar compared to the control, increased the pH, except at 24 h ($p < 0.05$). For malic acid, the pH was always higher than in the control ($p < 0.05$). In both the control and treated solutions, the pH increased overtime. However, a significant increase in pH was only observed in the treatment between 1 h and 24 h and 72 h and 120 h, respectively ($p < 0.05$). Lastly, for oxalic acid systems, no directional temporal trend for the control was found, but the pH at 1 h was significantly less than at 72 h ($p < 0.05$), but the pH between the 120 h was considerably higher compared to that at 72 h ($p < 0.05$). Compared to the control, the addition of biochar tended to increase the pH from 72 h onwards ($p < 0.05$). Furthermore, the pH of the biochar treated oxalic acid tended to increase with time, achieving significance from 72 h onwards ($p < 0.05$).

Concerning the EC of the solutions, the addition of biochar did not affect the EC of the water solution at any time point ($p > 0.05$). Neither was any temporal trends observed in either the control or biochar-treated water systems ($p > 0.05$). Irrespective of biochar treatment, the EC decreased to the order of oxalic acid > citric acid > malic acid > water. In the citric acid, biochar always significantly lowered the EC compared to the control ($p < 0.05$). In the control, the EC gradually increased with time, but this was not significant ($p > 0.05$). In the biochar treated citric acid system, the EC significantly decreased from 1 h to 24 h and then increased at 24 to 72 h ($p < 0.05$). No change in solution EC was observed after 72 h. In malic acid, the addition of biochar significantly decreased the solution EC at 72 h and 120 h, compared to the control ($p < 0.05$). For the control, no difference in EC was found with time ($p > 0.05$). However, the EC in the biochar treated systems fluctuated with time. From the 1 h to the 24 h, the EC of the solutions significantly increased, before declining again from the 24 h to the 72 h ($p < 0.05$). No further change was observed. For oxalic acid systems biochar always significantly decreased the EC compared to the control ($p < 0.05$). In the oxalic acid control, the EC ranged from 6.02 to 6.09 mS cm^{-1}. However, the increase was insignificant ($p > 0.05$). In contrast, the EC of biochar treated solutions typically decreased over time. These changes were significant from 1 h to 24 h and again at 72 h to 120 h ($p < 0.05$).

4.3.2.2 The effect of organic acids on biochar removal of cadmium

The results for the cadmium batch sorption study are shown in Table 4.13. In the water control, no temporal deviation from the theoretical initial concentration occurred ($p > 0.05$). The addition of biochar significantly decreased the soluble cadmium in water systems compared to the control at all intervals ($p < 0.05$). Contrastingly, the cadmium concentration in the water treatment significantly decreased over time and was significantly different at each time interval ($p < 0.05$). These results suggest biochar can remove soluble cadmium in water.

In the citric acid control, there was no temporal deviation from the theoretical initial concentration ($p > 0.05$). In the biochar treated citric acid, no changes in concentration occurred over time ($p > 0.05$). The concentrations were slightly less than the theoretical initial concentration, but no significant difference between the treatment and control was observed ($p > 0.05$). Furthermore, the concentration of soluble cadmium in the biochar treated citric acid compared to the water counterpart was significantly greater at 24 h, 72 h and 120 h, respectively ($p < 0.05$). The same trend that was found in citric acid systems was also observed in the malic acid systems. These results suggest that citric acid and malic acid inhibit the biochar-driven immobilisation of cadmium in aqueous systems.

Table 4.13 Cadmium concentrations (mmol L^{-1}) at various time intervals (h) in either water, citric, malic or oxalic acid-containing solutions with the addition of 0 or 1 g dL^{-1} RH700 biochar.

Solution	**Biochar (g dL^{-1})**	**Cadmium concentration (mmol L^{-1})**			
		1 h	**24 h**	**72 h**	**120 h**
Water	0	1.06±0.00Aa	1.06±0.01Aa	1.05±0.01Aa	1.06±0.00Aa
Citric	0	1.01±0.06Aab	1.01±0.06Aa	1.02±0.06Aa	1.01±0.06Aa
Malic	0	1.02±0.05Aab	1.02±0.06Aa	1.03±0.06Aa	1.02±0.05Aa
Oxalic	0	0.63±0.05Ac	0.62±0.04Ab	0.59±0.03Ab	0.56±0.05Ab
Water	1	0.91±0.00Ab	0.50±0.00Bc	0.31±0.00Cc	0.21±0.00Dc
Citric	1	0.98±0.05Aab	0.96±0.05Aa	0.96±0.05Aa	0.96±0.05Aa
Malic	1	0.98±0.05Aab	0.97±0.05Aa	0.97±0.05Aa	0.97±0.05Aa
Oxalic	1	0.73±0.03Ac	0.48±0.01Bc	0.48±0.03Bb	0.54±0.01Bb

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

In the oxalic acid systems, concentrations were less than the theoretical initial concentration in the control and biochar treatment. In the control, concentrations were over 40% less than the initial concentration, but biochar only appeared to remove soluble cadmium concentrations at 24 h ($p < 0.05$). Concentrations between 1 h and 24 h in the biochar treatment were significantly less with time ($p < 0.05$) but the concentration then stayed statistically similar until the experiment end ($p > 0.05$). The results suggest biochar was unable to remove soluble cadmium in the presence of oxalic acid. To summarise:

1. In water, biochar was able to remove around 79 % of soluble cadmium from solution.
2. In citric acid, biochar failed to remove soluble cadmium.
3. In malic acid, biochar failed to remove soluble cadmium.
4. In oxalic acid, biochar failed to remove soluble cadmium.

4.3.2.3 Cadmium adsorption kinetics in different solution types

The adsorption kinetics for cadmium onto biochar in water and the three model LMWOAs were studied (Table 4.14). Pseudo-first-order, pseudo-second-order and intraparticle diffusion models were applied to study rate kinetics and intraparticle diffusion. Cadmium adsorption kinetics in water showed a strong correlation to pseudo-first-order kinetics ($R^2 = 0.979$) but there was a disparity between the experimental Qe value of 0.079 mmol g^{-1} (Appendix A2) and the calculated Qe parameter for pseudo-second-order kinetics (0.064 mmol g^{-1}). An improved level of fit to pseudo-second-order order kinetics was found ($R^2 = 0.986$) and the model had Qe values like that of the experimental data (Appendix A2). The calculated parameters suggest that pseudo-second-order rate kinetics should be considered to describe cadmium retention in the studied systems. When applied to the intraparticle diffusion model, cadmium adsorption in water revealed a near-perfect-linear fit ($R^2 = 0.953$) and low *C* values suggesting intraparticle diffusion was not the sole rate-limiting step in cadmium adsorption kinetics. Equally, the level of linearity and closeness to passing through the origin confirm intraparticle diffusion was likely a factor in the cadmium adsorption process.

Table 4.14 Pseudo-first-order, pseudo-second-order and intraparticle diffusion kinetic model parameters for systems containing cadmium and RH700 biochar in the presence of either water, citric acid, malic acid, or oxalic acid

Kinetic model	**Parameter**	**Solution type**			
		Water	**Citric acid**	**Malic acid**	**Oxalic acid**
Pseudo-first order	Qe (mmol g^{-1})	0.064	n/a[1]	n/a	n/a
	k_1 (h^{-1})	0.027	n/a	n/a	n/a
	R^2	0.979	n/a	n/a	n/a
Pseudo-second order	Qe (mmol g^{-1})	0.081	0.004	0.003	0.047
	k_2 (g $mmol^{-1}$ h^{-1})	1.564	668.031	1788.481	17.240
	R^2	0.986	1.000	1.000	0.995
Intraparticle diffusion	k_i (mmol g^{-1} $h^{0.5}$)	0.009	0.009	0.009	0.005
	C (mmol g^{-1})	0.010	0.010	0.004	0.015
	R^2	0.953	0.953	0.986	0.755

[1] n/a; not applicable.

The small adsorption that took place in the place in the LMWOA-containing systems did not fit to pseudo-first-order kinetics. The calculated Qe parameter for pseudo-second-order kinetics was close to the experimental Qe values (Appendix A2), except for citric acid. However, there was a perfect linear fit for cadmium adsorption in citric and malic acid ($R^2 = 1$). Although cadmium fit to the intraparticle diffusion model, the lesser R^2 value suggests intraparticle diffusion kinetics do not describe the adsorption of cadmium to biochar in the presence of LMWOAs.

4.3.3 Lead-containing systems

4.3.3.1 pH and electrical conductivity of the lead-containing solutions

The pH and EC results can be seen in Table 4.15. For the water control, the addition of biochar increased the pH compared to the control ($p < 0.05$). Over time, the pH in the control tended to fluctuate, but no change was significant ($p > 0.05$). Contrastingly, with biochar addition, the water solution became more alkaline at each interval ($p < 0.05$). The LMWOAs acidified the solutions compared to water ($p < 0.05$). In the citric acid systems, the control pH was around 2.41, showing no temporal variations ($p > 0.05$). Except at 24 h, the addition of biochar to citric acid significantly increased the pH ($p < 0.05$). Considering temporal pH change, the pH tended to increase with time ($p < 0.05$) despite a small decrease between 24 h and 72 h ($p < 0.05$). For malic acid systems, the control was always more acidic that the biochar treatment, except at 24 h ($p < 0.05$). The control tended to become more acidic over time, significantly decreasing from 24 h and 72 h ($p < 0.05$). Whereas, the pH of the biochar treated malic acid increased with time. Initially, from 1 h to 24 h, the increase was significant ($p < 0.05$) before plateauing at 24 h and 72 h. However, a further significant increase in pH was observed from 72 h to 120 h. Regarding the oxalic acid systems, the pH of the control fluctuated with time. However, if comparing the pH at the 1 h and 120 h, the pH decreased by 0.10 units ($p < 0.05$). The addition of biochar increased the pH only after 72 h and 120 h of shaking ($p < 0.05$). Initially, biochar had a liming effect ($p < 0.05$), before the pH decreased to a similar level at 72 h to that observed at 1 h. By 120 h, there was no significant difference in pH compared to 1 h ($p > 0.05$).

Table 4.15 pH and electrical conductivity (EC) (mS cm^{-1}) results at various time intervals (h) in lead-containing solutions with either water, citric, malic or oxalic acid and the addition of 0 or 1 g dL^{-1} RH700 biochar

Parameter	**Solution**	**Biochar (g dL^{-1})**	**Sampling interval**			
			1 h	**24 h**	**72 h**	**120 h**
pH	Water	0	4.65±0.05Ab	4.29±1.02Ab	5.48±0.02Ab	5.09±0.01Ab
	Citric	0	2.40±0.01Ae	2.43±0.01Ac	2.40±0.03Af	2.42±0.02Ae
	Malic	0	2.57±0.01ABd	2.61±0.04Ac	2.53±0.02BCe	2.45±0.02Ce
	Oxalic	0	1.91±0.02Cf	1.95±0.06Ac	1.79±0.01Bh	1.81±0.01Ag
	Water	1	5.16±0.03Da	6.62±0.06Ca	6.94±0.03Ba	7.57±0.05Aa
	Citric	1	2.54±0.02Cd	2.68±0.02Ac	2.59±0.01Bd	2.7±0.01Ad
	Malic	1	2.7±0.01Cc	2.8±0.05Bc	2.83±0.02Bc	2.97±0.01Ac
	Oxalic	1	1.94±0.02BCf	2.03±0.05Ac	1.93±0.01Cg	2.02±0.02ABf
EC (mS cm^{-1})	Water	0	0.26±0.00Ad	0.26±0.00Ae	0.26±0.00Af	0.26±0.00Af
	Citric	0	1.63±0.01ABb	1.62±0.01Bc	1.65±0.02ABc	1.68±0.02Ac
	Malic	0	1.22±0.00Ac	1.23±0.02Acd	1.24±0.01Ad	1.25±0.02Ad
	Oxalic	0	5.9±0.32Aa	6.03±0.37Aa	5.93±0.28Aa	6.13±0.36Aa
	Water	1	0.26±0.00Bd	0.26±0.00Be	0.26±0.00Bf	0.28±0.00Af
	Citric	1	1.44±0.00Abc	1.14±0.02Bd	1.15±0.02Bde	1.15±0.01Bde
	Malic	1	1.08±0.01Ac	0.91±0.01Bd	0.88±0.01Be	0.79±0.01Ce
	Oxalic	1	5.65±0.02Aa	4.92±0.00Bb	4.53±0.02Cb	4.37±0.07Db

All values are presented as mean ± standard error ($n = 3$). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan’s post-hoc test ($p < 0.05$).

For the water control, no temporal variations in solution EC were observed in either the control or biochar-amended systems ($p > 0.05$). Nor did the addition of biochar affect the EC compared to the control ($p > 0.05$). For citric acid control, the EC tended to increase over time, but only the EC at 24 h and 120 h was different ($p < 0.05$). In comparison, the biochar treatment decreased with time. Initially, there was a sharp decrease from 1 h to 24 h ($p < 0.05$). After 24 h till the end of the experiment, the EC was stable and stayed around 1.15 mS cm^{-1}. The EC of the citric acid solutions were always less following the addition of biochar ($p < 0.05$). For the malic acid control, no change in EC was observed over time ($p > 0.05$). The EC was significantly lower following biochar application after 72 h or more 120 h contact with biochar ($p < 0.05$). In the treated malic acid systems, the EC decreased with time and was significant at 1 h compared to all other time points. However, there was no difference between pH in the biochar-malic acid systems between 24 and 72 h. The EC of the oxalic acid control was higher than the biochar treatment from 24 h onwards ($p < 0.05$). Although no temporal trend was found in the oxalic acid control, the oxalic acid with biochar decreased overtime from 5.65 mS cm^{-1} to 4.37 mS cm^{-1} and the EC was statistically different at each time point ($p < 0.05$).

4.3.3.2 The effect of organic acids on biochar removal of lead

The results for the lead batch sorption study are shown in Table 4.16. In the water control, the lead concentration maintained the theoretical initial concentration over time. In the biochar treatment, significantly less soluble lead was always found ($p < 0.05$). The concentration declined significantly at each interval ($p < 0.05$). Initially, around 20 % of biochar was removed after 1 h, and by 24 h, over 60 % of soluble lead had been removed by the biochar. Concentrations continued to decrease to a lesser extent by 72 h, but complete removal was observed at 120 h.

Table 4.16 Lead concentrations (mmol L^{-1}) at various time intervals (h) in either water, citric, malic or oxalic acid-containing solutions with the addition of 0 or 1 g dL^{-1} RH700 biochar

Solution	**Biochar (g dL^{-1})**	**Lead concentration (mmol L^{-1})**			
		1 h	**24 h**	**72 h**	**120 h**
Water	0	1.06±0.00Aa	1.06±0.00Aa	1.05±0.00Aa	1.06±0.00Aa
Citric	0	1.05±0.01Aa	1.05±0.01Aa	1.05±0.01Aa	1.05±0.01Aa
Malic	0	1.06±0.00Aa	1.06±0.01Aa	1.06±0.01Aa	1.06±0.00Aa
Oxalic	0	0.01±0.00Ae	0.01±0.00Ad	0.01±0.00Ad	0.01±0.00Ac
Water	1	0.80±0.01Ac	0.37±0.01Bc	0.12±0.00Cc	0.00±0.00Dc
Citric	1	0.95±0.01Ab	0.91±0.01Bb	0.91±0.01Bb	0.91±0.00Bb
Malic	1	0.96±0.00Ab	0.92±0.00Bb	0.92±0.01Bb	0.91±0.00Bb
Oxalic	1	0.03±0.00Ad	0.01±0.00Ad	0.01±0.00Ad	0.01±0.00Ac

All values are presented as mean ± standard error ($n = 3$). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

In the citric acid control, the initial experimental concentration was maintained over time ($p < 0.05$). In the biochar treatment, biochar significantly removed soluble lead at all occasions ($p < 0.05$). At 1 h the concentration was 0.95 mmol L^{-1} suggesting around 5 % lead removal by the biochar. This decreased significantly to 0.91 mmol L^{-1} at 24 h ($p < 0.05$). No further temporal change was found ($p > 0.05$). Similar findings were reported for malic acid control and the biochar treatment. The results suggest that both citric and malic acid limited the biochar-driven removal of soluble lead. In the oxalic acid systems, nearly all soluble lead had been removed from the control system indicating oxalic acid was able to remove lead. Although biochar was found to significantly impede the oxalic acid-driven removal of lead at 1 h ($p < 0.05$), this was not significant at later sampling intervals, and concentrations of lead were identical in the control and treatment systems ($p > 0.05$). This suggests oxalic acid was able to remove soluble lead rather than the biochar materials. In summary:

1. In water, biochar was able to remove all soluble lead from solution.
2. Citric acid impeded the biochar-driven removal of lead
3. Malic acid impeded the biochar-driven removal of lead
4. In oxalic acid treated with biochar, biochar did not affect soluble lead.

4.3.3.3 Lead adsorption kinetics in different solution types

The adsorption kinetics for lead onto biochar in water and the three model LMWOAs were studied (Table 4.17). Data was fit to pseudo-first-order, pseudo-second-order and intraparticle diffusion models to study rate kinetics and intraparticle diffusion. Lead adsorption in the water revealed a strong fit to pseudo-first-order kinetics (R^2 = 0.991). However, the experimental Qe value of 0.1 mmol g^{-1} (Appendix A2) was higher than the Qe values from the model. A good level of fit to the pseudo-second-order was also observed (R^2 = 0.986), and the experimental Qe matched that of the model (0.100 versus 0.102 mmol g^{-1}), indicating lead adsorption by rice husk biochar follows pseudo-second-order kinetics. When fitted to the intraparticle diffusion, a near-linear fit was reported (R^2 = 0.967) and small a *C* value. This indicates intraparticle diffusion was likely the sole rate-limiting step in lead adsorption kinetics. However, only one-step of linearity was observed when $t^{0.5}$ was plotted against t/Qt. This may imply only one process governed adsorption of lead in the water.

Table 4.17 Pseudo-first-order, pseudo-second-order and intraparticle diffusion kinetic model parameters for systems containing lead and RH700 biochar in the presence of either water, citric acid, malic acid, or oxalic acid

Kinetic model	**Parameter**	**Solution type**			
		Control	**Citric acid**	**Malic acid**	**Oxalic acid**
Pseudo-first order	Qe (mmol g^{-1})	0.077	n/a[1]	0.003	n/a
	k_1 (h^{-1})	0.026	n/a	0.019	n/a
	R^2	0.991	n/a	0.561	n/a
Pseudo-second order	Qe (mmol g^{-1})	0.102	0.009	0.009	0.099
	k_2 (g mmol^{-1} h^{-1})	1.555	371.717	55.432	1319.25
	R^2	0.986	1.000	0.995	1.000
Intraparticle diffusion	k_i (mmol g^{-1} h$^{0.5}$)	0.009	0.001	0.004	0.006
	C (mmol g$^{-1)}$	0.009	0.003	0.004	0.050
	R^2	0.967	0.684	0.781	0.378

[1] n/a; not applicable.

Lead adsorption in LMWOAs did not correspond to the pseudo-first-order model but fit the pseudo-second-order model. Qe values matched the experimental Qe for all LMWOAs, suggesting the limited adsorption observed is well described by the pseudo-second-rate order model (Appendix A2). For the LMWOA containing systems, the adsorption data did not report a good level of fit with the intraparticle diffusion model implying lead adsorption in these systems did not follow intraparticle diffusion kinetics. In brief, lead adsorption onto rice husk biochar in water is defined best by either pseudo-first-order or pseudo-second-order kinetics as both models had good levels of fit but the second-rate order better matched the experimental Qe. The pseudo-second-order model best predicts the adsorption kinetics of lead in LMWOAs.

4.3.4 Zinc-containing systems

4.3.4.1 pH and electrical conductivity of the zinc-containing solutions

The pH and EC of the zinc-containing solutions are shown in Table 4.18. The pH of the water-zinc systems was always significantly more alkaline following the addition of biochar ($p < 0.05$). The pH difference was most apparent at 120 h when biochar increased the pH by approximately 0.93 units. Over time, the water control significantly increased from 24 h onwards ($p < 0.05$) before decreasing from 72 h to 120 h by around 0.49 units. The biochar treatment significantly increased the pH between each sampling interval ($p < 0.05$). The citric acid control was always more alkaline than the treatment ($p < 0.05$) and slightly increased with time, achieving significance at 72 h. The temporal change in the citric acid treatment was more apparent, with a significant increase of over 1 unit from 1 h to 24 h ($p < 0.05$). After this point, the pH ranged from 2.69 to 2.75 but was not significantly different across time points ($p < 0.05$). The pH in the malic acid systems was always more alkaline after biochar application ($p < 0.05$). For instance, at 120 h the pH in the malic acid control was 2.53 opposed to 3.13. Over time, pH was inclined to increase, but significance was only achieved between 1 h compared to 120 h ($p < 0.05$). The pH in the oxalic acid systems was much more acidic than all other systems. The pH in the oxalic acid control was always more acidic compared to those amended with biochar ($p < 0.05$). The liming effect of the biochar was most apparent at 72 h and 120 h with an increase of around 0.21 units at each instance. Temporally the pH of the oxalic acid control increased but significance was only achieved from 72 h onwards ($p < 0.05$). In comparison, the oxalic acid treatment also increased with contact time, achieving significance from 24 h onwards ($p < 0.05$).

Table 4.18 pH and electrical conductivity (EC) (mS cm^{-1}) results at various time intervals (h) in zinc-containing solutions with either water, citric, malic or oxalic acid and the addition of 0 or 1 g dL^{-1} RH700 biochar

Parameter	**Solution**	**Biochar (g dL^{-1})**	**Sampling interval**			
			1 h	**24 h**	**72 h**	**120 h**
pH	Control	0	5.44±0.01Cb	6.55±0.04Ab	6.65±0.04Ab	6.16±0.04Bb
	Citric	0	2.36±0.00Bf	2.36±0.01Bf	2.41±0.00Af	2.42±0.02Af
	Malic	0	2.45±0.03Be	2.49±0.01ABe	2.50±0.01ABe	2.53±0.01Ae
	Oxalic	0	1.83±0.01Ah	1.82±0.01ABh	1.75±0.02Bh	1.87±0.03Ah
	Control	1	5.89±0.04Da	6.93±0.03Ba	6.83±0.00Ca	7.09±0.00Aa
	Citric	1	2.57±0.01Bd	2.69±0.01Ad	2.74±0.02Ad	2.75±0.04Ad
	Malic	1	2.72±0.02Cc	2.90±0.02Bc	2.90±0.04Bc	3.13±0.01Ac
	Oxalic	1	1.92±0.02BCg	1.90±0.01Cg	1.96±0.02Bg	2.08±0.02Ag
EC (mS cm^{-1})	Control	0	0.23±0.00Af	0.23±0.00Ae	0.23±0.00Af	0.23±0.00Af
	Citric	0	1.55±0.00Dc	1.62±0.01Ac	1.57±0.00Cc	1.59±0.01Bc
	Malic	0	1.20±0.01Ad	1.23±0.02Acd	1.20±0.01Ad	1.21±0.01Ad
	Oxalic	0	6.02±0.04Aa	6.03±0.37Aa	6.08±0.04Aa	6.09±0.03Aa
	Control	1	0.25±0.00Df	0.27±0.00Ce	0.28±0.00Bf	0.30±0.00Af
	Citric	1	1.32±0.03Ad	0.91±0.01Cd	1.10±0.04Bd	1.06±0.04Bd
	Malic	1	1.02±0.02Be	1.16±0.02Ad	0.84±0.02Ce	0.77±0.01De
	Oxalic	1	5.23±0.16Ab	4.92±0.00Ab	4.15±0.17Bb	3.76±0.14Bb

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The EC of the water control was 0.23 mS cm^{-1} throughout the experiment and was no different from the biochar treatment ($p > 0.05$). Contrastingly, biochar increased the EC compared to the control and was statistically significant at each interval ($p < 0.05$). In the citric acid systems, the control EC ranged from 1.55 mS cm^{-1} to 1.62 mS cm^{-1}. The EC tended to fluctuate over time, first increasing then decreasing, and was different at each time interval ($p < 0.05$). Regardless, the EC in the control was always higher than in the biochar treated systems ($p < 0.05$). With the addition of biochar, EC initially decreased from 1.32 mS cm^{-1} at 1 h to 0.91 mS cm^{-1} at 24 h ($p < 0.05$). Then, the EC increased by around 0.2 units at 72 h ($p < 0.05$) and a mean EC of around 1.08 was kept until the end of the experiment. In malic acid systems, the EC in the control was significantly higher than the biochar treatment at 1 h, 72 h and 120 h, respectively ($p < 0.05$). No temporal change was observed in the malic acid control. However, biochar initially increased the EC at 24 h, before sharply declining from 1.16 mS cm^{-1} to 0.77 mS cm^{-1} at 120 h. The EC at all intervals for the biochar treatment in malic acid was statistically different from each other ($p < 0.05$). Concerning oxalic acid systems, the EC of the control was the highest EC of all systems studied, ranging from 6.02 mS cm^{-1} to 6.09 mS cm^{-1}. There were no temporal variations in EC in the control, whereas in the treated systems, EC declined with time. The change was statistically significant between 24 h and 72 h, respectively ($p < 0.05$). Compared to the control, the biochar significantly decreased the EC of the treated solutions at all intervals ($p < 0.05$).

4.3.4.2 The effect of organic acids on biochar removal of zinc

The results for the zinc batch sorption study are shown in Table 4.19. In the water control, no deviation from the initial concentration was found. With the addition of biochar, the amount of zinc in solution significantly decreased after 24 h of incubation and was maintained until the experiment end ($p < 0.05$). At 1 h the removal of zinc was slow, but nearly 50 % of all soluble zinc was removed by 24 h. The concentration at each interval was significantly lower than at the earlier intervals ($p < 0.05$).

The behaviour of zinc in the presence of LMWOAs was erratic. In the citric acid control, the concentration of soluble zinc was between around 47 to 88 % less than the theoretical initial concentration, suggestive of interactions between zinc and citrate ions. From 1 h to 24 h, concentrations significantly decreased from 0.53 mmol L^{-1} to 0.12 mmol

L^{-1} and then at 72 h increased to a similar level to that observed at 1 h ($p < 0.05$). The amount of zinc was statistically the same at 72 h and 120 h indicating no further changes to zinc solubility in the presence of citric acid. The application of biochar was found to decrease soluble zinc at 1 h ($p < 0.05$). However, at 24 h, no effect was observed compared to control systems ($p > 0.05$). On the contrary, by 72 h and at 120 h, the concentration of zinc increased with the biochar treatment ($p < 0.05$). Temporal variations showed a significant decrease in concentration after 1 h and then like the control system, an increase was observed at 72 h ($p < 0.05$). Also, like the citric acid control, no further significant temporal trend was observed after 72 h.

Table 4.19 Zinc concentrations (mmol L^{-1}) at various time intervals (h) in either water, citric, malic or oxalic acid-containing solutions with the addition of 0 or 1 g dL^{-1} RH700 biochar

Treatment	**Biochar (g dL^{-1})**	**Zinc concentration (mmol L^{-1})**			
		1 h	**24 h**	**72 h**	**120 h**
Water	0	1.07±0.01Aa	1.07±0.01Aa	1.07±0.01Aa	1.07±0.01Aa
Citric	0	0.53±0.09Ab	0.12±0.02Bd	0.51±0.05Ad	0.45±0.03Ad
Malic	0	0.89±0.06Aa	0.27±0.07Cc	0.75±0.04ABb	0.61±0.04Bc
Oxalic	0	0.39±0.12Abc	0.13±0.03Bd	0.23±0.02ABe	0.14±0.01Be
Water	1	0.91±0.01Aa	0.52±0.01Bb	0.27±0.01Ce	0.17±0.00De
Citric	1	0.27±0.06Bc	0.13±0.01Cd	0.63±0.00Ac	0.69±0.01Ab
Malic	1	0.47±0.08Bbc	0.14±0.00Cd	0.65±0.00Ac	0.70±0.00Ab
Oxalic	1	0.23±0.10Ac	0.08±0.01Ad	0.24±0.01Ae	0.19±0.01Ae

All values are presented as mean ± standard error ($n = 3$). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

In the malic acid system, deviation from the theoretical initial concentration was observed as described for citric acid. Temporally, there was an initial decrease at 24 h ($p < 0.05$) followed by an increase at 72 h ($p > 0.05$), and lastly, a decline to less than that observed a 1 h ($p < 0.05$). The biochar treatment significantly removed soluble zinc at 1 h, 24 h and 72 h respectively. However, by 120 h, more zinc was released into solution, and this was significantly greater than the zinc found in the control ($p < 0.05$). Similar to the other acids, the fluctuation in concentrations was also found in the oxalic acid control and treatment. In the control, the concentration significantly decreased from 1 h to 24 h, but no further change was significant ($p < 0.05$). However, a comparison of the concentration at 1 h (0.39 mmol L^{-1}) compared to that at 120 h (0.14 mmol L^{-1}) confirms an overall decline in soluble zinc at the experiment end ($p < 0.05$). Biochar did not affect soluble zinc at any time point ($p > 0.05$), suggesting that zinc removal was inhibited. No changes over time in soluble zinc were observed throughout ($p > 0.05$). In summary, the behaviour of zinc in the presence of LMWOAs was complicated. The findings showed:

1. In water biochar removed around 83 % of soluble zinc from solution.
2. Biochar was initially able to decrease soluble zinc in citric acid, but no removal after 1 h was found.
3. Malic acid did not impede removal of zinc at 1 h, 24 h and 72 h but did prevent removal at 120 h.
4. In oxalic acid, biochar was unable to remove soluble zinc.

4.3.4.3 Zinc adsorption kinetics in different solution types

The adsorption kinetics for zinc onto biochar in water and the three model LMWOAs were studied (Table 4.20). In the water, zinc adsorption showed a poor level of fit to pseudo-first-order kinetics ($R^2 = 0.650$), and the experimental Qe (Appendix A2) was higher than predicted by the model.

Table 4.20 Pseudo-first-order, pseudo-second-order and intraparticle diffusion kinetic model parameters for systems containing zinc and RH700 biochar in the presence of either water, citric acid, malic acid, or oxalic acid

Kinetic model	**Parameter**	**Solution type**			
		Control	**Citric acid**	**Malic acid**	**Oxalic acid**
Pseudo-first order	Qe (mmol g^{-1})	0.047	n/a[1]	n/a	n/a
	k_1 (h^{-1})	0.220	n/a	n/a	n/a
	R^2	0.650	n/a	n/a	n/a
Pseudo-second order	Qe (mmol g^{-1})	0.086	0.031	0.030	0.080
	k_2 (g $mmol^{-1}$ h^{-1})	1.274	-5.854	-6.227	-33.692
	R^2	0.980	0.980	0.981	0.998
Intraparticle diffusion	k_i (mmol g^{-1} $h^{0.5}$)	0.009	0.009	0.009	0.005
	C (mmol g^{-1})	0.010	0.010	0.004	0.015
	R^2	0.953	0.953	0.986	0.755

[1] n/a; not applicable.

When fitted to the pseudo-second-order model, a good level of fit was found ($R^2 = 0.980$), and experimental Qe values were nearly identical to those predicted by the model (0.083 mmol g^{-1} versus 0.086 mmol g^{-1}) (Appendix A2). There was also a relatively good level of fit to the intraparticle diffusion model ($R^2 = 0.953$). However, the lack of correlation between the experimental Qe and predicted Qe values (Appendix A2) suggests it is not appropriate to describe zinc adsorption kinetics onto biochar by using this model.

The adsorption of zinc in the presence of LMWOAs did not fit to the pseudo-first-order model. Equally, the pseudo-second-order rate parameter (k_2) were all negative for all acids, which is both experimentally and practically impossible. This is likely due to increased concentrations from 1 h onwards. Lastly, the lack of correlation between the experimental Qe and predicted Qe values for the zinc fitted to the intraparticle diffusion model (Appendix A2) suggests it is not appropriate to describe zinc adsorption kinetics onto biochar using intraparticle diffusion. In summary, pseudo-second-order kinetics best described the adsorption of zinc onto biochar in water systems. Zinc adsorption onto biochar in the presence of LMWOAs was not well described by any of the three models examined.

4.3.5 pH vs. metal concentration bivariate correlations

The pH of the solution may affect metal solubility. To test the relationship, a bivariate correlation was carried out (Table 4.21). For cadmium in water, there was a significant strong negative linear relationship between the solution pH and the cadmium concentration ($R^2 = -0.965$) ($p < 0.01$). This shows that as pH increased, the concentration of cadmium decreased. There were no significant relationships between the pH in either of the LMWOA-containing solutions and the cadmium concentrations in the respective solutions ($p > 0.05$). For lead in water, there was a significant strong negative linear relationship between the solution pH and the lead concentration (R^2 = -0.982) ($p < 0.01$). Likewise, in the citric acid solution, there was a significant strong negative linear relationship between solution pH and the lead concentration (R^2 = -0.760) ($p < 0.01$). The pH in the malic acid-containing solution showed a moderate significant relationship with lead concentration (R^2 = - 0.661) ($p < 0.05$). Therefore, as the pH of the solutions increased, the lead concentration decreased. There was no significant relationship between the pH of the oxalic acid-containing solution and the lead concentration. Finally, for zinc in the water, there was a significant strong negative linear relationship (R^2 = -0.913) ($p < 0.01$) between solution pH and zinc concentration, indicated by the reduced zinc concentration with increasing pH. In the citric acid solution, there was a significant moderate positive linear relationship between solution pH and zinc concentration suggestive of increasing zinc concentrations with increased alkalinity (R^2 = 0.604) ($p < 0.05$). There was no significant relationship between the pH of the malic and oxalic acid-containing solutions and the zinc concentration available.

Table 4.21 Bivariate correlation results for pH in the different metal containing-solutions versus the metal concentrations in the water, citric, malic or oxalic acid-containing treatments

Metal	**Solution**	**pH vs. Concentration bivariate correlation**		
		Pearson's Correlation (R^2)	**Sig. (2-tailed)**	***n***
Cadmium	Water	-.965**	< 0.001	12
	Citric	-0.222	0.488	12
	Malic	-0.109	0.735	12
	Oxalic	-0.072	0.825	12
Lead	Water	-0.982**	< 0.001	12
	Citric	-0.760**	0.004	12
	Malic	-0.661*	0.019	12
	Oxalic	-0.400	0.197	12
Zinc	Water	-0.913**	< 0.001	12
	Citric	0.604*	0.038	12
	Malic	0.358	0.253	12
	Oxalic	0.320	0.311	12

*Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

4.4 Results for hypothesis 2

4.4.1 Systems with biochar and no-added nutrients

The pH and EC results for these systems were shown in Table 4.11 and described in section 4.3.1. The amount of nitrate and phosphate released is presented in Table 4.22. Small levels of biochar-borne nitrate was released in all four systems (0 to 0.01 mmol L^{-1}). For the water and malic acid-containing systems, biochar-borne nitrate was present from 1 h. In citric acid, no nitrate was detected after the 1 h interval. For oxalic acid-containing systems, values fluctuated from 0 to 0.01 mmol L^{-1}. The four systems were always statistically similar ($p > 0.05$).

Table 4.22 Nitrate and phosphate (mmol L^{-1}) concentrations at various time intervals (h) in either water, citric, malic or oxalic acid-containing solutions with the addition of 1 g dL-1 RH700 biochar and no added nutrients

Parameter	Solution	Sampling interval			
		1 h	24 h	72 h	120 h
Nitrate (mmol L^{-1})	Water	0.01±0.00Aa	0.01±0.00Aa	0.01±0.00Aa	0.01±0.00Aa
	Citric	0.01±0.00Aa	0.00±0.00Ba	0.00±0.00Ba	0.00±0.00Ba
	Malic	0.01±0.00Aa	0.01±0.00Aa	0.01±0.00Aa	0.01±0.00Aa
	Oxalic	0.01±0.00Aa	0.00±0.00Ba	0.01±0.00Aa	0.00±0.00Ba
Phosphate (mmol L^{-1})	Water	0.03±0.00Cb	0.03±0.00Cb	0.04±0.00Bb	0.05±0.00Aa
	Citric	0.07±0.00Ba	0.08±0.00Aa	0.04±0.00Cb	0.01±0.00Da
	Malic	0.07±0.00Ba	0.08±0.00Aa	0.08±0.00Aa	0.08±0.00Aa
	Oxalic	0.07±0.00Ca	0.08±0.00Ba	0.08±0.00Ba	0.09±0.00Aa

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

Biochar-borne phosphate was released in all four systems. In water, values ranged from 0.03 to 0.05 mmol L^{-1} and increased with time. Except at 72 h, biochar-borne phosphate in water was significantly less than those in the acid-containing systems ($p < 0.05$). Furthermore, at 72 h, citric acid released significantly less phosphate than biochar in the other two LMWOAs. No statistical difference was found at the other intervals between the LMWOAs. Notably, the amount of extracted phosphate decreased in citric acid systems but increased in the other LMWOAs systems over time.

4.4.2 Nitrate-containing systems

4.4.2.1 pH and electrical conductivity of the nitrate-containing solutions

The pH and EC results for the nitrate-containing systems can be seen in Table 4.23. In the water control, the pH ranged from 4.13 to 4.63, but there was no significant temporal variation ($p > 0.05$). In the biochar treated systems, the pH was significantly higher compared to the control ($p < 0.05$). For instance, the pH in the water biochar treatment ranged from 8.70 to 9.63. Initially, the pH increased sharply from 1 h to 24 h ($p < 0.05$). Between 72 h and 120 h, the pH fell slightly but the pH was still alkaline (9.23). At 24 h and 72 h, the pH remained similar.

Table 4.23 pH and electrical conductivity (EC) (mS cm^{-1}) results at various time intervals (h) in nitrate-containing solutions with either water, citric, malic or oxalic acid and the addition of 0 or 1 g dL^{-1} RH700 biochar

Parameter	Solution	Biochar (g dL^{-1})	Sampling interval			
			1 h	**24 h**	**72 h**	**120 h**
pH	Water	0	4.63±0.02Ab	4.13±0.20Ab	4.33±0.21Ab	4.13±0.20Ab
	Citric	0	2.52±0.03Ae	2.56±0.07Ad	2.49±0.02Ad	2.56±0.07Ad
	Malic	0	2.63±0.02Ade	2.62±0.01Ad	2.64±0.02Ad	2.62±0.01Ad
	Oxalic	0	1.88±0.02Ag	1.89±0.02Ae	1.90±0.02Ae	1.89±0.02Ae
	Water	1	8.70±0.09Ca	9.55±0.08Aa	9.63±0.14Aa	9.23±0.01Ba
	Citric	1	2.66±0.03Bd	2.74±0.02Acd	2.76±0.01Acd	2.74±0.02Ad
	Malic	1	2.83±0.03Bc	2.98±0.02Ac	3.03±0.01Ac	2.98±0.02Ac
	Oxalic	1	2.03±0.06Af	2.02±0.02Ae	2.09±0.06Ae	2.02±0.02Ae
EC (mS cm^{-1})	Water	0	0.11±0.00Be	0.13±0.02Bg	0.13±0.00Bf	0.35±0.09Af
	Citric	0	1.44±0.01Ac	1.41±0.02Ac	1.30±0.13Ac	1.41±0.02Ac
	Malic	0	1.05±0.01Acd	1.03±0.01Ad	1.04±0.01Ad	1.03±0.01Ad
	Oxalic	0	6.07±0.03Aa	5.99±0.04Aa	6.08±0.06Aa	5.99±0.03Aa
	Water	1	0.17±0.00De	0.25±0.00Af	0.23±0.01Bf	0.21±0.00Cg
	Citric	1	0.88±0.38Ad	1.06±0.01Ad	1.03±0.01Ad	1.06±0.02Ad
	Malic	1	0.91±0.00Ad	0.78±0.01Be	0.76±0.01Be	0.78±0.01Be
	Oxalic	1	5.50±0.02Ab	4.72±0.06Bb	4.22±0.06Cb	4.72±0.02Bb

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

In the citric acid control, the pH was expectedly acidic, ranging from 2.49 to 2.56. There was no temporal trend seen ($p > 0.05$). The biochar treatment only had a significantly higher pH at 1 h ($p < 0.05$). All other changes were not significant compared to the control ($p > 0.05$). The malic acid control kept a pH of around 2.62 throughout ($p > 0.05$), while in the biochar treatment, the pH was higher at all intervals ($p < 0.05$). In the treatment, the pH initially increased from 2.83 at 1 h to 2.98 at 24 h ($p < 0.05$). After this time, there was no significant pH change ($p > 0.05$). The oxalic acid control was the most acidic compared to the other LMWOAs. No temporal changes occurred. Similarly, no temporal changes were observed in the presence of biochar ($p > 0.05$). However, the pH significantly increased at 1 h compared to the control ($p < 0.05$) but no further changes in pH was observed in the presence of biochar ($p > 0.05$).

In the nitrate-containing water control, the EC ranged from 0.11 to 0.35 mS cm^{-1} significantly increasing at 120 h ($p < 0.05$). The presence of biochar tended to increase the EC, but this was only significant at 24 h and 120 h ($p < 0.05$). In the citric acid control, no change in EC was seen over time ($p > 0.05$). In the presence of biochar, the EC was always significantly lower than the control ($p < 0.05$). Like the control, no temporal variation in EC was seen in the biochar-containing counterpart ($p > 0.05$). In the malic acid control, the EC stayed constant with time ($p > 0.05$). Systems containing biochar tended to have a lower EC, but this was only significant at 24 h onwards ($p < 0.05$). The EC in oxalic acid systems was much higher than the other systems. The EC in the control ranged from 5.99 mS cm^{-1} to 6.08 mS cm^{-1}, but the increase was not significant over time ($p > 0.05$). Following the biochar application, the EC of the oxalic acid solutions significantly decreased at all intervals ranging from 4.22 to 5.50 mS cm^{-1} ($p < 0.05$). The EC initially fell gradually from 1 h to 72 h ($p < 0.05$), before returning to a similar level to that observed at 24 h (4.72 mS cm^{-1}) ($p > 0.05$).

4.4.2.2 The effect of organic acids on biochar removal of nitrate

The results for the nitrate batch sorption study are shown in Table 4.24. In the water control, the theoretical initial concentration was maintained over time, and the presence of biochar did not affect nitrate concentrations ($p > 0.05$). The results show that biochar was unable to adsorb nitrate in water.

Table 4.24 Nitrate concentrations (mmol L^{-1}) at various time intervals (h) in either water, citric, malic or oxalic acid-containing solutions with the addition of 0 or 1 g dL^{-1} RH700 biochar

Treatment	**Biochar (g dL^{-1})**	**Nitrate concentration (mmol L^{-1})**			
		1 h	**24 h**	**72 h**	**120 h**
Water	0	1.01±0.00Aa	1.01±0.00Aa	1.01±0.00Aa	1.00±0.01Aa
Citric	0	1.01±0.03Aa	1.01±0.01Aa	1.01±0.01Aa	1.02±0.01Aa
Malic	0	0.94±0.01Aa	0.95±0.01Ab	0.96±0.02Ab	0.97±0.01Ab
Oxalic	0	0.97±0.01Ba	1.00±0.00ABa	1.02±0.01Aa	1.03±0.02Aa
Water	1	1.01±0.00Aa	1.01±0.01Aa	1.00±0.01Aa	1.00±0.01Aa
Citric	1	0.77±0.01Ac	0.37±0.02Be	0.13±0.01Ce	0.04±0.01Dd
Malic	1	0.87±0.06Ab	0.45±0.01Bc	0.17±0.01Cd	0.03±0.00Dd
Oxalic	1	0.75±0.01Ac	0.4±0.01Bd	0.40±0.01Bc	0.39±0.01Bc

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

In the citric acid control, the theoretical initial concentration was maintained ($p > 0.05$). However, the nitrate concentrations significantly decreased at all intervals to nearly 100% removal at 120 h, following the addition of biochar ($p < 0.05$). The concentration initially fell sharply between 1 h and 24 h (0.77 mmol L^{-1} to 0.37 mmol L^{-1}) ($p < 0.05$). From 24 h onwards, the concentration continued to decline but at a more gradual rate ($p < 0.05$). In the malic acid systems, the control was slightly less than the theoretical initial concentration but was maintained throughout ($p > 0.05$). In malic acid with added biochar, the nitrate concentration followed a similar temporal variation to that of the citric acid with added biochar ($p < 0.05$). These suggest biochar affected soluble nitrate concentrations. In the oxalic acid control, the initial concentration was more or less maintained. In the system with added biochar, nitrate concentrations decreased at all intervals compared to the control ($p < 0.05$). After a drop from 0.75 mmol L^{-1} to 0.40 mmol L^{-1} between 1 h and 24 h (p <

0.05), the concentration then stayed the same throughout the experiment ($p > 0.05$). In summary:

1. In water with added biochar, nitrate concentrations were unaffected.
2. In citric and malic acid with added biochar, nearly 100 % of nitrate was removed.
3. In oxalic acid with added biochar, a reduction in nitrate was observed but to a lesser extent than observed for citric and malic acid counterparts.

4.4.2.3 Nitrate adsorption kinetics in different solution types

No nitrate adsorption by biochar in water was observed; therefore, the kinetics could not be determined. The adsorption kinetics for nitrates onto biochar in the LMWOAs were studied. Pseudo-first-order, pseudo-second-order and intraparticle diffusion models were applied to study rate kinetics and intraparticle diffusion (Table 4.25). The adsorption of nitrate to biochar in the presence of all LMWOAs does not fit to pseudo-first-order kinetics. A low R^2 and a disparity between the experimental Qe and model Qe values occurred (Appendix A2). For pseudo-second-order kinetics, there was a strong positive R^2 for all three of the LMWOA-containing systems indicating that the sorption of nitrate may follow second-order rate kinetics. The pseudo-second-order rate constant (k_2) suggests the rate order to be as follows: oxalic acid < citric acid < malic acid. When comparing the pseudo-second-order parameters to the experimental data, it is apparent that in the presence of oxalic acid, the removal of nitrate ions by the biochar materials was slower, as compared to the citric and malic acid systems. This is reflected by the lower estimated kinetic order Qe values (Table 4.25)

Nitrate adsorption in citric acid and malic acid-containing systems showed a good level of fit to the intraparticle diffusion model ($R^2 = > 0.953$) and had low C values, suggesting that intraparticle diffusion was not the sole rate-limiting step in nitrate adsorption. However, the level of linearity and closeness to passing through the origin suggests that intraparticle diffusion was present in nitrate adsorption. A more inadequate fit was observed for nitrate adsorption in oxalic acid (R^2 = > 0.755), and a slightly lower intraparticle diffusion model rate constant was also found. Furthermore, in oxalic acid, plots were not linear and could be separated into two regions. The initial linear steps for both biochar materials were steep, indicating that nitrate ions moved quickly towards the biochar surfaces. The second linear steps with lower slopes suggest another rate-limiting step was controlling the removal kinetics. Given the weak correlation to the intraparticle diffusion model, it can be concluded that intraparticle diffusion kinetics were not the rate-limiting step for nitrate adsorption in the presence of oxalic acid.

Table 4.25 Pseudo-first-order, pseudo-second-order and intraparticle diffusion kinetic model parameters for systems containing nitrate and RH700 biochar in the presence of citric acid, malic acid, or oxalic acid

Kinetic model	**Parameter**	**Solution type**		
		Citric acid	**Malic acid**	**Oxalic acid**
Pseudo-first order	Qe (mmol g^{-1})	0.071	0.082	0.013
	k_1 (h^{-1})	0.029	0.025	0.043
	R^2	0.660	0.632	0.559
Pseudo-second order	Qe (mmol g^{-1})	0.098	0.100	0.061
	k_2 (g $mmol^{-1}$ h^{-1})	1.903	1.146	21.79
	R^2	0.989	0.977	1.000
Intraparticle diffusion	k_i (mmol g^{-1} $h^{0.5}$)	0.009	0.009	0.005
	C (mmol g^{-1})	0.010	0.004	0.015
	R^2	0.953	0.986	0.755

4.4.3 Phosphate-containing systems

4.4.3.1 pH and electrical conductivity of the phosphate-containing solutions

The pH and EC results for the phosphate-containing systems can be seen in Table 4.26. In the water systems, the control pH ranged from 5.22 to 5.64. The pH was significantly higher from 1 h to 24 h and from 72 h to 120 h ($p < 0.05$). In the biochar treatment, the pH was significantly more alkaline, ranging from 7.11 to 9.24 ($p < 0.05$). The pH increased significantly until 72 h ($p < 0.05$), after which there was no change. The pH in the citric acid control was unaffected by time ($p > 0.05$). The pH of the biochar-amended citric acid solution was higher than the control ($p < 0.05$), but not at 72 h ($p > 0.05$). The malic acid control was also unaffected by time ($p > 0.05$). The pH of the malic acid system with added biochar was higher than the control at all intervals ($p < 0.05$). However, the increase in pH for each interval over time was not significant at 72 h ($p > 0.05$). The pH of the oxalic acid systems was more acidic than the other LMWOAs, and no change occurred in the control over time ($p > 0.05$). In contrast, in the biochar treatment, the pH slowly increased, with the pH being significantly more alkaline at 72 h and 120 h compared to the pH at 1 h ($p < 0.05$). The pH of the oxalic acid systems with added biochar was significantly higher than the control solution pH at all intervals, except for 72 h ($p < 0.05$).

Table 4.26 pH and electrical conductivity (EC) (mS cm^{-1}) results at various time intervals (h) in phosphate-containing solutions with either water, citric, malic or oxalic acid and the addition of 0 or 1 g dL^{-1} RH700 biochar

Parameter	Solution	Biochar (g dL^{-1})	Sampling interval			
			1 h	24 h	72 h	120 h
pH	Water	0	5.22±0.00Cb	5.61±0.07ABb	5.40±0.04BCb	5.64±0.11Ab
	Citric	0	2.44±0.02Ae	2.43±0.01Ae	2.45±0.03Ad	2.48±0.03Ae
	Malic	0	2.59±0.02Ad	2.57±0.00Ad	2.58±0.00Ad	2.62±0.02Ade
	Oxalic	0	1.85±0.02Ag	1.84±0.02Ag	1.85±0.01Ae	1.86±0.02Ag
	Water	1	7.11±0.04Ca	8.19±0.07Ba	9.07±0.18Aa	9.24±0.09Aa
	Citric	1	2.57±0.02Cd	2.63±0.02Bd	2.64±0.00ABd	2.67±0.01Ad
	Malic	1	2.73±0.02Cc	2.86±0.01Bc	2.89±0.00ABc	2.91±0.01Ac
	Oxalic	1	1.92±0.03Cf	1.97±0.02BCf	2.01±0.02ABe	2.10±0.03Af
EC (mS cm-1)	Water	0	0.88±0.00Af	0.71±0.01BCg	0.79±0.02ABh	0.67±0.05Ce
	Citric	0	2.38±0.01ABb	2.4±0.01Ac	2.37±0.01ABd	2.36±0.02Bc
	Malic	0	2.3±0.01BCc	2.33±0.00Ad	2.31±0.00Be	2.28±0.01Ccd
	Oxalic	0	2.7±0.01Aa	2.72±0.01Aa	2.7±0.01Ab	2.55±0.14Ab
	Water	1	1.63±0.03Ce	2.65±0.01Bb	3.04±0.00Aa	3.12±0.06Aa
	Citric	1	2.33±0.01Ac	2.29±0.01Be	2.27±0.01Cf	2.25±0.01Ccd
	Malic	1	2.23±0.01Ad	2.17±0.01Bf	2.14±0.00Bg	2.16±0.03Bd
	Oxalic	1	2.67±0.01Aa	2.66±0.01Ab	2.61±0.01Bc	2.57±0.01Bb

All values are presented as mean ± standard error ($n = 3$). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The EC of the water control ranged from 0.67 mS cm^{-1} to 0.88 mS cm^{-1} and decreased over time. The EC of the systems with added biochar was always significantly higher than the control ($p < 0.05$). For instance, the EC of the control at 120 h was 0.67 mS cm^{-1} compared to 3.12 mS cm^{-1} for the biochar treatment. The EC in the treatment tended to significantly increase with time until 72 h ($p < 0.05$). The EC in citric acid control showed no inclining or declining trend. In contrast, the EC of the biochar treatment tended to decrease with time until 72 h ($p <0.05$). Likewise, the EC in the citric acid with added biochar was lower than that of the control for the first three intervals ($p < 0.05$). The EC of the malic acid control significantly decreased from 24 h onwards ($p < 0.05$). In the biochar treatment, the EC significantly decreased from 1 h to 24 h but was constant afterwards ($p < 0.05$). The EC of the malic acid solutions with added biochar was less than the control at the first three intervals ($p < 0.05$). Lastly, the EC of the oxalic acid control decreased with time, but this was not significant ($p > 0.05$). In the biochar treatment, the EC was significantly lower compared to the control at 24 h and 72 h only ($p < 0.05$). In the treatment, the EC tended to decrease and significance was achieved between 24 h and 72 h ($p < 0.05$).

4.4.3.2 The effect of organic acids on biochar removal of phosphate

The results for the phosphate batch sorption study are shown in Table 4.27. In the water system control, the theoretical initial concentration tended to decrease at 24 h to 0.91 mmol L^{-1} before increasing from 72 h to 120 h, back to the theoretical initial concentration ($p < 0.05$). The pH of the water was always significantly higher with added biochar present ($p < 0.05$) and did not change with time ($p > 0.05$). In the citric acid control, the initial concentration followed the same pattern as the water control. This was also observed for the malic acid and oxalic acid controls ($p < 0.05$). The phosphate concentration significantly increased with biochar amendment ($p < 0.05$). Compared to the first three sampling occasions, the concentration of soluble phosphate increased significantly at 120 h ($p < 0.05$).

Table 4.27 Phosphate concentrations (mmol L^{-1}) at various time intervals (h) in either water, citric, malic or oxalic acid-containing solutions with the addition of 0 or 1 g dL^{-1} RH700 biochar

Solution	**Biochar (g dL^{-1})**	**Phosphate concentration (mmol L^{-1})**			
		1 h	**24 h**	**72 h**	**120 h**
Water	0	1.01±0.01Ae	0.91±0.00Be	0.91±0.00Be	1.01±0.01Ae
Citric	0	0.97±0.01Af	0.89±0.00Bef	0.89±0.00Bef	0.96±0.01Af
Malic	0	0.95±0.00Af	0.86±0.01Bf	0.86±0.01Bf	0.95±0.00Af
Oxalic	0	1.04±0.00Ad	0.95±0.01Bd	0.95±0.01Bd	1.04±0.00Ad
Water	1	1.05±0.01Ad	1.05±0.01Ac	1.04±0.01Ac	1.05±0.01Ad
Citric	1	1.20±0.01Bb	1.18±0.01Bb	1.18±0.01Bb	1.32±0.02Ab
Malic	1	1.17±0.01Bc	1.17±0.01Bb	1.17±0.01Bb	1.26±0.02Ac
Oxalic	1	1.30±0.01Ba	1.28±0.01Ba	1.28±0.01Ba	1.36±0.01Aa

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

In malic acid with added biochar, the phosphate concentrations also increased at all intervals ($p < 0.05$). The concentration released tended to stay constant until 72 h, after which a significant increase in phosphate released was observed ($p < 0.05$). Finally, in the oxalic acid systems, the same temporal trend was observed in the biochar treatment as was described for malic acid. However, in the oxalic acid biochar treatment, significantly more phosphate was released compared to the other acids ($p < 0.05$). In summary:

1. In water with added biochar, phosphate was released at all intervals.

2. In citric, malic and oxalic acid with added biochar, phosphate was released at all intervals. This was greater than the amount of phosphate released in the water systems.

4.4.3.3 Phosphate release in different solution types

To contextualise the observed release of phosphate, the nutrient release percentage was calculated using the formula provided in Equation 3.8. As shown in Figure 4.6, the biochar in the control solutions released 2 % of the total amount of phosphate added after 1 h and released no more phosphate ions afterwards. The amount released in the LMWOAs solutions was constant to the order of oxalic acid > citric acid > malic acid-containing systems, until 72 h when a more acute increase of released phosphate was observed.

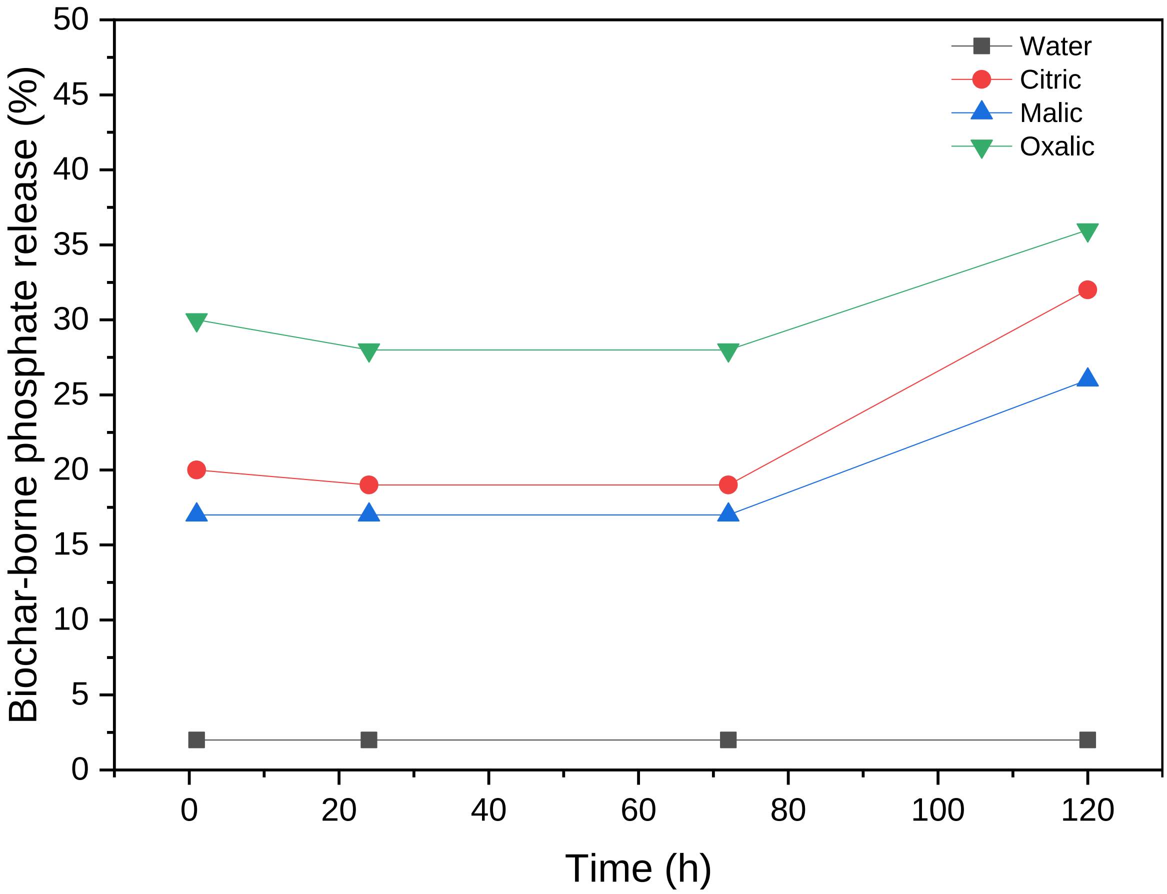

Figure 4.6 The amount of phosphate leached into water or the different LMWOA solutions (%) from rice husk biochar over 120 h contact time.

4.4.4 pH vs. nutrient concentration bivariate correlations

Bivariate correlation results between the pH and nutrient concentrations are presented in Table 4.28. There was no significant correlation between the pH of the control or oxalic acid-containing systems and the relative soluble nitrate concentration. However, there was a significant negative linear relationship between pH of the citric and malic acid-containing systems and the amount of nitrate or phosphate in these systems (R^2 = -0.732 and -0.814 respectively) ($p < 0.01$). Therefore, lower pH was associated with higher soluble nitrate concentrations.

For the phosphate, there was no significant correlation between solution pH and the concentrations in any system, except for phosphate in oxalic acid. A moderate positive linear relationship existed between the pH of the oxalic acid systems and the concentration of phosphate in solution (R^2 = 0.662), which means, that when the pH of the system increased, so did the concentration of phosphate in solution.

Table 4.28 Bivariate correlation results for pH in the different nutrient containing-solutions versus the nutrient concentrations in the water, citric, malic or oxalic acid-containing treatments

Nutrient	**System**	**pH vs. Concentration bivariate correlation**		
		Pearson's Correlation (R^2)	**Sig. (2-tailed)**	***n***
Nitrate	Water	-0.103	0.751	12
	Citric	-0.732**	0.007	12
	Malic	-0.814**	0.001	12
	Oxalic	-0.107	0.741	12
Phosphate	Water	-0.119	0.713	12
	Citric	0.542	0.068	12
	Malic	0.415	0.18	12
	Oxalic	0.662*	0.019	12

*Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

4.5 Results for hypothesis 3

4.5.1 Cadmium-containing soils

4.5.1.1 pH and electrical conductivity of the cadmium-containing soils

The pH and EC results for the cadmium-containing soils are presented in Table 4.29. In the LMWOA-free system without added biochar, the pH ranged from 5.85 to 6.32. The pH initially decreased between 1 h and 24 h, before increasing again between 72 and 120 h ($p < 0.05$). In the treated water systems, the pH ranged from 6.65 to 7.24, and despite an initial decrease in pH at 24 h and 72 h ($p < 0.05$), the system became continually more alkaline between 72 h and 120 h ($p < 0.05$). When comparing the water control to the biochar-added treatment, the pH was continually more alkaline in the presence of biochar ($p < 0.05$).

Table 4.29 pH pH and electrical conductivity (EC) (mS cm^{-1}) of the overlying water layer at various time intervals (h) in either water, citric, malic or oxalic acid-containing cadmium-containing soils. Systems contain either 0 or 1 g dL^{-1} RH700.

Parameter	**Treatment**	**Biochar (g dL^{-1})**	**Sampling interval**			
			1 h	**24 h**	**72 h**	**120 h**
pH	Water	0	6.32±0.03Ab	5.88±0.05Bb	5.85±0.01Bb	6.27±0.04Ac
	Citric	0	2.73±0.01Dd	3.00±0.02Cd	3.26±0.05Be	3.74±0.02Ag
	Malic	0	2.92±0.01Dc	3.15±0.08Cd	3.55±0.02Bd	4.46±0.05Af
	Oxalic	0	2.11±0.01Ce	2.3±0.04BCe	2.77±0.03Bf	5.03±0.37Ae
	Water	1	7.24±0.01Aa	7.01±0.03Ba	6.65±0.09Da	6.89±0.02Ca
	Citric	1	2.89±0.05Dc	3.02±0.00Cd	3.34±0.02Be	4.09±0.05Afg
	Malic	1	2.13±0.02De	2.53±0.01Ce	3.31±0.01Be	6.48±0.01Ab
	Oxalic	1	2.94±0.01Dc	3.24±0.01Cc	3.79±0.01Bc	5.57±0.02Ad
EC (mS cm^{-1})	Water	0	0.71±0.02Bf	0.72±0.00Bd	0.74±0.01Be	0.78±0.01Ad
	Citric	0	1.37±0.00Ad	1.29±0.01Bc	1.23±0.01Cb	1.20±0.01Da
	Malic	0	1.19±0.01Be	1.86±0.28Aab	1.11±0.00Bd	0.98±0.01Bbc
	Oxalic	0	3.49±0.08Ab	2.16±0.04Ba	1.31±0.01Ca	0.71±0.02Dd
	Water	1	0.75±0.01Df	0.79±0.00Cd	0.81±0.01Be	0.85±0.00Acd
	Citric	1	1.35±0.01Ad	1.25±0.01Bc	1.16±0.01Cc	0.98±0.03Dbc
	Malic	1	3.27±0.01Ac	1.71±0.00Bb	0.90±0.00De	1.02±0.01Cb
	Oxalic	1	1.20±0.00Ae	1.15±0.00Bc	1.09±0.00Cd	0.93±0.02Dc

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The pH of the citric acid control soil ranged from 2.73 to 3.74, increasing over time. Each sampling interval was significantly more alkaline than the previous pH ($p < 0.05$). In the treated system, the pH was only different at 1 h, where an increase of around 0.16 units was observed ($p < 0.05$). The pH in the biochar treatment also increased over time from 2.89 to 4.09 and each sampling point was significantly more acidic than the previous ($p < 0.05$). In the malic acid control, soil pH ranged from 2.92 to 4.46, increasing in pH with each sampling interval ($p < 0.05$). The biochar treatment pH was significantly more acidic at the first three intervals but was significantly more alkaline compared to the control at 120 h (6.48 versus 4.46) ($p < 0.05$). Like the malic acid control, the pH also increased with time and was more alkaline at each interval ($p < 0.05$). Lastly, the pH in the oxalic acid control was initially very acidic (2.11 to 2.77). However by 120 h, the pH increased to 5.03. The pH only achieved temporal significance from 72 h onwards ($p < 0.05$). In the treated oxalic acid systems, the pH was more alkaline at all intervals compared to the control pH ($p < 0.05$). The pH in the oxalic acid biochar treatment also increased with time and was significantly more alkaline at each interval ($p < 0.05$).

Turning now to the EC results, the EC for the water control ranged from 0.71 mS cm^{-1} to 0.78 mS cm^{-1} and achieved temporal significance at 120 h ($p < 0.05$). The EC of the control was no different from the EC of the biochar treated system ($p > 0.05$). In the biochar treated system, the EC significantly increased at each interval ($p < 0.05$), ranging from 0.75 mS cm^{-1} to 0.85 mS cm^{-1}. The EC in the citric acid control soil significantly decreased at each time interval time, ranging from 1.37 mS cm^{-1} to 1.20 mS cm^{-1} ($p < 0.05$). Initially, the EC was no different in the control compared to the treatment, but at 72 h and 120 h, the EC was significantly lower in the biochar-amended system ($p < 0.05$). Like the control, the EC in the treatment decreased with time and was significantly lower at each interval ($p < 0.05$).

In the malic acid treatment, the control tended to decrease with time, but significance was only achieved at 24 h ($p < 0.05$). Initially, the EC was higher in the biochar-amended malic acid system by around 2.08 mS cm^{-1} at 1 h ($p < 0.05$). However, at each successive interval, the EC was significantly lower in the treatment as opposed to the control ($p < 0.05$). The EC in the malic acid biochar treatment initially fell sharply from 1 h through to 72 h ($p < 0.05$). The EC then increased slightly but was lesser than that observed at 24 h ($p < 0.05$). The EC in the oxalic acid control was initially high (3.49 mS cm^{-1}) before gradually decreasing at each interval ($p < 0.05$). In the treated oxalic acid system, the EC significantly decreased the EC compared to the control, at 1 h, 24 h and 72 h ($p < 0.05$). However, at 120 h, the EC was significantly higher following biochar application ($p < 0.05$). The temporal variation in the treatment was like the control, decreasing overtime at each interval ($p < 0.05$).

4.5.1.2 Biochar and LMWOA dynamics in spiked cadmium-containing soils

The results for the cadmium soil incubation are presented in Figure 4.7 and Figure 4.8. Following the soil incubation, the amount of added cadmium was low in both systems. In the control system, there was always significantly more soluble cadmium compared to that of the biochar treated system ($p < 0.05$). Over time, the soluble cadmium in the control reduced slightly but this was not significant ($p > 0.05$). In the biochar treated water system, the amount of cadmium immobilised reduced significantly from 1 h to the 24 h ($p < 0.05$). There was no further significant change to concentration with incubation time ($p < 0.05$). The biochar systems appeared to enhance the immobilisation of the added cadmium, compared to the control.

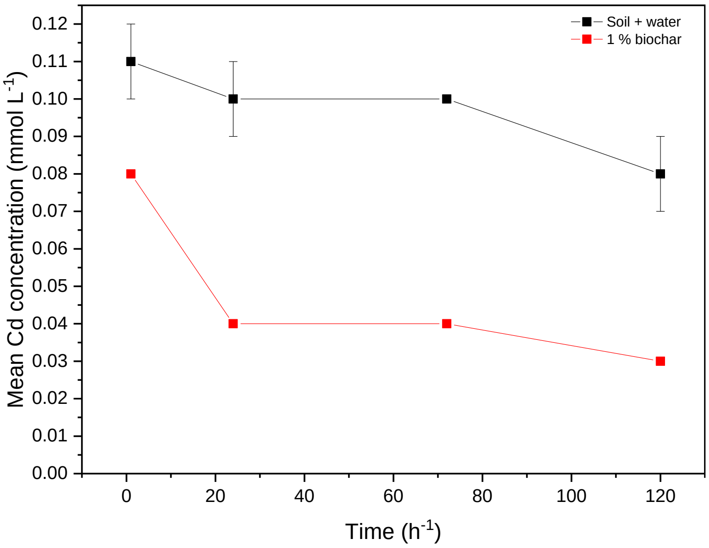

Figure 4.7 Temporal variations of soil solution-borne cadmium in the presence or absence of RH700. Error bars represent standard error (n = 3).

In the citric acid control, the cadmium concentration ranged from 0.32 to 0.56 mmol L^{-1}. Initially, the concentration increased from 0.32 mmol L^{-1} to 0.56 mmol L^{-1} at 72 h ($p < 0.05$). From 72 h to120 h, the concentration then fell slightly ($p < 0.05$). In the biochar treated system, cadmium was reduced as opposed to in the control, at 1 h, 72 h and 120 h respectively ($p < 0.05$). A similar temporal observation as the control was found in the treatment, an initial increase until 72 h followed by a decrease by 120 h ($p < 0.05$). In the malic acid control, the concentration continued to follow this pattern. All intervals were significantly different, except 1 h and 120 h ($p < 0.05$). In the malic acid with added biochar, cadmium solubilisation increased at 1 h ($p < 0.05$) but did not affect concentrations at the 24 h ($p > 0.05$). At the latter two sampling points, malic-acid driven solubilisation of cadmium reduced following biochar treatment when compared to the control ($p < 0.05$). There was no difference in concentrations at 1 h and 24 h, but from 24 h, the cadmium concentration was significantly lower at each interval ($p < 0.05$). Concerning soil systems with oxalic acid, less cadmium was present compared to the other acid systems. In the oxalic acid control, concentrations ranged from 0.07 to 0.16 mmol L^{-1}. The same pattern of an increase until 72 h was found, followed by a decrease at 120 h ($p < 0.05$). The amount of oxalic acid-mobilised cadmium was reduced at 1 h and 24 h in the biochar treated systems, as opposed to the control ($p < 0.05$). At later time points, there was no difference in concentration between the control and biochar treated systems ($p > 0.05$). The temporal variation in concentration was only observed with the higher concentration found at 72 h compared to the other intervals ($p < 0.05$). To review:

1. Citric acid driven-solubilisation of cadmium was lower in the presence of biochar, except at 24 h.

2. Malic acid driven-solubilisation of cadmium was impeded by biochar at 72 h and 120 h.

3. Oxalic driven-solubilisation of cadmium was not evident after 24 h.

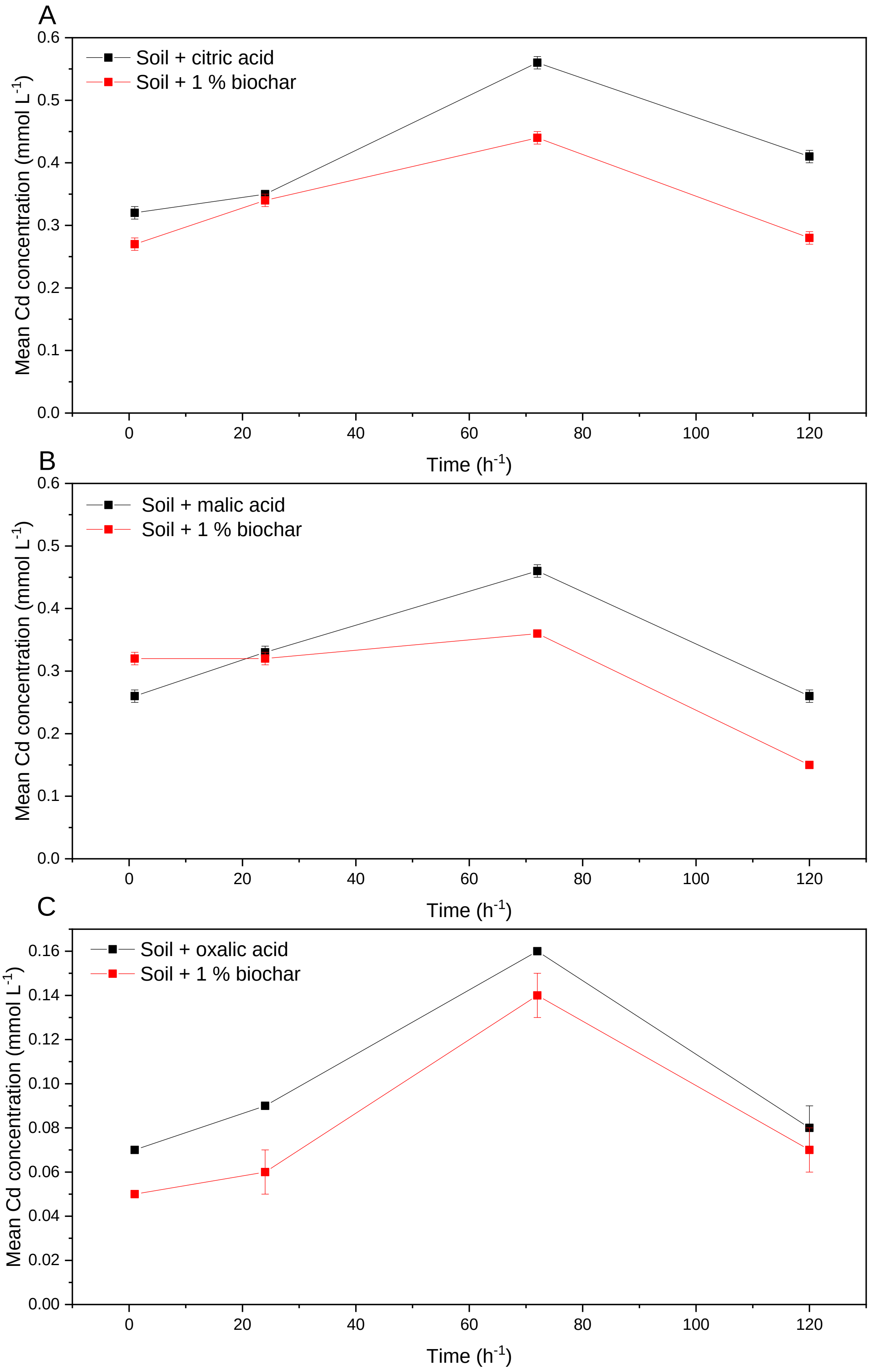

Figure 4.8 Temporal variations in soil solution-borne cadmium in the presence or absence of RH700 in (A) citric acid, (B) malic acid and (C) oxalic acid. Error bars represent standard error (n = 3).

4.5.2 Zinc-containing soils

4.5.2.1 pH and electrical conductivity of the zinc-containing soils

The pH and EC results for the zinc-containing soils are presented in Table 4.30. In the LMWOA-free control, the pH ranged from 5.71 to 6.42. Initially, there was a decline in pH between 1 h and 24 h ($p < 0.05$). However, from 24 h onwards, the pH became more alkaline at each interval ($p < 0.05$). The pH was lower in the control compared to the biochar-treated water system at 24 h and 72 h, respectively ($p < 0.05$). In the biochar treatment, the pH initially declined between the first two intervals ($p < 0.05$). Then, between 72 h and 120 h, the pH increased to a level less than that recorded at 1 h ($p < 0.05$). In citric acid, the control system pH was initially acidic (2.73 and 2.95 at 1 h and 24 h). However, the pH significantly increased at 72 h and 120 h ($p < 0.05$). The same temporal variation was found for the citric acid biochar treatment ($p < 0.05$). The pH of the biochar treated citric acid systems was more alkaline as opposed to the control, but this was only significant at 72 h ($p < 0.05$). Regarding the pH of the malic acid systems, the pH in the control ranged from 2.64 to 4.65. The pH initially fell from 2.97 to 2.64 at the first two intervals, afterwards becoming more alkaline at each occasion ($p < 0.05$). In comparison to the biochar treated malic acid system, the pH was more acidic without biochar amendment from 24 h onwards ($p < 0.05$). In the biochar treated system, the pH became significantly more alkaline with time ($p < 0.05$). Finally, in the oxalic acid system, the control pH always increased with time ($p < 0.05$). The pH was significantly more acidic in the control as opposed to the biochar treated at 72 h and 120 h only ($p < 0.05$). At 120 h, there was nearly 1-unit difference. The temporal change in the oxalic acid treatment was like the control, increasing significantly with time ($p < 0.05$).

Table 4.30 pH and electrical conductivity (EC) (mS cm^{-1}) of the overlying water layer at various time intervals (h) in either water, citric, malic or oxalic acid-containing zinc-containing soils. Systems contain either 0 or 1 g dL^{-1} RH700.

Parameter	**Treatment**	**Biochar (g dL^{-1})**	**Sampling interval**			
			1 h	**24 h**	**72 h**	**120 h**
pH	Water	0	6.42±0.02Aa	5.71±0.05Db	6.02±0.01Ca	6.22±0.01Bb
	Citric	0	2.73±0.10Ce	2.95±0.07Cc	4.26±0.05Bf	5.07±0.04Ad
	Malic	0	2.97±0.07Ccd	2.64±0.17Dd	3.63±0.04Bg	4.65±0.02Ae
	Oxalic	0	2.03±0.03Df	2.33±0.03Ce	4.57±0.08Be	5.72±0.10Ac
	Water	1	6.84±0.02Aa	6.35±0.13Ca	6.30±0.01Ca	6.74±0.02Ba
	Citric	1	2.80±0.03Cde	2.89±0.04Cc	4.77±0.03Bd	5.24±0.00Ad
	Malic	1	3.05±0.02Dc	3.13±0.00Cc	5.03±0.02Bc	6.22±0.02Ab
	Oxalic	1	2.14±0.05Df	2.39±0.02Ce	5.29±0.00Bb	6.62±0.02Aa
EC (mS cm^{-1})	Water	0	0.71±0.00Ae	0.73±0.00Ae	0.74±0.00Ae	0.77±0.01Ad
	Citric	0	1.38±0.00Ad	1.29±0.00Bc	1.10±0.00Cb	0.88±0.02Db
	Malic	0	1.24±0.00Ae	1.14±0.01Bcd	0.96±0.02Cd	0.83±0.01Dc
	Oxalic	0	3.67±0.03Aa	2.41±0.13Ba	1.01±0.00Cc	0.69±0.02De
	Water	1	0.73±0.01Be	0.76±0.02Be	0.81±0.00Ae	0.84±0.00Ac
	Citric	1	1.38±0.01Ad	1.29±0.00Bc	1.00±0.01Cc	0.68±0.01De
	Malic	1	2.67±0.01Ac	1.11±0.02Bd	0.90±0.00Cd	0.76±0.01Dd
	Oxalic	1	2.73±0.10Ac	2.95±0.07Ba	4.26±0.05Ca	5.07±0.04Da

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The EC of the LMWOA-free control ranged from 6.02 mS cm^{-1} to 6.42 mS cm^{-1}. There was initially a decrease in EC from 1 h to 24 h ($p < 0.05$), but then the EC significantly increased at each interval ($p < 0.05$). In comparison to the biochar treated water system, the EC was significantly lower in the control at 24 h and 120 h, respectively ($p < 0.05$). There was no significant difference at the other time intervals ($p > 0.05$). The temporal changes in the biochar-treated system showed an initial decrease from 1 h to 24 h, then an increase between 72 h and 120 h ($p < 0.05$). In all LMWOA-containing zinc-soil systems, both the control and biochar-treated systems had a significantly lower EC with increased incubation time ($p < 0.05$).

In citric acid, the EC of the biochar treatment compared to the control was significantly lower at 72 h and 120 h ($p < 0.05$) In the malic acid control, the EC at 1 h was less than the treatment system EC, but at 120 h the EC in the control was significantly higher than in the treated system ($p < 0.05$). For oxalic acid systems, the EC of the control was significantly higher than in the treatment at 1 h ($p < 0.05$); however, this was not maintained. At 72 h and 120 h, the EC of the control was significantly lower than in the treatment, by a difference of around 3.25 mS cm^{-1} and 4.38 mS cm^{-1}, respectively ($p < 0.05$).

4.5.2.2 Biochar and LMWOA dynamics in spiked zinc-containing soils

The results of the zinc soil incubation are shown in Figure 4.9 and Figure 4.10. For the LMWOA-free control, the zinc concentration was lower than in the control at 1 h and 72 h, respectively ($p < 0.05$). At 120 h, there was significantly less soluble zinc in the control opposed to the biochar-treated system ($p < 0.05$). With time, the concentration in the control decreased, but only the decline at 24 h and 120 h were significant from the other intervals ($p < 0.05$). For the biochar treated system, the concentration tended to decrease up until 72 h, when significance was achieved ($p < 0.05$). A sharp increase in mobilised zinc then occurred at 120 h ($p < 0.05$). It appears the biochar initially had some efficacy to reduce zinc solubilisation.

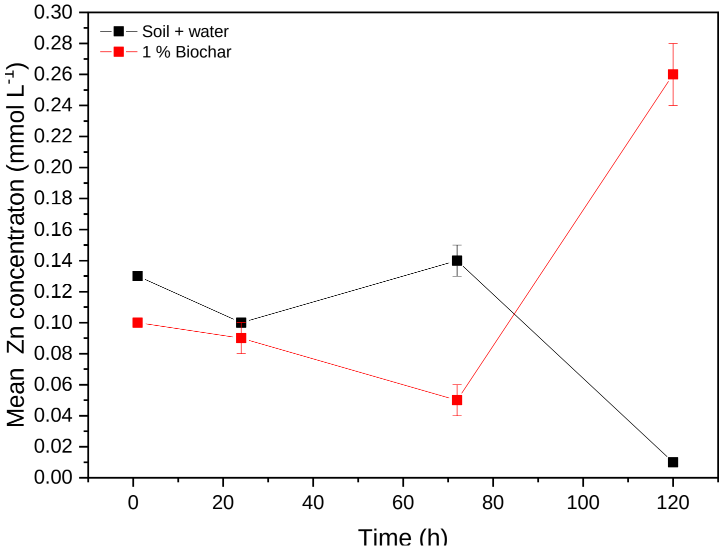

Figure 4.9 Temporal variations of soil solution-borne cadmium in the presence or absence of RH700. Error bars represent standard error (n = 3)

In the citric acid control, the concentrations ranged from 0.07 mmol L^{-1} to 0.67 mmol L^{-1}. Initially, the concentration increased until 72 h, before falling considerably between the 72 h and 120 h. The zinc concentration was significantly different at each interval ($p < 0.05$). The same temporal trend was observed for the citric acid biochar treatment ($p < 0.05$). However, there was always significantly less soluble zinc in the biochar treated system ($p < 0.05$). The malic acid control also showed a rise in concentrations until 72 h ($p < 0.05$). At 120 h, the zinc concentration returned to the level observed at 24 h ($p > 0.05$). Biochar initially showed limited ability to limit malic acid solubilisation of zinc at 1 h and 24 h. On the contrary, the zinc concentration was enhanced at these intervals ($p < 0.05$). By 72 h onwards, there was a significantly lower amount of zinc in the biochar treated system as opposed to the control ($p < 0.05$). Despite a significant increase in concentrations from 1 h through to 72 h ($p < 0.05$) the decrease from 72 h to 0.36 mmol L^{-1} at 120 h was not significantly different from that observed at 1 h ($p > 0.05$).

Oxalic acid systems also showed an increased concentration until 72 h, followed by a decrease. Regardless, all concentrations were different at each interval ($p < 0.05$). The oxalic acid biochar treatment also followed a similar temporal behaviour, but there was no difference between concentrations in the first 24 h ($p > 0.05$). The amount of oxalic acid-driven solubilised zinc was always less in the treatment as opposed to the control ($p < 0.05$). To summarise:

1. Biochar was always able to limit the citric acid-driven solubilisation of zinc.
2. Biochar initially enhanced the malic acid-driven solubilisation of zinc but limited solubilisation from 72 h onwards.
3. Biochar limited the oxalic acid-driven solubilisation of zinc throughout.

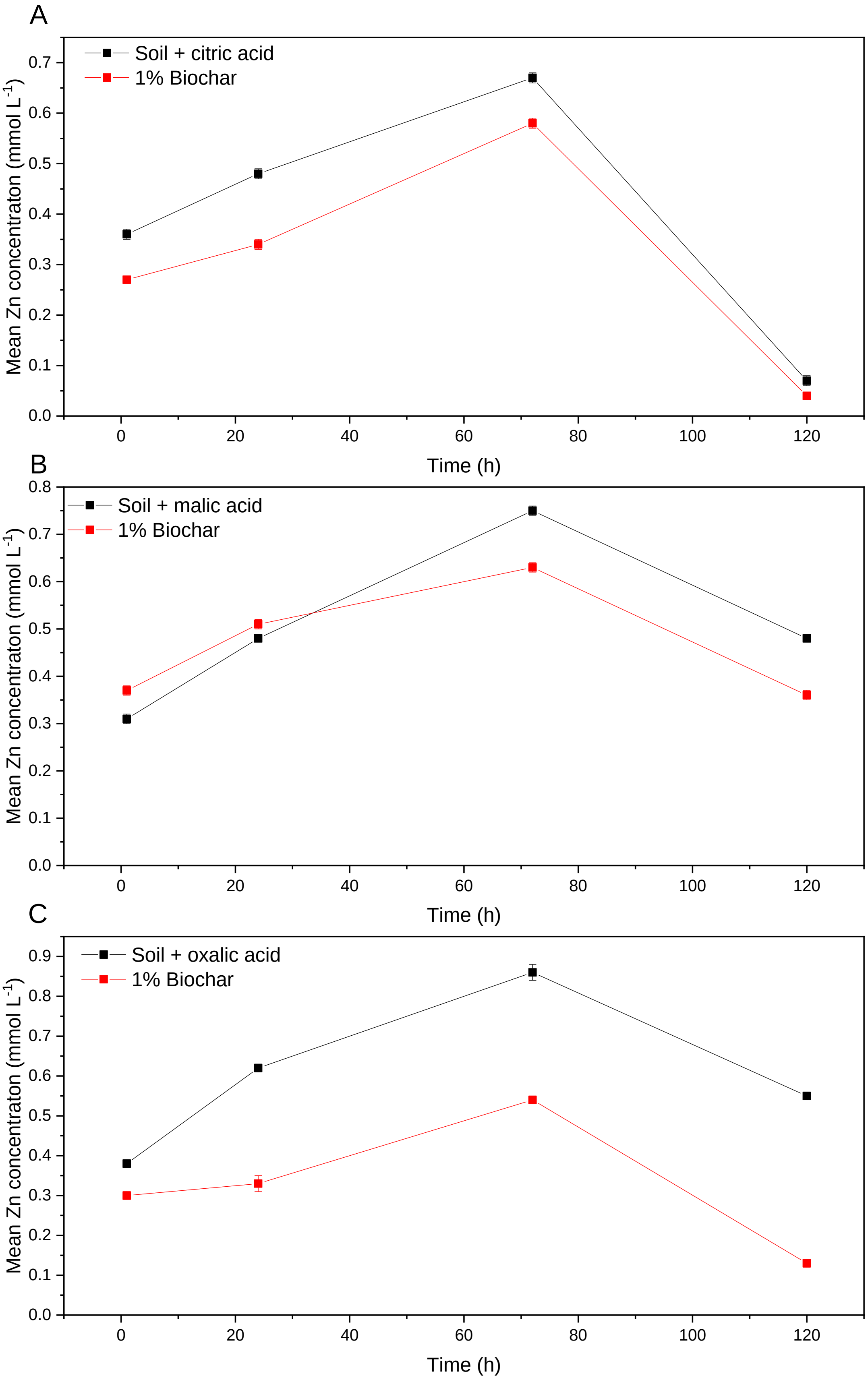

Figure 4.10 Temporal variations in soil solution-borne zinc in the presence or absence of RH700 in (A) citric acid, (B) malic acid and (C) oxalic acid. Error bars represent standard error (n = 3).

4.5.3 Nitrate-containing soils

4.5.3.1 pH and electrical conductivity of the nitrate-containing soils

To examine the hypothesis that biochar will reduce the LMWOA-driven solubilisation of nitrate in soils, the pH and EC of the nitrate-containing soils were analysed (Table 4.31). The pH of the water system without biochar ranged from 6.24 to 6.35. Initially, the pH rose from 6.35 to 6.50 from 1 h to 24 h. However, this was not statistically significant ($p > 0.05$). The pH then gradually decreased with time, yet only the pH at 24 h and 120 h were statistically different ($p < 0.05$). When likened to the biochar-containing counterpart, the pH was always significantly less without biochar ($p < 0.05$). The biochar-added system pH ranged from 6.37 to 7.49. The temporal change in pH was a significant increase between 1 h and 24 h ($p < 0.05$), and a significant decrease between 72 h and 120 h, respectively ($p < 0.05$).

Table 4.31 pH and electrical conductivity (EC) (mS cm^{-1}) of the overlying water layer at various time intervals (h) in either water, citric, malic or oxalic acid-containing nitrate-containing soils. Systems contain either 0 or 1 g dL^{-1} RH700.

Parameter	**Treatment**	**Biochar (g dL^{-1})**	**Sampling interval**			
			1 h	**24 h**	**72 h**	**120 h**
pH	Water	0	6.35±0.02ABb	6.50±0.13Ab	6.30±0.01ABd	6.24±0.01Be
	Citric	0	2.97±0.01Df	3.61±0.01Cd	6.02±0.01Bd	6.76±0.02Ad
	Malic	0	3.25±0.00De	3.64±0.01Cd	6.10±0.00Bd	6.98±0.02Ab
	Oxalic	0	2.81±0.01Dg	3.21±0.01Ce	5.76±0.00Be	6.82±0.00Ac
	Water	1	6.55±0.02Ba	7.49±0.02Aa	7.27±0.01Aa	6.37±0.17Be
	Citric	1	4.47±0.01Cc	3.73±0.00Dd	6.86±0.02Bc	7.04±0.02Ab
	Malic	1	4.17±0.00Cd	4.12±0.02Dc	7.03±0.01Bb	7.36±0.01Aa
	Oxalic	1	2.54±0.01Dh	3.03±0.01Cf	6.61±0.01Bc	6.99±0.01Ab
EC (mS cm^{-1})	Water	0	0.86±0.02Cf	0.92±0.01Be	1.03±0.01Af	1.06±0.02Ah
	Citric	0	5.20±0.06Ab	5.15±0.01Ad	5.24±0.01Ab	5.24±0.00Ab
	Malic	0	5.14±0.01Bb	5.33±0.11Ab	5.04±0.01Bd	4.68±0.00Cf
	Oxalic	0	6.32±0.00Aa	5.23±0.00Bbcd	5.22±0.02Bb	5.16±0.00Cc
	Water	1	0.97±0.02De	1.01±0.00Ce	1.07±0.01Be	1.13±0.01Ag
	Citric	1	5.16±0.01Bb	5.30±0.01Abc	5.03±0.01Cd	4.74±0.01De
	Malic	1	4.92±0.00Dc	5.18±0.00Acd	5.10±0.00Bc	4.82±0.00Cd
	Oxalic	1	3.28±0.00Cd	5.92±0.01Aa	5.54±0.01Ba	5.45±0.00Da

All values are presented as mean ± standard error ($n = 3$). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The pH of the citric acid control ranged from 2.97 to 6.76, becoming significantly more alkaline at each interval and was always significantly more acidic compared to the biochar-containing counterpart ($p < 0.05$). This was clear at 1 h where the pH in the biochar-added system was greater by around 1.50 units. At 24 h, the pH decreased from 4.47 at 1 h to 3.73 ($p < 0.05$). However, the pH then significantly increased at each latter interval ($p < 0.05$). The pH of the malic acid control ranged from 3.25 to 6.98, increasing with time ($p < 0.05$). Like the citric acid systems, the pH of the malic acid system was always significantly higher following biochar application ($p < 0.05$). The pH in the treated malic acid ranged from 4.17 to 7.36. As described for citric acid, there was a decrease in pH at 24 h, but the pH then increased until the end of the experiment ($p < 0.05$). The temporal pH change in the oxalic acid control was like the other acids, increasing at all intervals ($p < 0.05$). The pH of the control at 1 h and 24 h was significantly more alkaline than in the biochar treatment ($p < 0.05$). However, at 72 h and 120 h, the pH was more alkaline in the presence of biochar ($p < 0.05$).

The EC of the water control was low, ranging from 0.86 mS cm^{-1} to 1.06 mS cm^{-1}, significantly increasing with time ($p < 0.05$). The same temporal variation was seen for the biochar treatment ($p < 0.05$). In the biochar-containing system, the EC increased to greater than control at all intervals ($p < 0.05$). The EC of the citric acid system control ranged from 5.15 mS cm^{-1} to 5.24 mS cm^{-1} and there was no significant change in EC over time ($p > 0.05$). Compared to the control, the EC in the citric acid biochar treatment was significantly higher at 24 h and then significantly lower at 72 h and 120 h, respectively ($p < 0.05$). The EC initially increased in the biochar treatment and then declined at each interval after 24 h ($p < 0.05$). In the malic acid control the EC ranged from 4.68 mS cm^{-1} to 5.33 mS cm^{-1} initially increasing at 24 h by around 0.19 mS cm^{-1} ($p < 0.05$). Afterwards, the EC tended to decline at each interval ($p < 0.05$). Compared to the malic acid control, the treated malic acid system had a lower EC at 1 h and 24 h and a higher the EC at 72 h and 120 h ($p < 0.05$). The same temporal trend as the control was observed in the treatment ($p < 0.05$). The EC of the oxalic acid systems was higher than the other LMWOAs. The control EC ranged from 5.16 mS cm^{-1} to 6.32 mS cm^{-1}, decreasing at 24 h and 120 h respectively ($p < 0.05$). Compared to the control, the EC in the biochar treated oxalic acid was significantly lower at the first two intervals and then significantly higher at the last two ($p < 0.05$). As observed for the other acids, the EC tended to increase between 1 h and 24 h, then decrease at each subsequent interval ($p < 0.05$).

4.5.3.2 Biochar and LMWOA dynamics in spiked nitrate-containing soils

To support the third hypothesis of this thesis, the effects of biochar on nitrate immobilisation in soil without LMWOA was first determined (Figure 4.11). In the systems without LMWOAs the behaviour of biochar on nitrate immobilisation was unclear. Compared to the biochar-free control, the concentration of soluble nitrate in the biochar-amended system was significantly lower at 1 h and 24 h and then significantly higher at 72 h and 120 h ($p < 0.05$). Over time the control system slightly decreased, where the nitrate at 1 h was significantly higher and at 120 h was significantly lower than all other values ($p < 0.05$). There was no difference in concentrations at 24 h and 72 h respectively. For the system amended with biochar, there was no temporal variation in concentration ($p > 0.05$). Results for systems containing LMWOAs are shown in Figure 4.12. In citric acid systems, there was an overall decrease in soluble nitrate over time, achieving significance from 72 h onwards ($p < 0.05$). At 1 h and 24 h, there was less soluble nitrate in the control compared to the biochar-containing counterpart ($p < 0.05$). However, at 72 h, there was more soluble nitrate compared to the treated citric acid system ($p < 0.05$). There was no difference between the control and treatment at 120 h ($p > 0.05$). Importantly, the control system performed better overall in terms of nitrate immobilisation than the system with added biochar.

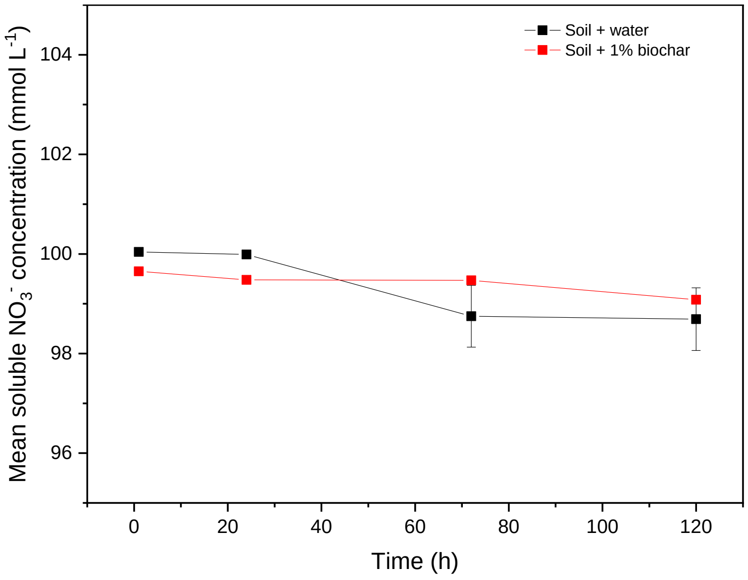

Figure 4.11 Temporal variations of soil solution-borne cadmium in the presence or absence of RH700. Error bars represent standard error (n = 3).

In the malic acid systems, a similar trend to citric acid systems was found. However, soluble nitrate concentrations in the malic acid control were kept constant ($p > 0.05$). In the biochar treated malic acid system, the concentration significantly increased at 24 h, before significantly reducing further at each interval ($p < 0.05$). The amount of soluble nitrate was significantly lower in the control compared to the biochar-containing counterpart at 1 h, 24 h and 72 h, respectively ($p < 0.05$). There was no difference in nitrate concentrations at 120 h in either the control or treated system ($p > 0.05$). As found for citric acid systems, the malic acid control system performed better overall in terms of nitrate immobilisation, compared to the system with added biochar. In the oxalic acid control, the amount of soluble nitrate was constant throughout ($p > 0.05$). In comparison to the biochar treated system, there was significantly less nitrate at 1 h and 120 h, respectively ($p < 0.05$).

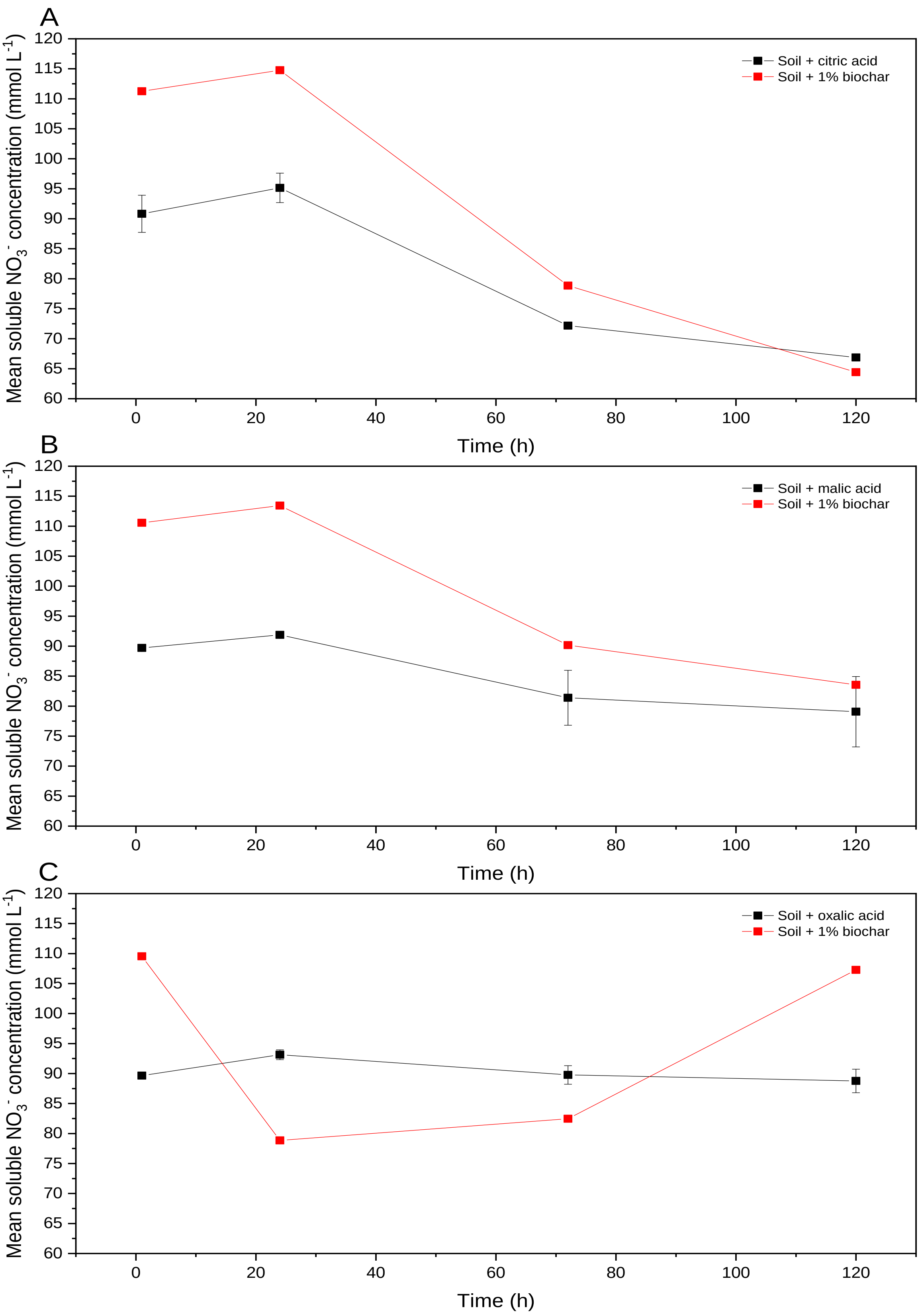

Figure 4.12 Temporal variations in soil solution-borne nitrate in the presence or absence of RH700 in (A) citric acid, (B) malic acid and (C) oxalic acid. Error bars represent standard error (n = 3).

Likewise, at 24 h and 72 h, there was significantly more soluble nitrate in the control compared to the biochar-treated counterpart ($p < 0.05$). In summary:

1. In citric and malic acid, the biochar immobilised nitrate over time but to a lesser-extent than biochar-free systems.
2. In oxalic acid, biochar was able to reduce the LMWOA-driven solubilisation of nitrates in soils at 24 h and 72 h only.

4.5.4 Phosphate-containing soils

4.5.4.1 pH and electrical conductivity of the phosphate-containing soils

The pH and EC of the phosphate-containing soils are displayed in Table 4.32. In the soil system without added LMWOAs, the pH ranged from 6.55 to 7.04. There was no statistical difference in pH when comparing the values at 1 h and 72 h ($p > 0.05$). However, statistical significance was achieved at the other intervals ($p < 0.05$). Compared to the biochar-containing counterpart, there was no statistical difference in pH ($p > 0.05$). In citric acid, the control pH ranged from 3.74 to 4.72, significantly increasing at each interval ($p < 0.05$). At 1 h and 24 h, the pH was more alkaline in the control than in the biochar treatment ($p < 0.05$). By 72 h and 120 h, the pH was more alkaline in the biochar-treated system ($p < 0.05$). Over time, the pH in the citric acid biochar treatment significantly increased at each interval ($p < 0.05$). For the malic acid systems, the pH of the control ranged from 3.78 to 5.42 and significantly increased at each interval ($p < 0.05$). The pH of the biochar treated malic acid system was more acidic at 1 h compared to the control ($p < 0.05$). However, at 24 h and 72 h, the pH was more alkaline in the biochar treatment ($p < 0.05$). There was no difference in pH between the control and treatment at 120 h ($p > 0.05$). For the oxalic acid systems, the pH in the control ranged from 3.02 to 5.71, significantly increasing with time ($p < 0.05$). The pH in the treated system compared to the control was more alkaline at the first three intervals ($p < 0.05$), but there was no difference in pH by 120 h ($p > 0.05$).

Table 4.32 pH and electrical conductivity (EC) (mS cm^{-1}) of the overlying water layer at various time intervals (h) in either water, citric, malic or oxalic acid-containing phosphate-containing soils. Systems contain either 0 or 1 g dL^{-1} RH700.

Parameter	**Treatment**	**Biochar (g dL^{-1})**	**Sampling interval**			
			1 h	**24 h**	**72 h**	**120 h**
pH	Water	0	6.55±0.02Bb	6.85±0.07Aa	6.53±0.01Bb	7.04±0.01Ca
	Citric	0	3.74±0.01Db	4.04±0.01Cc	4.24±0.01Bd	4.72±0.01Ac
	Malic	0	3.78±0.01Da	4.21±0.01Cb	4.55±0.01Bc	5.42±0.01Ab
	Oxalic	0	3.02±0.00De	3.52±0.00Ce	4.95±0.01Bb	5.71±0.01Aa
	Water	1	6.53±0.02Ab	7.95±0.02Ba	6.51±0.01Ab	9.01±0.01Aa
	Citric	1	3.53±0.01Dc	3.82±0.01Cd	5.33±0.11Ba	5.60±0.01Aa
	Malic	1	3.74±0.01Db	4.32±0.00Ca	5.20±0.01Ba	5.45±0.01Ab
	Oxalic	1	3.06±0.01Dd	3.35±0.00Cf	5.31±0.01Ba	5.72±0.10Aa
EC (mS cm^{-1})	Water	0	1.54±0.01Af	1.54±0.00Af	0.96±0.01Be	0.72±0.01Ce
	Citric	0	2.97±0.01De	5.18±0.01Aa	3.35±0.02Cc	3.54±0.00Bb
	Malic	0	3.06±0.01Cd	3.34±0.01Bc	3.51±0.01Ab	3.51±0.00Ab
	Oxalic	0	3.40±0.00Ab	3.27±0.00Cde	3.32±0.01Bc	3.34±0.01Bc
	Water	1	1.21±0.01Dg	1.31±0.01Cg	1.35±0.01Bd	1.42±0.01Ad
	Citric	1	3.15±0.01Dc	3.25±0.01Ce	3.52±0.00Bb	3.68±0.01Aa
	Malic	1	3.16±0.01Bc	3.27±0.01Bd	3.51±0.00Ab	3.54±0.07Ab
	Oxalic	1	3.43±0.01Da	3.64±0.01Ab	3.61±0.01Ba	3.55±0.01Cb

All values are presented as mean ± standard error (n = 3). Means with different uppercase letters in the same row (time) and lowercase letters in the same column (treatment) are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

Regarding the EC of the phosphate-containing soils, the EC of systems without added LMWOAs and no biochar initially stayed the same at 1 h and 24 h (1.54 mS cm^{-1}) ($p > 0.05$). Then at each later interval, a significant decrease occurred ($p < 0.05$), resulting in an endpoint EC at 120 h of 0.72 mS cm^{-1}. Compared to the biochar-containing counterpart, the EC was significantly higher at the first two intervals and then lower at the last two intervals in the biochar free system ($p < 0.05$). In the biochar added systems, the temporal trend revealed an increasing EC with time, statistically significant at all sampling occasions ($p < 0.05$). The citric acid control had an EC ranging from 2.97 mS cm^{-1} to 5.18 mS cm^{-1}. Initially, the EC increased sharply between 1 h and 24 h ($p < 0.05$). After this point, the EC significantly decreased at all intervals until reaching 3.54 mS cm^{-1} at 120 h ($p < 0.05$). In the biochar treated citric acid, the EC was first higher than the control ($p <$ 0.05). However, at the latter three sampling occasions, the EC was less than the control (p

< 0.05). Over time the EC of the citric acid biochar treatment increased from 3.15 mS cm^{-1} to 3.68 mS cm^{-1} ($p < 0.05$). For malic acid, the EC of the control ranged from 3.06 mS cm^{-1} to 3.51 mS cm^{-1}. The EC significantly increased until 72 h ($p < 0.05$). The addition of biochar in malic acid-containing systems first increased the EC, then at 24 h decreased the EC compared to the control ($p < 0.05$). Neither the EC at 72 h nor 120 h was significantly different in the control and biochar treatment ($p > 0.05$). With time, the EC in the biochar treatment increased, but the change was only significant between 24 h and 72 h, respectively ($p < 0.05$). In the oxalic acid systems, the control EC ranged from 3.27 mS cm^{-1} to 3.40 mS cm^{-1}. Between 1 h and 24 h, the EC significantly fell, before increasing slightly at 72 h ($p < 0.05$). There was no difference in EC after 72 h ($p > 0.05$). The EC was always significantly higher with added biochar than in the control ($p < 0.05$). A clear trend showing an initial increase in EC between 1 h and 24 h occurred, followed by a steady decline in EC. Each change over time was significant at the $p < 0.05$ level.

4.5.4.2 Biochar and LMWOA dynamics in spiked phosphate-containing soils

The dynamics in un-amended and amended phosphate spiked soils were determined. In water (Figure 4.13), enhanced phosphate immobilisation was observed in systems with added biochar ($p < 0.05$). This indicates that biochar was able to immobilise soluble phosphate at sampling occasions. For the system without biochar, the amount of soluble phosphate was significantly higher at 24 h compared to all other intervals, likewise, at 120 h the amount of soluble phosphate was significantly less than at other time points ($p < 0.05$). There was no difference for soluble phosphate at either 1 h or 72 h respectively. In the added biochar system, the temporal trend was similar, and there was a significantly higher amount of soluble phosphate at 24 h compared to the other time intervals ($p < 0.05$).

However, the amount of phosphate at 1 h compared to 72 h and 120 h was significantly higher ($p < 0.05$), indicative of increased biochar immobilisation over time.

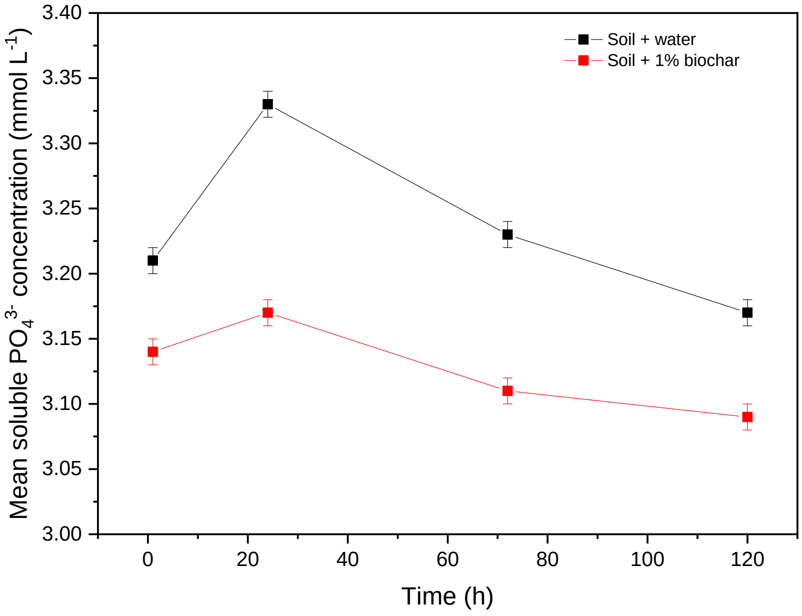

Figure 4.13 Temporal variations in soil solution-borne phosphate in the presence or absence of RH700 in water. Error bars represent standard error (n = 3).

To see if biochar will reduce the LMWOA-driven solubilisation of phosphate in soils, citric, malic and oxalic acid systems were studied (Figure 4.14). In the citric acid control, the soluble phosphate concentration ranged from 3.32 mmol L^{-1} to 3.94 mmol L^{-1}, and the concentration showed a steady yet significant increase with time ($p < 0.05$). At 1 h and 24 h, more soluble phosphate was available in the biochar-treated citric acid system compared to the control ($p < 0.05$). However, at 120 h, biochar significantly decreased the citric acid-driven mobilisation of phosphate ($p < 0.05$). The temporal variations in phosphate in the biochar treatment show an increase in soluble phosphate until 24 h ($p < 0.05$). After 24 h, the available phosphate decreased to 3.73 mmol L^{-1} at 120 h ($p < 0.05$). In the malic acid control, the soluble phosphate ranged from 3.50 mmol L^{-1} to 3.77 mmol L^{-1}. The only significant increase in concentration occurred at 24 h ($p < 0.05$).

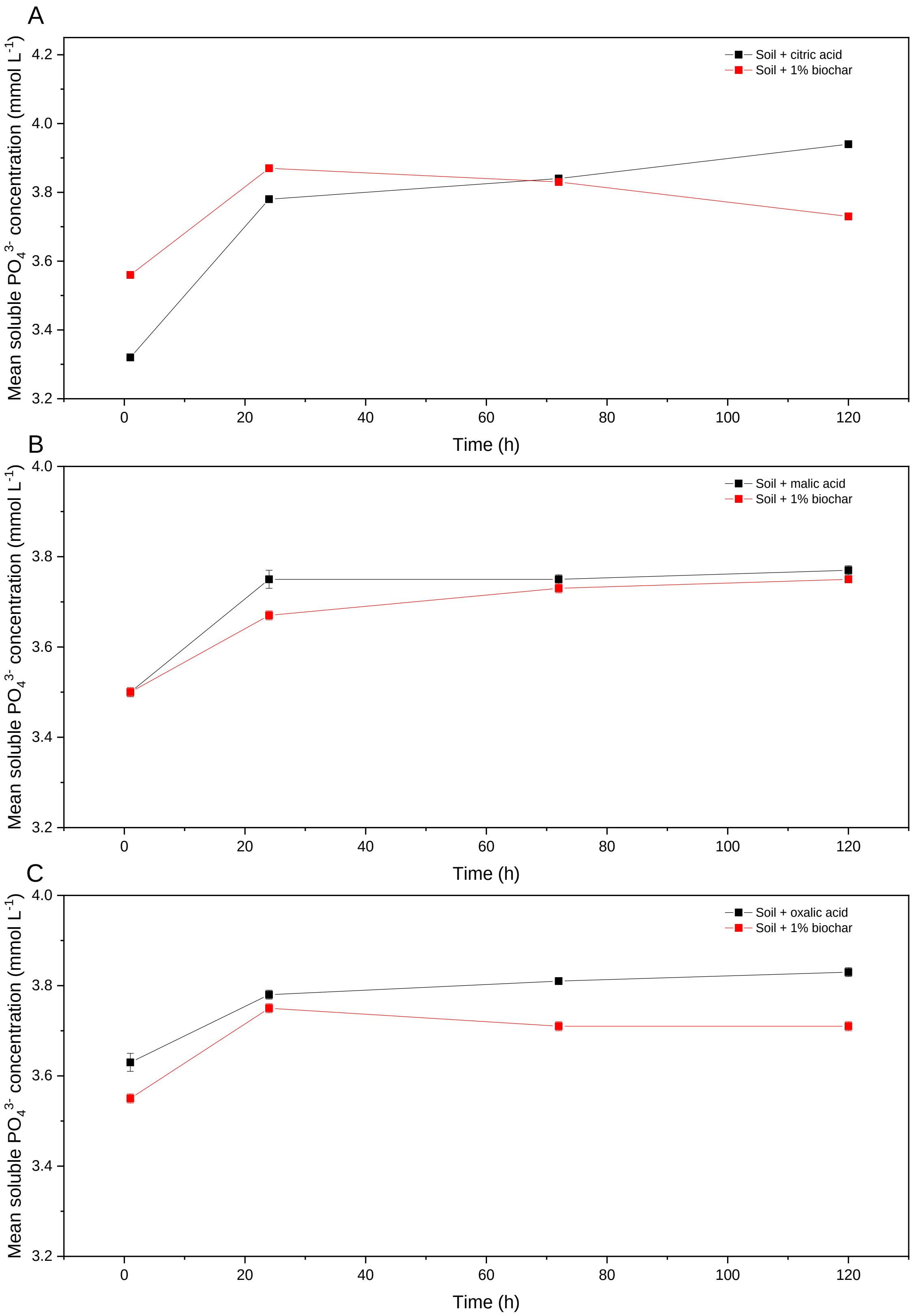

Figure 4.14 Temporal variations in soil solution-borne phosphate in the presence or absence of RH700 in (A) citric acid, (B) malic acid and (C) oxalic acid. Error bars represent standard error (n = 3).

The presence of biochar initially had no effect on soluble phosphate in the malic acid system ($p > 0.05$). However, at 24 h, soluble phosphate was less than in the control ($p < 0.05$). At 72 h and 120 h, soluble phosphate concentrations were unaffected ($p > 0.05$). Finally, in the oxalic acid control, the concentration of soluble phosphate increased with time. This was a significant change between 1 h and 24 h, and, 1 h, 24 h and 120 h ($p < 0.05$). The amount of soluble phosphate in oxalic acid was less in the biochar treated counterpart at the 1 h, 72 h and 120 h ($p < 0.05$). After the initial significant increase between 1 h and 24 h ($p < 0.05$), the amount of available phosphate then significantly decreased at 72 h ($p < 0.05$). No further change in concentration was observed after 72 h ($p < 0.05$). In summary:

1. In citric acid, the biochar-phosphate dynamics were unclear.
2. In malic and oxalic acid, biochar was able to reduce the LMWOA-driven solubilisation of phosphate but to a lesser extent to that seen in the water systems.

4.6 Results for hypotheses 4a to 4d

In this section, separate plant parameters are discussed separately. As no tomato germinated during the growth period, these were omitted from the results section. Reasons behind unsuccessful germination are discussed chapter 5.

4.6.1 Germination and growth parameter assay

The results for hypotheses 4a to 4d are presented in Table 4.33. Hypothesis 4a stated that biochar and LMWOA-activated biochar would increase the germination percentage of different edible plant species. Of the four species that germinated, biochar and LMWOA-activated biochar had no effect on seedling germination for the different edible plant species sown ($p > 0.05$).

Table 4.33 Germination rate (%),Total biomass (g^{-1} FW), shoot height (mm) and root length (mm) for lettuce, mustard, radish and pea after fourteen days growth

Plant species	**Treatment**	**Germination rate (%)**	**Total biomass (g^{-1} FW^{1})**	**Shoot height (mm)**	**Root length (mm)**
Lettuce	0%	75 ± 14a	0.41±0.01a	50.41±4.28a	7.79±1.65a
Lactuca	1% biochar	100 ± 0a	0.23±0.01c	59.66±1.32a	9.02±1.07a
sativa	1% activated biochar	92 ± 8a	0.31±0.01b	54.41±3.47a	11.52±1.30a
Mustard	0%	100 ± 0a	1.34±0.06b	90.61±3.90a	11.19±2.65a
Sinapis	1% biochar	100 ± 0a	1.73±0.16a	87.00±8.36a	11.19±1.97a
alba	1% activated biochar	100 ± 0a	1.60±0.02ab	99.49±2.86a	17.31±1.76a
Radish	0%	100 ± 0a	2.06±0.01a	69.21±5.24a	22.31±3.21a
Raphanus	1% biochar	92 ± 8a	1.25±0.03c	65.94±3.85a	20.32±3.87a
sativus	1% activated biochar	100 ± 0a	1.46±0.01b	69.26±5.99a	21.97±3.25a
Pea	0%	92 ± 8a	1.62±0.05b	89.43±6.29a	142.29±16.31a
Pisum	1% biochar	92 ± 8a	1.69±0.18a	80.32±8.19ab	124.03±13.38ab
sativum	1% activated biochar	75 ± 14ab	1.53±0.08b	67.18±7.28a	89.26±8.12b

[1]FW; Fresh weight [2]All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

Hypothesis 4b stated that biochar and LMWOA-activated biochar would increase the total fresh biomass of different edible plant species. For lettuce, the presence of either biochar significantly reduced biomass with a lesser effect for the activated biochar amendment compared to the normal biochar ($p < 0.05$). Fresh mustard biomass was significantly greater when grown in biochar-amended soils when compared to mustard grown in soil without biochar ($p < 0.05$). However, there was no difference between mustard biomass when grown in either the unamended and soils amended with activated biochar ($p > 0.05$). Radish biomass was significantly reduced in the presence of either biochar; however, the biomass was higher in the presence of activated biochar compared to biochar ($p < 0.05$). Fresh pea biomass was significantly increased in the presence of biochar ($p < 0.05$) whereas activated biochar had no significant effect on the biomass compared to peas grown in biochar amended soils ($p > 0.05$). However, significantly more biomass was grown in biochar-amended soils compared to the activated biochar-amended soils ($p < 0.05$).

Hypothesis 4c was that biochar and LMWOA-activated biochar would increase the shoot height of different edible plant species. The shoot heights of all the studied plant species were unaffected by the presence or absence of biochar ($p > 0.05$). The final hypothesis (4d) was that biochar and LMWOA-activated biochar would increase the root length of different edible plant species. The root lengths of lettuce, mustard and radish were not affected by either biochar amendment. However, pea root lengths were significantly shorter in activated biochar-amended soils compared to the control ($p < 0.05$). Notably, there was no difference between pea roots grown in biochar and the activated-biochar-amended soils.

4.7 Results for hypotheses 5a and 5b

4.7.1 Post-experiment soil analysis

Post-experimental analysis of the greenhouse soils was carried out (Table 4.34). Concerning total concentrations of trace elements, a decreasing order of iron (2845.06 mg kg^{-1}) > aluminium (765.21 mg kg^{-1}) > copper (110.06 mg kg^{-1}) > lead (82.60 mg kg^{-1}) > manganese (76.46 mg kg^{-1}) > zinc (57.27 mg kg^{-1}) > nickel (17.69 mg kg^{-1}) > arsenic (5.96 mg kg^{-1}) > chromium (2.95mg kg^{-1}) > cadmium (2.01 mg kg^{-1}) > cobalt (0.09 mg kg^{-1}) was observed in soils with 0 % biochar. A similar order was found for 1 % biochar soils, which followed a decreasing order of iron (5512.04 mg kg^{-1}) > aluminium (734.44 mg kg^{-1}) > copper (201.62 mg kg^{-1}) > manganese (134.28 mg kg^{-1}) > lead (110.23 mg kg^{-1}) > zinc (95.22 mg kg^{-1}) > nickel (41.06 mg kg^{-1}) > chromium (4.39 mg kg^{-1}) > arsenic (3.53 mg kg^{-1}) > cadmium (2.97 mg kg^{-1}) > cobalt (0.25 mg kg^{-1}). Total concentrations for the 1 % activated biochar soils followed a decreasing order of iron (3006.48 mg kg^{-1}) > aluminium (719.97 mg kg^{-1}) > copper (137.78 mg kg^{-1}) > manganese (115.81 mg kg^{-1}) > lead (87.52 mg kg^{-1}) > zinc (65.19 mg kg^{-1}) > nickel (26.04 mg kg^{-1}) > chromium (3.89 mg kg^{-1}) >

cadmium (2.33 mg kg^{-1}) > arsenic (2.27 mg kg^{-1}) > cobalt (0.17 mg kg^{-1}). Compared to either biochar-amended soil, iron, manganese, cadmium, cobalt, chromium, copper, nickel, lead and zinc was significantly less in the control ($p < 0.05$). For these elements, concentrations were always higher in the 1 % biochar treatment compared to those of the 1 % activated biochar treatment ($p < 0.05$). Nickel also had the highest total concentration in the 1 % biochar treatment however no statistical difference was found between the control and the 1 % activated biochar treatment ($p > 0.05$). In contrast to these elements, aluminium and arsenic had significantly higher total concentrations in the control opposed to the 1 % biochar treatment and the 1 % activated biochar treatment ($p < 0.05$). In all instances, total metal concentration was greater than the bioavailable fraction.

Table 4.34 Results for the total nitric acid-digestible and 0.01 mol L^{-1} calcium chloride-extractable (CaCl$_2$) trace elements from the greenhouse soils following the application of either 1% w/w biochar or LMWOA-activated biochar amendment after fourteen days pea growth

Element	**Post-experimental technique**	**Treatment**		
		0% biochar	**1% biochar**	**1 % activated biochar**
Aluminium	Total (mg kg^{-1})	765.21±11.11^{a}	734.44±5.42^{b}	719.97±7.06^{c}
	CaCl$_2$ extractable (mg kg^{-1})	< LOD[1]	< LOD	< LOD
Iron	Total (mg kg^{-1})	2845.06±2.52^{c}	5512.04±2.96^{a}	3006.48±22.03^{b}
	CaCl$_2$ extractable (mg kg^{-1})	< LOD	< LOD	< LOD
Manganese	Total (mg kg^{-1})	76.460±0.77^{c}	134.28±1.07^{a}	115.81±4.25^{b}
	CaCl$_2$ extractable (mg kg^{-1})	12.05±0.40^{c}	16.24±0.19^{a}	13.12±0.08^{b}
Arsenic	Total (mg kg^{-1})	5.96±0.32^{a}	3.53±0.02^{b}	2.27±0.07^{c}
	CaCl$_2$ extractable (mg kg^{-1})	< LOD	0.88±0.03^{a}	< LOD
Cadmium	Total (mg kg^{-1})	2.01±0.10c	2.97±0.68^{a}	2.33±1.42^{b}
	CaCl$_2$ extractable (mg kg^{-1})	< LOD	< LOD	< LOD
Cobalt	Total (mg kg^{-1})	0.09±0.02^{c}	0.25±0.04^{a}	0.17±0.07^{b}
	CaCl$_2$ extractable (mg kg^{-1})	< LOD	< LOD	< LOD
Chromium	Total (mg kg^{-1})	2.95±1.43^{c}	4.39±1.33^{a}	3.89±0.18^{b}
	CaCl$_2$ extractable (mg kg^{-1})	< LOD	< LOD	< LOD
Copper	Total (mg kg^{-1})	110.06±0.27^{c}	201.62±0.44^{a}	137.78±4.86^{b}
	CaCl$_2$ extractable (mg kg^{-1})	29.66±0.21^{b}	42.78±1.78^{a}	29.99±0.20^{b}
Nickel	Total (mg kg^{-1})	17.69±7.55^{b}	41.06±2.20^{a}	26.04±0.73^{b}
	CaCl$_2$ extractable (mg kg^{-1})	28.50±0.59^{a}	27.35±2.09^{a}	26.09±1.73^{a}
Lead	Total (mg kg^{-1})	82.60±14.46^{c}	110.23±4.29^{a}	87.52±2.07^{b}
	CaCl$_2$ extractable (mg kg^{-1})	4.03±0.02^{a}	2.04±0.04^{b}	4.10±0.08^{a}
Zinc	Total (mg kg^{-1})	57.27±1.08^{c}	95.22±41.87^{a}	65.19±30.78^{b}
	CaCl$_2$ extractable (mg kg^{-1})	27.95±0.36^{a}	27.21±0.32^{a}	22.14±0.24^{b}

[1]<LOD indicated concentration was less than the limit of detection [2]All values are presented as the mean ± standard error (n = 3). Means with different superscript letters in the same row are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

The bioavailable trace elements of the 0 % biochar soils without biochar as measured by a 0.01 mol L^{-1} calcium chloride extraction are presented in Table 4.34. No aluminium, arsenic, cadmium, cobalt, chromium or iron was extracted. Concentrations of trace elements followed a decreasing order of copper (29.66 mg kg^{-1}) > nickel (28.5 mg kg^{-1}) > zinc (27.95 mg kg^{-1}) > manganese (12.05 mg kg^{-1}) > lead (4.03 mg kg^{-1}) > aluminium (0 mg kg^{-1}) = arsenic (0 mg kg^{-1}) = cadmium (0 mg kg^{-1}) = cobalt (0 mg kg^{-1}) = chromium (0 mg kg^{-1}) = iron (0 mg kg^{-1}). The application of normal biochar increased the bioavailable copper, manganese and arsenic. The increase of copper and manganese compared to the other treatments was significant ($p < 0.05$). On the other hand, bioavailable lead significantly decreased after biochar amendment ($p < 0.05$). Although a slight change in concentration was observed between other elements, none of these were significantly different from the 0 % biochar soil. The decreasing trend of trace elements in the 1 % biochar-amended soils followed the order of copper (42.78 mg kg^{-1}) > nickel (27.35 mg kg^{-1}) > zinc (27.21 mg kg^{-1}) > manganese (16.24 mg kg^{-1}) > lead (2.04 mg kg^{-1}) > arsenic (0.88 mg kg^{-1}) > aluminium (0 mg kg^{-1}) = cadmium (0 mg kg^{-1}) = cobalt (0 mg kg^{-1}) = chromium (0 mg kg^{-1}) = iron (0 mg kg^{-1}). In the LMWOA-activated biochar-amended soils, copper and lead were significantly less in the LMWOA-activated biochar treatment compared to the normal biochar treatment ($p < 0.05$), but were not significantly different from the control ($p > 0.05$). Zinc and manganese were lower in the LMWOA-activated biochar treatment compared to the control and biochar treated soils ($p < 0.05$). There was no difference between nickel concentrations in either of the three treatments. In the LMWOA-activated soils, elements followed the decreasing order of copper (29.99 mg kg^{-1}) > nickel (26.09 mg kg^{-1}) > zinc (22.14 mg kg^{-1}) > manganese (13.12mg kg^{-1}) > lead (4.10 mg kg^{-1}) > aluminium (0 mg kg^{-1}) = arsenic (0 mg kg^{-1}) = cadmium (0 mg kg^{-1}) = cobalt (0 mg kg^{-1}) = chromium (0 mg kg^{-1}) = iron (0 mg kg^{-1}).

4.7.2 Growth performance of the pea plants during the experiment

Figure 4.15 provides photographic evidence of the pea growth throughout the experiment. It is clear that growth increased throughout the experiment but the difference between treatments is not obvious. Growth parameters are shown in Table 4.35. The shoot biomass results for both biochar materials were not significantly different ($p > 0.05$) but peas grown in activated biochar-amended soils had significantly larger biomass compared to the control ($p < 0.05$). Root biomass and shoot heights was only significantly greater in the activated biochar-amended soils ($p < 0.05$). Pea root lengths were unaffected by the presence of either biochar ($p > 0.05$).

Figure 4.15 Variations in pea (*Pisum sativum*) growth one month after sowing. The corresponding treatment name is above the image (top line) and the time is to the left of the image. BC refers to biochar and ABC refers to LMWOA-activated biochar application.

Table 4.35 Shoot biomass (g^{-1} DW), root biomass (g^{-1} DW), shoot height (mm) and root length (mm) for pea plants after one month growth

Biochar (w/w)	Shoot biomass (g^{-1} DW[1])	Root biomass (g^{-1} DW[1])	Shoot height (mm)	Root length (mm)
0%	0.56±0.01b	0.08±0.01b	145.80±10.59b	134.42±11.47a
1% biochar	0.59±0.01ab	0.08±0.01b	163.25±8.8b	134.90±12.7a
1% activated biochar	0.66±0.01a	0.14±0.01a	179.37±5.68a	142.06± 9.63a

[1]DW; Dry weight [2]All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

4.7.3 Chlorophyll a, b and total chlorophyll content

Chlorophyll content in the pea leaves was determined using UV-VIS after one month of growth (Table 4.36). Neither biochar nor activated biochar-amended soils appeared to affect chlorophyll a, chlorophyll b or total chlorophyll content ($p > 0.05$).

Table 4.36 Chlorophyll a, chlorophyll b and total chlorophyll content of fresh plant pea leaves, determined by 80% acetone extraction, after one month growth

Biochar (w/w)	Chlorophyll a (µg g^{-1} FW[1])	Chlorophyll b (µg g^{-1} FW)	Total Chlorophyll (µg g^{-1} FW)
0%	85.51±25.71a	28.63±8.86a	114.15±34.53a
1% biochar	71.93±15.97a	28.36±7.52a	100.29±23.48a
1% activated biochar	60.12±13.38a	21.92±4.59a	82.04±17.84a

[1]FW; Fresh weight [2]All values are presented as the mean ± standard error (n = 3). Means with different letters in the same column are significantly different according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

4.7.4 Trace element concentration in the root tissues

The experimental results for root trace element concentrations are shown in Figure 4.16 and Figure 4.17. Root aluminium concentrations were significantly reduced in the presence of either biochar ($p < 0.05$) but there was no difference between biochar types ($p > 0.05$). The same trend was found for iron and manganese root concentrations. Biochar did not affect arsenic, cadmium, chromium, cobalt or zinc found in root tissues ($p > 0.05$). However, significantly less cobalt was found in pea roots when grown in the presence of activated biochar compared to the unamended soils ($p < 0.05$). There was no difference between cobalt from roots grown in either biochar-amended soils ($p > 0.05$). The copper concentrations found in pea roots grown in soils amended with either biochar were significantly reduced compared to those grown in unamended soils, to the order of activated biochar > biochar treatments ($p < 0.05$). There was significantly less lead in pea roots grown in soils amended with activated biochar compared to the control soils ($p < 0.05$). Finally, both biochar affected the nickel concentrations in pea roots ($p < 0.05$) but activation did not cause a difference in concentration ($p > 0.05$). To summarise, it was hypothesised that biochar will reduce trace element uptake by pea plants and that LMWOA-activated biochar will not affect trace element uptake by a selected plant species. Concerning root concentrations, the results found:

1. Biochar and LMWOA-activated biochar reduced root-borne aluminium, iron, manganese and nickel in pea plants. There was no difference between biochar materials.

2. LMWOA-activated biochar reduced root-borne cobalt and lead in pea plants. There was no difference between biochar materials.

3. Biochar and LMWOA-activated biochar reduced root-borne copper in pea plants. Activated biochar caused less root accumulation.

4. Neither biochar nor LMWOA-activated biochar affected root-borne arsenic, cadmium, chromium, or zinc in pea plants grown in both soils.

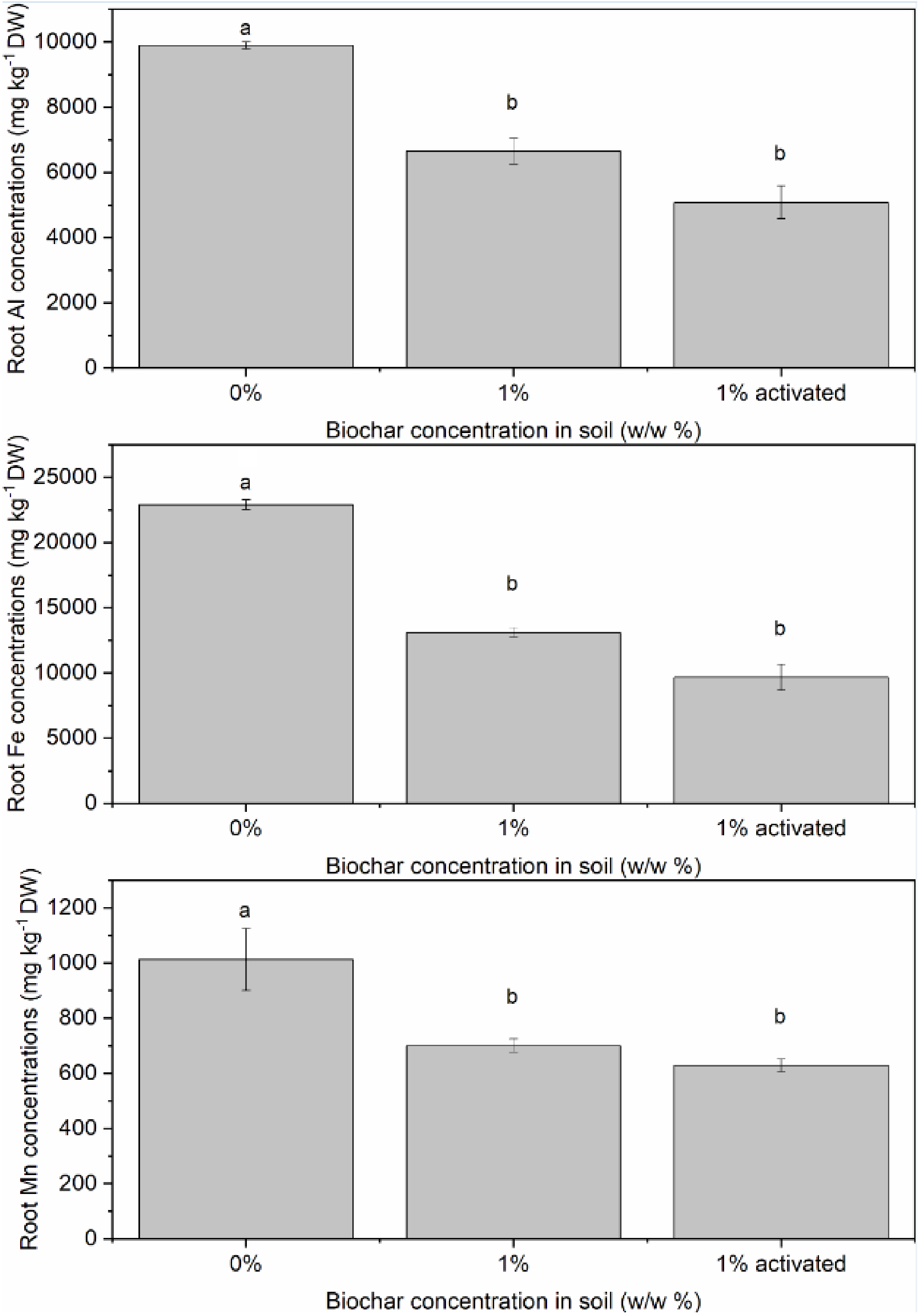

Figure 4.16 Concentration of (A) aluminium, (B) iron and (C) manganese in the roots of pea plants grown on the amended and unamended soils after one months growth. Values are presented as mean values ± standard error. Different lowercase letters denote a statistically significant difference according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

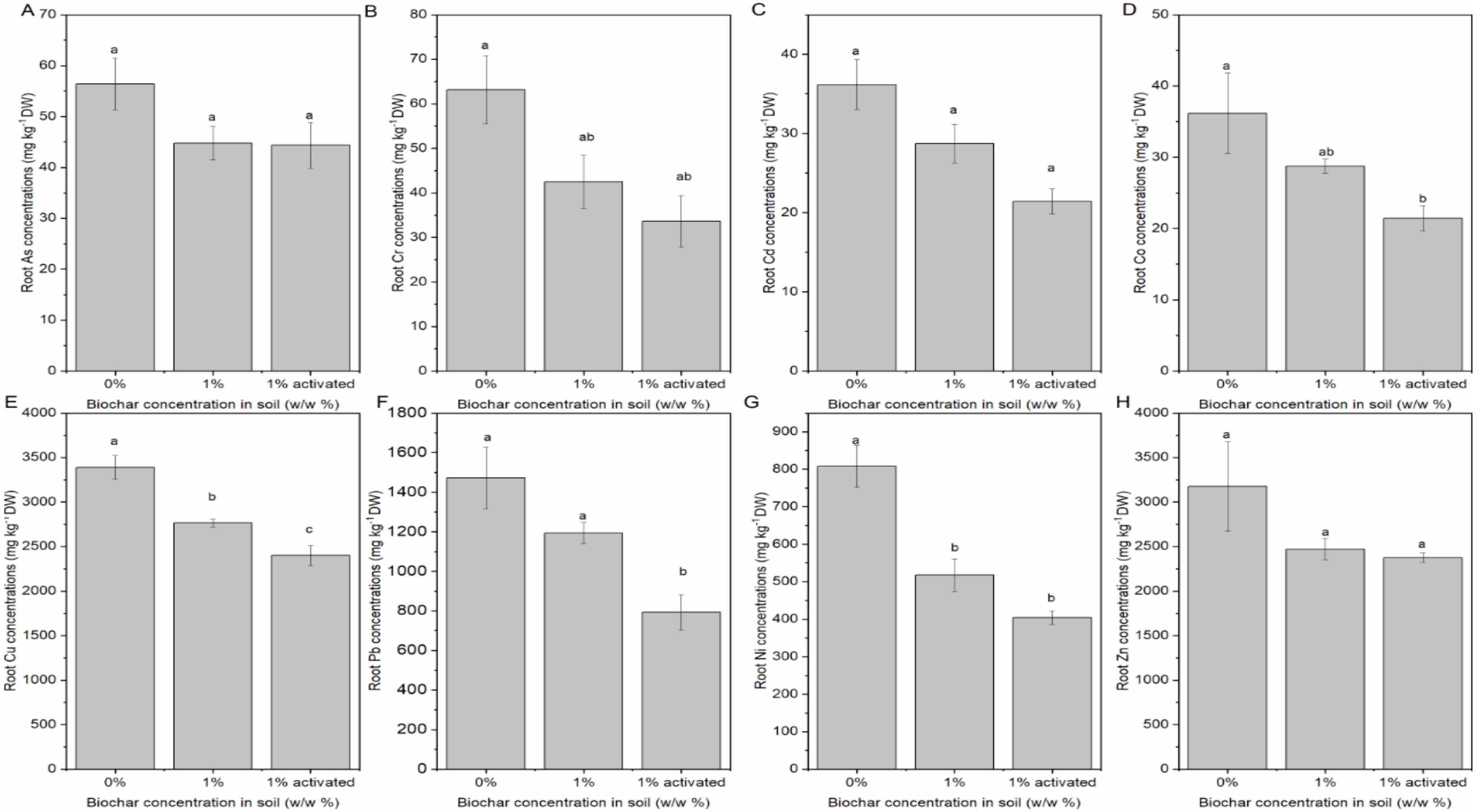

Figure 4.17 Concentration of (A) arsenic (B) chromium (C) cadmium (D) cobalt (E) copper (F) lead (G) nickel and (H) zinc in the roots of pea plants grown on the amended and unamended soils after one month's growth. Values are presented as mean values ± standard error. Different lowercase letters denote a statistically significant difference according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

4.7.5 Trace element concentration in the aerial foliage

The experimental results for shoot trace element concentrations are shown in Figure 4.18 and Figure 4.19. Neither biochar nor LMWOA-activated biochar affected the shoot accumulated concentrations of iron, manganese, arsenic, cadmium, chromium, copper, cobalt and zinc ($p > 0.05$). However, pea plants grown in activated biochar-amended soils showed significantly reduced aluminium accumulation in the aerial biomass compared to the control ($p < 0.05$). The same trend was found for nickel. However, there was no difference between foliage concentrations between either biochar types ($p > 0.05$). The foliage concentrations of lead were significantly reduced following the application of either biochar ($p < 0.05$). The reduction was greater after activated biochar application and was significantly different from plants grown in cells amended with normal biochar ($p < 0.05$). To summarise, it was hypothesised that biochar will reduce trace element uptake by pea plants and that LMWOA-activated biochar will not affect trace element uptake by a selected plant species. Concerning shoot concentrations, the results found:

1. LMWOA-activated biochar reduced aluminium and nickel in the aerial foliage of pea plants compared to biochar-amended and unamended soils. There was no difference between biochar materials.

2. Biochar and LMWOA-activated biochar reduced lead in the aerial foliage of pea plants grown in contaminated soils after one-month. LMWOA-activated biochar significantly reduced foliage concentrations compared to normal biochar.

3. Shoot-borne iron, manganese, arsenic, cadmium, chromium, copper, cobalt and zinc was not affected by the presence of biochar in either substrate studied.

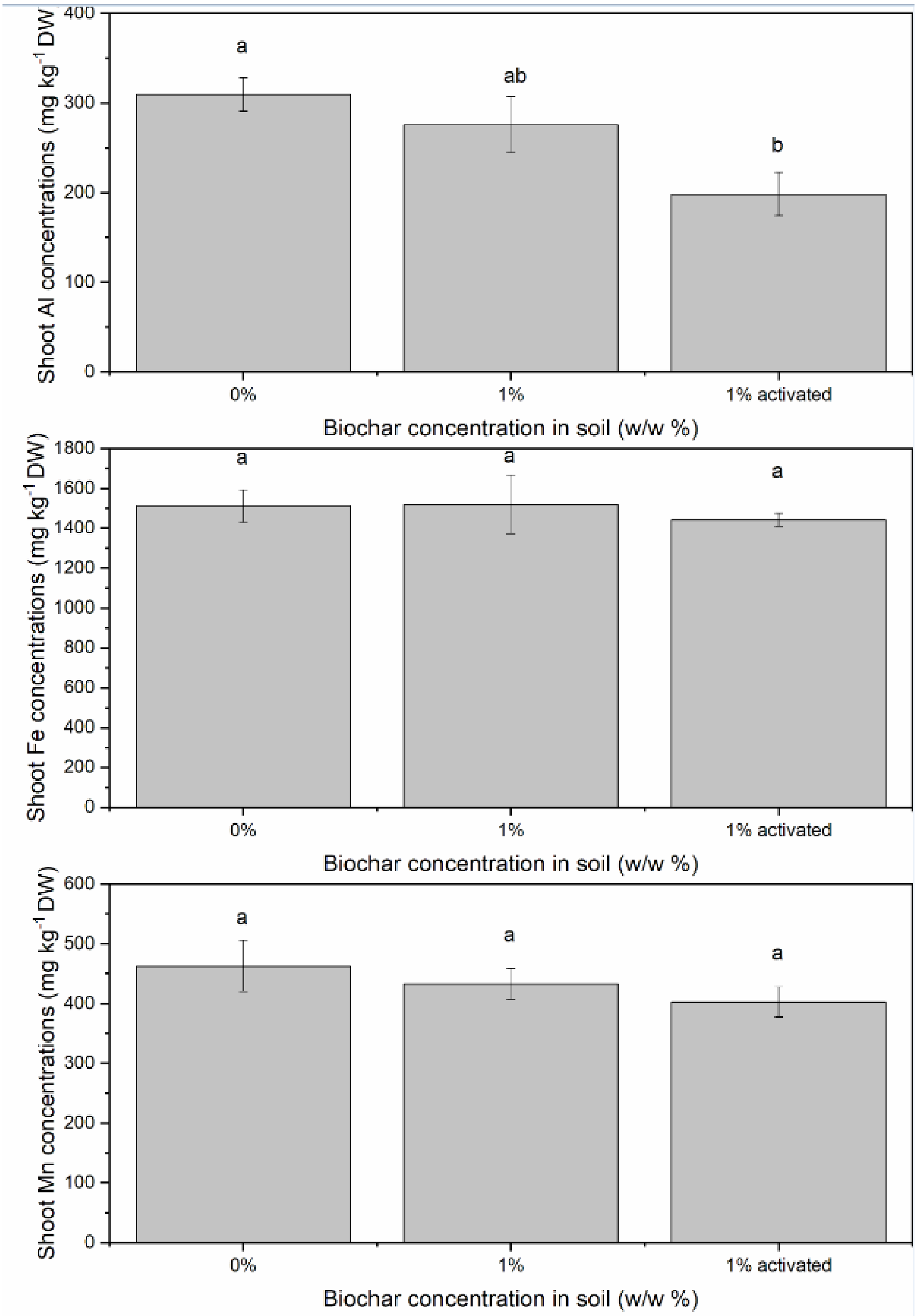

Figure 4.18 Concentration of (A) aluminium, (B) iron and (C) manganese in the shoots of pea plants grown on the amended and unamended soils after one month’s growth. Values are presented as mean values ± standard error. Different lowercase letters denote a statistically significant difference according to one-way ANOVA and Duncan’s post-hoc test ($p < 0.05$).

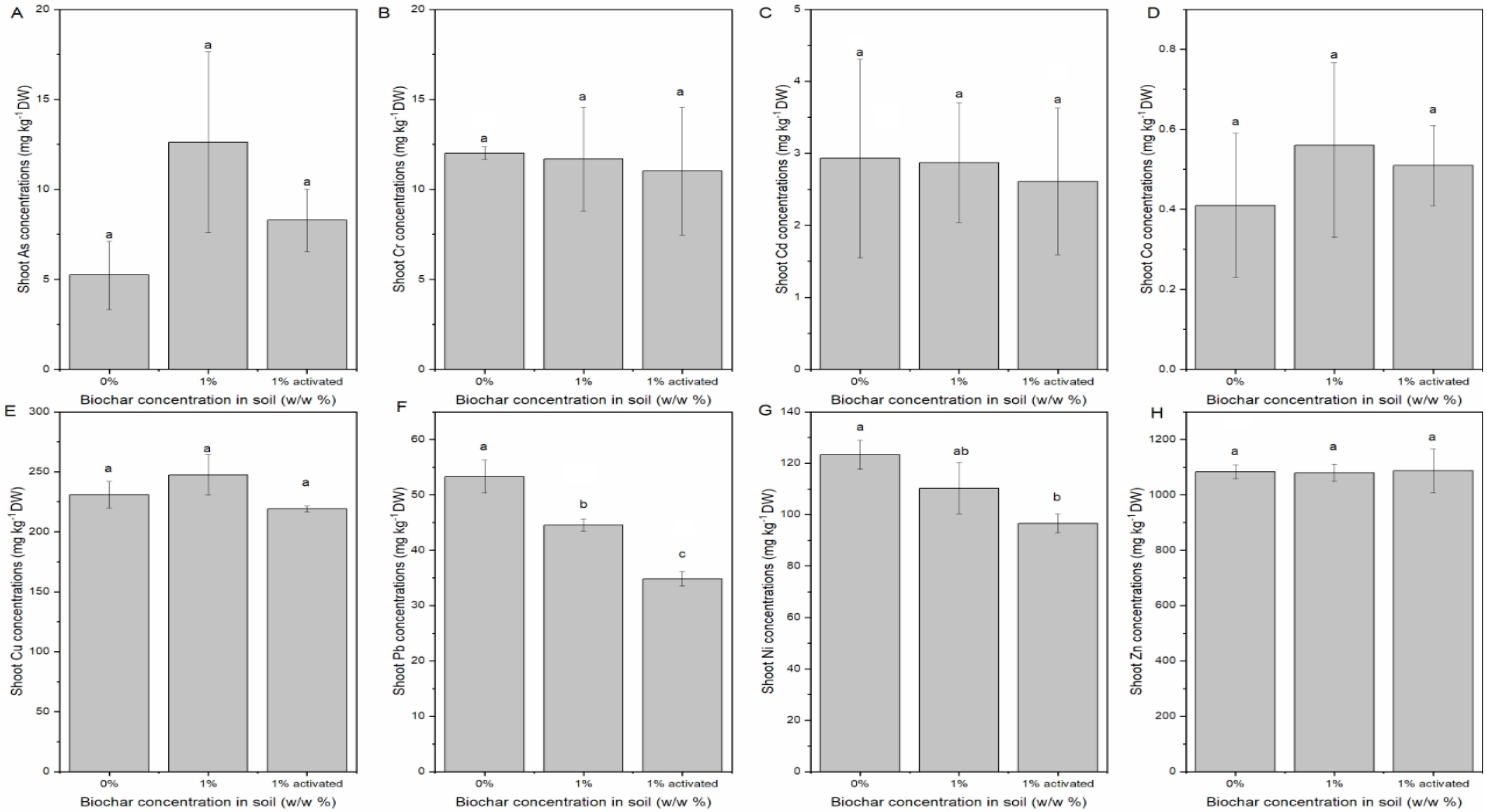

Figure 4.19 Concentration of (A) arsenic (B) chromium (C) cadmium (D) cobalt (E) copper (F) lead (G) nickel and (H) zinc manganese in the shoots of pea plants grown on the amended and unamended soils after one month's growth. Values are presented as mean values ± standard error. Different lowercase letters denote a statistically significant difference according to one-way ANOVA and Duncan's post-hoc test ($p < 0.05$).

4.8 Summary

In this chapter, the results of each experiment conducted to meet the thesis aim have been reported. The twelve biochar materials from the UKBRC standard biochar materials set were characterised and the results for each hypothesis were presented. Concerning the biochar characteristics, the study revealed the biochar materials are alkaline (Table 4.1); contained different functional groups (Figure 4.2 and Table 4.3), and generally displayed a porous surface area (Figure 4.3 and Figure 4.4). Some of the biochar materials such as SS550 and SS700 were found to be high in total heavy metal content (Table 4.4).

Hypothesis 1 was that LMWOAs will inhibit the biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems. The main findings were that rice husk biochar could successfully immobilise cadmium, lead and zinc in water (Table 4.13, Table 4.16 and Table 4.19). The biochar favourably retained lead removing it entirely from solution. LMWOAs impeded the biochar-driven removal of cadmium and lead solutions but the behaviour of zinc in the presence of LMWOAs was unclear. Hypothesis 2 then theorised that LMWOAs will facilitate the biochar-driven immobilisation of nitrate and phosphate in aqueous systems. Major findings were that the addition of LMWOAs facilitated the adsorption of nitrate (Table 4.24). Without LMWOAs, biochar was unable to remove soluble nitrate. Nitrate adsorption by rice husk biochar in the presence of LMWOAs followed pseudo-second-order kinetics (Table 4.25). However, nitrate adsorption appeared to be kinetically slower in the presence of oxalic acid. A second significant finding for Hypothesis 2 was the inability of biochar to remove phosphate from solution (Table 4.27). On the contrary, LMWOAs increased the amount of soluble phosphate in solution (Table 4.27 and Figure 4.6).

Hypothesis 3 stated that biochar will reduce the LMWOA-driven solubilisation of trace elements and nutrients in soils. The main findings were that rice husk biochar inhibited the LMWOA-driven mobilisation of soil-borne both cadmium and zinc (Figure 4.8 and Figure 4.10). However, unlike the enhanced effects of LMWOAs on nitrate immobilisation by biochar in solution (Table 4.24), this was not observed for the soil systems (Figure 4.12). The addition of citric and malic acid into the soil caused the immobilisation of nitrate over time but the no-added biochar soil performed better in terms of nitrate immobilisation (Figure 4.11 and Figure 4.12). The addition of biochar reduced the immobilisation rate of the added nitrate. Regarding phosphate, for the systems without added LMWOAs, biochar enhanced phosphate immobilisation (Figure 4.13) but in the presence of LMWOAs, the enhanced effect appeared reduced (Figure 4.14).

Hypothesis 4 was that biochar and LMWOA-activated biochar will affect various growth parameters of several plant species. The results found that biochar and LMWOA-activated biochar unanimously showed no effect on germination percentage but had variable effects on plant growth (Table 4.33). Hypothesis 5a stated that biochar will reduce trace element uptake by a selected plant species. Findings showed that pea root concentrations of aluminium, iron, manganese, cobalt, copper, lead and nickel in soil amended with normal biochar was reduced and the shoot concentrations of aluminium and lead were reduced in normal biochar-amended soils (Figure 4.16 and Figure 4.17).

Finally, the last hypothesis stated that LMWOA-activated biochar will not affect trace element uptake by a selected plant species. The findings showed that root-borne aluminium, iron, manganese, cobalt, copper, lead and nickel were reduced in the presence of LMWOA-activated biochar. (Figure 4.18 and Figure 4.19). More copper was reduced than in the presence of normal biochar. Shoot-borne aluminium and lead also decreased;

however, lead foliage reduction was more than in the normal biochar-amended soils. The next chapter in this thesis will discuss these key findings within the limitations of the methods outlined in Chapter 3, and ascertain whether to accept or reject each hypothesis.

CHAPTER 5

Discussion

5.1 Overview

In the last chapter, the UKBRC biochar materials were characterised (section 4.2) and the results for each experiment conducted to test the individual hypotheses were presented (section 4.3 to section 4.7) using the methods outlined in Chapter 3. This thesis aimed to examine the effect of biochar on trace element and nutrient solubility in the presence of LMWOAs. To meet this aim, this chapter will now provide a summary of the studies completed and a critical discussion of the key findings for the biochar characterisation studies (section 5.2) and hypotheses 1 to 5 (section 5.3 to 5.7) to help support or disconfirm the hypotheses stipulated in Chapter 2. Recommendations for future research are also proposed where necessary.

5.2 Biochar characterisation

Before work on the research hypotheses, the various UKBRC standard biochar materials were characterised. The findings confirm that the biochar materials studied have different physicochemical properties. Notable results will now be discussed in-depth.

5.2.1 Varying pH values for UKBRC standard biochar and implications

Earlier studies have highlighted the importance of biochar characterisation before application (Aller, 2016; Cantrell et al., 2012; Jindo et al., 2014; Keiluweit et al., 2010; Mukome et al., 2013). This thesis aimed to characterise the twelve UKBRC standard biochar using commonly applied methods and a novel LMWOA-extraction procedure. The current study found that all the biochar materials were alkaline (Table 4.1) ranging from 7.58 to 11.17 and had high points of zero charge (Table 4.2). The values for biochar pH agree with previous work (Cantrell et al., 2012; Singh et al., 2010). Biochar pH is typically alkaline because of the alkaline functional groups, ash content and alkaline mineral content of the material. However, biochar feedstock, pyrolysis temperature and ash content are thought to be the primary factors to govern final pH (Enders et al., 2012; Yakout, 2017). This study found lignocellulosic biochar to have higher pH values compared to SS550, SS700, SWP550 and SWP700 (Table 4.1), as previously reported (Gao et al., 2019; Kloss et al., 2012; Rajkovich et al., 2012). To confirm any association between pH and ash content, a bivariate correlation was conducted (Table 5.1). This study has been unable to demonstrate that there is a significant correlation between biochar pH and ash content (as provided by the UKBRC) ($R^2 = -0.109$, $p = 0.735$).

These results may be explained in part by the presence of alkaline functional groups which were less prevalent for lower pH biochar (Table 4.3 and Figure 4.2). A further reason may be the hydrolysis of biochar-borne goethite which could release protons and acidify the biochar materials, as found in sewage sludge-derived biochar studies (Gao et al., 2019; Xu, Kan, Zhao, & Cao, 2016). For softwood biochars (SWP550 and SWP700), the high lignin content (around 25 %) is more likely to cause a lower pH, whereas the lesser lignin content is likely to increase pH for the other lignocellulosic feedstock (Huang, Li, Meng, & Chen, 2018). In addition to the alkaline pH, the high point of zero charge suggests that in lower pH soils such as Ultisols and Oxisols the biochar is likely to be protonated, which may reduce its efficacy for cation retention and increase the efficacy for anion retention (Alozie et al., 2018). This would also occur in rhizospheric soils where the pH can be considerably lower than bulk soil (Sun et al., 2019). The implications of these biochar characteristics suggest that higher pH biochar materials from the UKBRC may act as a liming agent in low pH soils, which may decrease exchangeable aluminium and improve nutrient availability in contaminated soils. However, caution should be exercised if more circumneutral pH biochar were applied to alkaline soils, as this may affect nutrient and trace element solubility (Laghari et al., 2016).

Table 5.1 Pearson's correlation coefficients amongst the UKBRC standard biochar properties

	Ash^1	**pH**^{2}	**EC**^{2}
Ash^1	1		
pH^2	-0.109	1	
EC^2	0.711**	-0.675*	1

pH: pH value; Ash: Ash content (%); EC: Electrical conductivity (mS cm^{-1}); Correlation is significant at *$p < 0.05$; **$p < 0.01$ (two-tailed).
^{1}UKBRC provided value (2018); ^{2}Values produced from the current research.

5.2.2 Surface functionality and morphological differences

Another important finding was the functionality and porosity of the biochar materials. Many of the biochar materials shared similar functional groups (Figure 4.2 and Table 4.3) and displayed surface porosity (Figure 4.3 and Figure 4.4). Biochar materials can have both acidic (e.g. carboxyl and hydroxyl groups) and basic (e.g. carbonyl, quinone, heterocyclic nitrogen) functional groups (Kumar et al., 2018; Uchimiya, Wartelle, Klasson, Fortier, & Lima, 2011). Depending on solution pH these functional groups can either become protonated with free H^+ ions in low pH environments or deprotonated at higher pH. As such, surface functional groups play an essential role in biochar-contaminant interactions via surface complexation (Ahmad et al., 2014; Liu & Zhang, 2009; Mireles, Parsons, Trad, Cheng, & Kang, 2019). The identified functional groups and their associated wavelengths for the biochar materials were aromatic C=C stretching (1500 to 1580 cm^{-1}), C-O-C stretching vibrations of hemicellulose and cellulose (1035 to 1080 cm^{-1}), aliphatic ether C-O (1000 to 1020 cm^{-1}) and aromatic C-H bending (700 to 900 cm^{-1}). Biochar materials created at 550 °C showed much more functionality than those created at 700 °C, which relates to the decreased amount of oxygen-containing functional groups (Shaaban, Se, Mitan, & Dimin, 2013; Shen, Zhang, Jin, Alessi et al. 2018). To further support the FTIR findings in the current study, the manufacturer-supplied information regarding oxygen content (per cent) and elemental ratios. Oxygen/carbon element ratios are often used as an indicator of biochar characteristics. The manufacturers reported the majority of the biochar materials produced at 700 °C to have an O/C ratio ≤ 0.07, apart from OSR700 (0.09). Whereas all those biochar materials produced at 550 °C had an O/C ratio of ≥ 0.08. Particularly, SS550 which had an O/C of 0.17. A low oxygen/carbon element ratio is suggestive of a low number of functional groups due to decarboxylation and increasing aromaticity. By plotting H/C against O/C element ratios, a visualisation of the difference in aromaticity and polarity of the twelve standard biochar materials is provided (Figure 5.1).

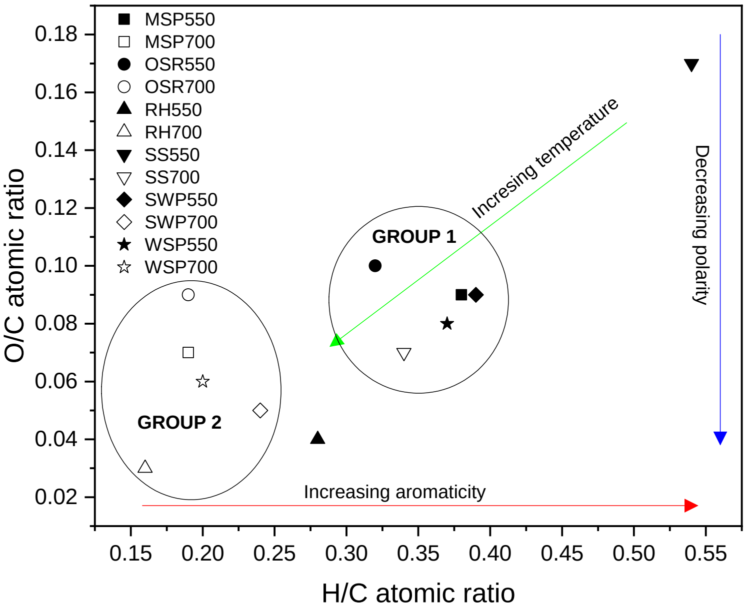

Figure 5.1 A comparison of the Van Krevelen plots for the twelve UKBRC standard biochar produced from miscanthus straw pellet (MSP) oil seed rape straw pellet (OSR), rice husk (RH), sewage sludge (SS), softwood pellet (SWP) and wheat straw pellet (WSP) produced at either 550 °C or 700 °C, respectively. The two circles represent two distinct groupings of H/C vs O/C ratio, leaving two outliers.

The functional groups present on the 550 ^{o}C biochar materials and the overall higher O/C ratio would confirm the presence of oxygen-containing functional groups.

Previous studies have suggested lower pyrolysis temperature may relate to complexation mechanisms (Dong, Ma, Zhu, Li, & Gu, 2013; Harvey, Herbert, Rhue, & Kuo, 2011). It is possible the functional groups found in this study may be beneficial for the immobilisation of heavy metal via complexation mechanisms. This would be dependent on factors such as soil pH, speciation for the available metals and co-existing trace elements and contaminants that may preferentially bind and/or compete for complexation with the active functional groups present. The biochar with less functionality would still be able to immobilise heavy metals via other sorption mechanisms such as electrostatic attraction or precipitation (see Chapter 2)

A pivotal point to the success of functional group identification is the type of preparation method. In the current study, a diamond plate was used on the FTIR. Johnston (2017, cited in Singh et al., 2017) compared the use of several FTIR techniques on spectral output. When comparing the technique applied here, and preparation with potassium bromide, the author found a difference in spectral output. In a demonstration, a comparative spectrum with and without the use of potassium bromide was performed. The outcome of which is displayed in Figure 5.2 The recalcitrance of high pyrolysis temperature biochar means that using incorrect methods may report lowered functionality. It is important to note, FTIR spectroscopy is a qualitative and complementary characterisation technique. As such, the process is subjective and cannot indefinitely identify functional groups present on the surface of the biochar materials. A more detailed study using the UKBRC standard biochar and different infrared spectroscopy techniques may be required before generalisation of biochar functionality can be made.

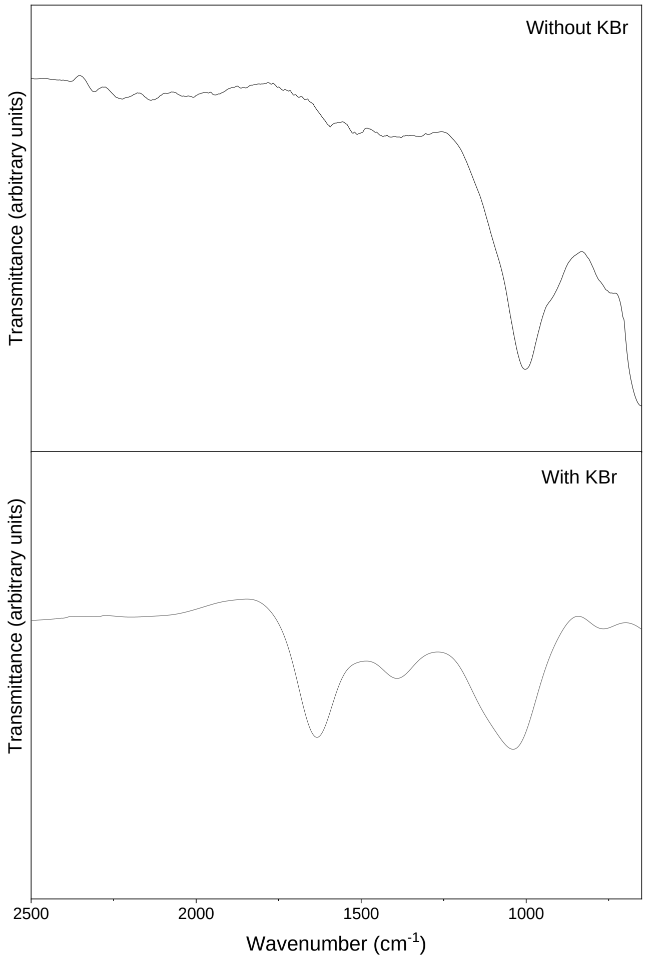

Figure 5.2 A comparison of different FTIR spectroscopy techniques on SS550 biochar either with or without the use of KBr to increase optical reflectance.

The SEM imagery (Figure 4.3 and 4.4) revealed the twelve biochar materials used in this study had porous and diverse surface morphologies. For instance, many of the images showed macroporous and mesoporous surfaces, whereas SS550 and SS700 showed limited porosity. The formation of pores occurs throughout pyrolysis. Mass is lost via dehydration and volatilisation and leaves behind a structure reflective of the original biomass that is more apparent with plant-derived biochar that leaves behind evidence of capillary networks (Downie et al., 2009; Novak & Johnson, 2019). This would explain why all the plant and wood-derived biochar from the UKBRC Standard Set had visible surface porosity compared to SS550 and SS700. In the current study, where imagery demonstrated pore networks this coincided with the manufacturers BET surface area measurements (Appendix A3). This relationship is supported by several recent studies (Regmi et al., 2012; Roy, Kumar, & Bhattacharyya, 2019; Vu et al., 2017).

Porosity is an essential characteristic of biochar materials. A larger porosity increases the surface area of the biochar. This may increase the number of active sites available for biochar-contaminant interactions. Macropores also provide a refuge for soil-borne microorganisms and help aerate soils, improving soil quality (Aller, 2016). In the same way as FTIR analysis, SEM imagery without the addition of BET surface area or other porosity measurements are qualitative analysis. As shown in SEM imagery and BET measurements by Q. Zhang et al. (2019), ball-milling increases the surface area of the biochar. The heterogeneous nature of biochar requires a suite of characterisation studies. So too is the importance of preparation techniques. To summarise, the SEM and FTIR data supplied in this research should be used to complement the existing information regarding the UKBRC standard biochar and cannot be used to make quantitative assumptions regarding the biochar characteristics.

5.2.3 Total metal content values

In addition to the pH, point of zero charge, surface functionality and porosity of the UKBRC standard biochar, it is interesting to note that certain biochar materials from the standard set contained a high total metal content (Table 4.4). Biochar total metal content primarily relates to the original mineral content of the original feedstock material (Cantrell et al., 2012; Yuan, Xu, & Zhang, 2011). During pyrolysis, the degradation of carbon, hydrogen, oxygen and organic content leads to the concentration of minerals and heavy metals as these only volatilise at high temperatures (> 600 ^{o}C) (Pituello et al., 2015; Zielińska & Oleszczuk, 2015). This study has been unable to demonstrate that biochar total heavy metal content increases with pyrolysis temperature as found by others (Cantrell et al., 2012; Jin et al., 2016; Zielińska & Oleszczuk, 2015). In this study, where total metals were detected, no general trend could be identified for any of the biochar materials examined. For instance, copper values decreased between MSP550 and MSP700 with pyrolysis temperature but increased between SWP550 and SWP700.

Notably, in the current study, SS550 and SS700 consistently contained detectable arsenic, chromium, copper, lead, molybdenum, nickel, selenium and zinc which were generally higher than the other biochar materials studied (straw, grass and wood-derived biochar materials). This is consistent with recent work by Zheng, Liu, Liu, Cai, and Zhang (2019). However, the high metal content of SS550 and SS700 does not necessarily mean an increased bioavailability of these biochar-borne metals (Domínguez, Menendez, Inguanzo, Bernad, & Pis, 2003; Domínguez, Menéndez, & Pis, 2006; Liu et al., 2014). The process of pyrolysis can stabilise minerals within feedstock into the carbonaceous fraction of the biochar material. Such stabilisation of biochar-borne minerals makes them recalcitrant to leaching (Inguanzo, Domínguez, Menéndez, Blanco, & Pis, 2002; Pituello et al., 2015).

In some instances, within this study total metal contents are less than the extracted metal contents. This is likely caused by metal content values below the limit of detection for XRF. It is plausible that the biochar materials employed in the current study may contain small levels of total metals that have not been detected by the XRF. It is essential to bear in mind the different techniques used to assess total and bioavailable metal content throughout biochar-related research. The current study used the method previously outlined in section 3.3.2.4, and the limitations such as biochar particle size, scanning time and reference material may have affected the data in this research. A more traditional and commonly used approach is that of ICP-OES with total digestion (such as used by the UKBRC, see Appendix A3). For instance, some works used either single or a mixture of acids to digest the biochar materials before analysis on ICP-OES. Commonly used digestants are nitric acid and hydrochloric acid, or a mix like that used by Liu et al. (2014) who utilised a mix of nitric, hydrochloric and hydrofluoric acids to digest biochar materials before analysis. ICP-OES is often favoured for analysis due to its acute limits of detection. However, these methods often require a lengthy sample preparation-to-analysis time and are increasingly cited as an expensive and non-eco-friendly laboratory practice (Arenas, Ortega, García-Martínez, Querol, & Llamas, 2011). XRF studies have been frequently put forward as cheaper, quicker and eco-friendlier compared to ICP-OES methods (Chojnacka & Mikulewicz, 2019). Although these methods have a less sensitive limit of detection for elements, environmental scientists for various applications increasingly, adopt them (Mullen et al., 2010; Shuttleworth, Clay, Evans, Hutchinson, & Rothwell, 2017; Sterk, Gazley, Wood, Collins, & Collis, 2018). Given that the total metal content of biochar materials does not necessarily reflect that which is bioavailable and therefore of potential toxicity, it was deemed suitable to use XRF in the current study for total analysis and ICP-OES for the analysis of extractable and possibly bioavailable metals. Furthermore, this decision decreased analysis costing and time.

The UKBRC uses three standards to compare the total metal content of biochar materials. These are the International Biochar Initiative (IBI), European Biochar Standard (EBC) and Biochar Quality Mandate (BQM). Importantly, these standards are not enforceable but act as a guideline for biochar producers and environmental practitioners. A summary of the guideline values can be seen in Table 5.2. As shown, the threshold values between guidelines can be widely varying in range. In most instances, the BQM is the most stringent of the guidelines. A summary of the biochar materials from the UKBRC standard biochar compared with the thresholds is set out in Table 5.3.

Table 5.2 A summary of the recommended standard threshold values for total heavy metal content in biochar materials as provided by the UKBRC (2018).

Element	**Biochar Quality Mandate**	**European Biochar Certificate**	**International Biochar Initiative**
Arsenic (mg kg^{-1})	10	n/a	12–100
Cadmium (mg kg^{-1})	3	1	1.4–39
Chromium (mg kg^{-1})	15	80	64–1200
Cobalt (mg kg^{-1})	n/a	n/a	40–150
Copper (mg kg^{-1})	40	100	63–1500
Lead (mg kg^{-1})	60	120	70–500
Mercury (mg kg^{-1})	1	1	1–17
Molybdenum (mg kg^{-1})	10	n/a	5–20
Nickel (mg kg^{-1})	10	30	47–600
Selenium (mg kg^{-1})	5	n/a	1–36
Zinc (mg kg^{-1})	150	400	200–7000

MSP550 breaches the BQM thresholds for copper and zinc. Likewise, SS550 and SS700 breach many of the BQM and EBC thresholds for the elements listed. Notably, none of the twelve biochar breaches the IBI thresholds. It is imperative to remind the reader that although these biochar materials breach some of the guideline values, this is based on total metal content and not that which is instantly available in the soluble pool within soils. Therefore, further work is required to examine the extractability of biochar-borne elements using different extractants against. For instance, the weathering of the biochar materials, microbial consumption and LMWOA-aging of the biochar materials may liberate elements of potential toxicity (Liu et al., 2017; Liu et al., 2014; Vause et al., 2018). Furthermore, soil pH, plant presence and climate regime may influence the rate in which these elements become available. As such, the data in this study should be used to help environmental practitioners make informed decisions regarding biochar choice for the intended application. It must be noted that the total metal content results derived by XRF and therefore the comparison to guideline values are subject to the limitations of the XRF methodology presented in section 3.3.2.4, so should be interpreted with caution.

Table 5.3 A summary of the different biochar materials and their metal contents in relation to the standard threshold values. Where the guideline acronym is inputted, the corresponding biochar had total metal values greater than the related guideline values

Element ($mg\ kg^{-1}$)	**MSP550**	**MSP700**	**OSR550**	**OSR700**	**RH550**	**RH700**	**SS550**	**SS700**	**SWP550**	**SWP700**	**WSP550**	**WSP700**
Arsenic	√	√	√	√	√	√	BQM	BQM	√	√	√	√
Cadmium	√	√	√	√	√	√	√	√	√	√	√	√
Chromium	√	√	√	√	√	√	BQM, EBC	BQM, EBC	√	√	√	√
Cobalt	√	√	√	√	√	√	√	√	√	√	√	√
Copper	BQM	√	√	√	√	√	BQM, EBC	√	√	√	√	√
Lead	√	√	√	√	√	√	BQM, EBC	BQM, EBC	√	√	√	√
Mercury	√	√	√	√	√	√	√	√	√	√	√	√
Molybdenum	√	√	√	√	√	√	√	√	√	√	√	√
Nickel	√	√	√	√	√	√	BQM, EBC	BQM, EBC	√	√	√	√
Selenium	√	√	√	√	√	√	√	√	√	√	√	√
Zinc	BQM	√	√	√	√	√	BQM, EBC	BQM, EBC	√	√	√	√

BQM; Biochar Quality Mandate: EBC; European Biochar Certificate: IBI; International Biochar Initiative: √; Tick indicates levels within discussed guidelines.

5.2.4 Extractable elements

The experiments also showed that many of the standard biochar had high levels of water extractable-nutrients (Figure 4.5). Biochar materials receive their nutrient-rich status as they are usually derived from nutrient-rich plant materials (Chan & Xu, 2012). However, these are usually lower in nutrient content compared to manures and sewage sludge-derived chars (Yuan, Lu, Wang, Chen, & Lei, 2016). Phosphorus and nitrogen are major components of inorganic materials, so a high amount of phosphorus and nitrogen-containing ions are expected within biochar materials. Notably, MSP700 released significantly higher amounts of nitrate and phosphate compared to other biochar materials ($p < 0.05$). To a lesser extent, RH700 also released ≥ 100 mg kg^{-1} of phosphate. The results reflect findings by others (Kloss et al., 2012; Prakongkep, Gilkes, & Wiriyakitnateekul, 2015; Yuan et al., 2016). Generally, all biochar materials also released elevated chloride and sulphate ions.

Studies have found positive improvements to soil nutrient status with biochar applications to the order of 10 t ha^{-1} (Jeffery et al., 2011; Limwikran, Kheoruenromne, Suddhiprakarn, Prakongkep, & Gilkes, 2018). A conversion of the water-extracted nitrate and phosphate content from all biochar materials in the current study to reflect an application rate of 10 t ha^{-1} is presented in Figure 5.3. This shows that all the biochar materials used in the study contain values much lower than the standard rates of fertiliser application rates reported in the UK (Department for Environment, Food and Rural Affairs [DEFRA], 2017). In summary, the nutrient content of the biochar materials may be suitable for smaller, more localised nutrient deficiencies, but not for more extensive application to tillage soils. This does not undermine the results of the current study. Recent research has identified that the co-existence of ions in solution can affect the contaminant removal efficacy of biochar materials (Hodgson et al., 2016; Wan et al., 2018). This is because of the release of biochar- borne ions into solution via dissolution. Released ions may compete for active sites on the biochar surface, slowing or completely inhibiting removal of target contaminants. Therefore, a quantitative evaluation of extractable ions of intended biochar should be evaluated before application.

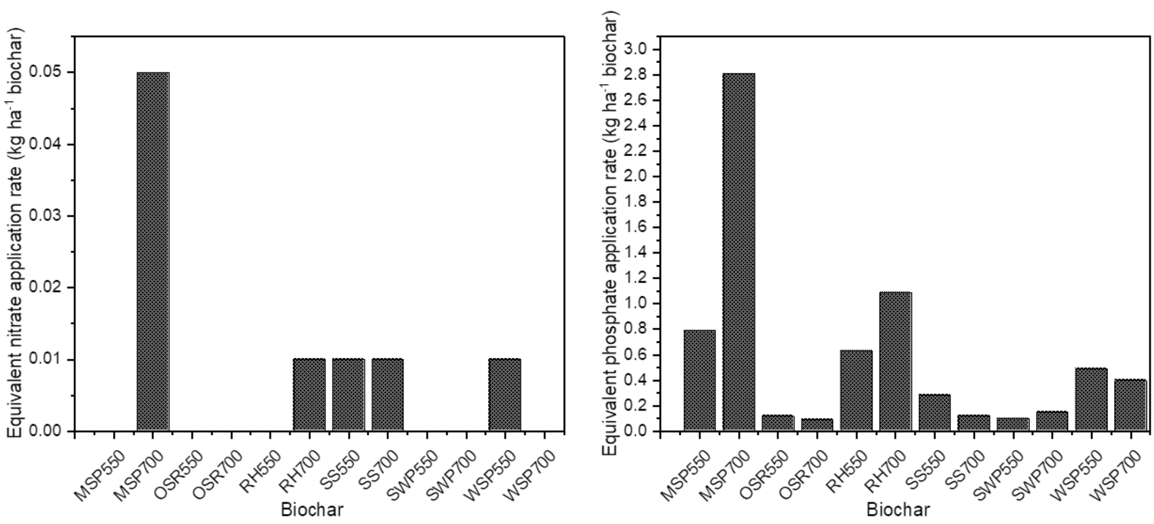

Figure 5.3 Equivalent nitrate (left) and phosphate (right) application rates based on a biochar application value of 10 t ha^{-1} as proposed by Jeffery et al. (2011). Values are calculated from water-extracted biochar-borne ions within this thesis.

5.2.5 Low-molecular-weight organic acid liberation of biochar-borne elements

This study also found evidence of differential LMWOA-released biochar-borne major elements and elements of potential toxicity (Table 4.7, Table 4.8 and Table 4.9). For common heavy metals of environmental importance (cadmium, cobalt, chromium, copper, nickel, lead and zinc), all except cobalt were detected. Arsenic was released in the control solutions for SWP700 and WSP700 but the standard error reported for SWP700 was high relative to the mean value. This is most likely down to analytical error. Chromium was only released in the presence of LMWOAs. All biochar materials released slight amounts of chromium; however, SS550 (1.33 mg kg^{-1}) and SS700 (0.49 mg kg^{-1}) released significantly more than any of the other biochar materials. This result is easily explained, given the large amount of total chromium detected in SS550 and SS700 (Table 4.4). The pH of the extracting solutions was ~2.47. Therefore, the soluble chromium may exist as $Cr_2O_7^{2-}$ or CrO_4^{2-}. Like chromium, copper was only released in LMWOAs and peaked for SS700 which had the highest total copper content of all twelve biochar (Table 4.4) which may be attributed to copper readily undergoing complexation with organic ligands. Further liberation of trace elements (boron and barium) was also observed.

Remarkably, SS550 and SS700 consistently released potentially toxic elements (e.g. chromium, copper, nickel, lead and zinc). As shown in Table 4.10, release rates for the various potentially toxic elements were between 0.1 to 16.1 %. These are lower release rates than those in previously published studies (Vause et al., 2018). However, the release rate followed a similar order. The high total metal content of the sewage sludge biochar materials compared to the other biochar explains the higher amount of LMWOA-extractable potentially toxic elements. This study has successfully provided new information that can be used to evaluate the phytoavailability of potentially toxic elements from UKBRC standard biochar materials.

Several caveats need to be noted regarding the present study. It is reported that rhizospheric concentrations of LMWOAs are typically within 0 to 20 mmol L^{-1} (Ash et al., 2016; Jones, 1998; van Hees, Jones, Jentschke, & Godbold, 2005). The concentration of LMWOAs used in the current study was an equimolar solution of 0.01 mol L^{-1} citric, malic and oxalic acid as previously used by Qin et al. (2018). Other studies have also used concentrations higher than the upper limit of LMWOA concentrations reported to understand the mechanisms occurring at the soil-root interface between trace elements and LMWOAs (Jia, Hou, Dai, Lu, & Yan, 2018; Joner, Corgie, Amellal, & Leyval, 2002). Under high concentrations of potentially toxic elements in the soil, roots can elevate root exudation to alleviate stress (Bruun, Van Rossum, & Ström, 2001). As such, it is plausible that such concentrations may occur in extreme circumstances of excessive contamination. Conversely, studies have shown root exudation to work on a concentration gradient spatially from the root itself (Gao, Yang, Ling, Kong, & Zhu, 2011). Gao et al. (2011)

found that the concentration of root exudation decreased with distance from the soil as LMWOAs were diffused and degraded throughout layers in the rhizosphere. Increased encounters with soil-borne microbes may also reduce the number of molecular acids due to microbial consumption as the distance from soil-plant interface increases. It can, therefore, be theorised that the concentration of LMWOAs at the point of secretion at the soil-root interface would be within the practical limits of this research. Further work using a range of concentrations and organic acid combinations should be considered.

Secondly, the release rates for the various potentially toxic elements (Table 4.10) were calculated using the biochar total element concentrations based on XRF results (Table 4.4). In section 3.3.2.4 of this thesis, the XRF method used to collect the biochar total element concentrations was presented as a research limitation. As such, the release rates that are given herein must be applied with caution. Furthermore, release rates calculated with biochar total metal content from acid digestion and ICP-OES analysis would better clarify the release of potentially toxic elements because of improved limits of detection and should be applied in future research (Kilbride, Poole, & Hutchings, 2006; Radu & Diamond. 2009).

5.3 Discussion for hypothesis 1

5.3.1 Retention of metals by RH700 in water

To start, it was tested if LMWOAs would inhibit the biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems. Firstly, the removal of these elements in water was realised. The current study found that rice husk biochar could significantly immobilise 100 % of soluble lead (Table 4.16), 83 % of soluble zinc (Table 4.19) and 79 % of soluble cadmium (Table 4.13) from solution ($p < 0.05$). These results are in accord with previous studies in terms of removal efficacy and selectivity sequence (Park et al., 2015; Xu et al., 2013). There are several possible explanations for favoured lead retention. A higher electronegativity and lower ionic radius would lead to a greater electrostatic attraction to the biochar surface and a greater potential to access the active sites in biochar micropores. In contrast, a larger ionic radius would lead to longer times to permeate biochar pores, decreasing adsorption (Gondek & Mierzwa-Hersztek, 2016). However, cadmium and zinc removal did not follow either the electronegativity constants or ionic radii (Table 5.4).

Table 5.4 Electronegativity constants, ionic radii and ionic potentials for cadmium, lead and zinc

Element	Electronegativity Constant[1]	Ionic Radius[2]	Ionic Potential (charge/radius)[2]
Cadmium	1.69	-0.426nm	1.9
Lead	2.33	-0.410nm	3.3
Zinc	1.65	-0.430nm	2.4

[1]Park et al. (2015) [2] Petrov, Budinova, & Khavesov (1992).

Ionic potential refers to an ion’s charge and radius quantifying the charge density of the ion, which is an important determining factor for the adsorption of ions by carbonaceous materials (Petrov, Budinova, & Khavesov, 1992). The adsorption selectivity of the three elements by the biochar materials is described by their ionic potentials (Table 5.4). Therefore, the greater retention of lead may be explained by a greater electrostatic attraction to the biochar active sites and greater accessibility to active sites within the biochar pores. The retention of cadmium and zinc is likely credited to ionic potential given the similar ionic radii.

To help quantify this, the theoretical sorption mechanisms for cadmium, lead and zinc can be assumed with biochar characterisation paired with sorption kinetic modelling. In the first instance, the point of zero charge of the biochar was 8.59 (Table 4.2). In the current study, the pH of the three biochar-metal-containing systems ranged from 7.09 to 7.57 (Table 4.12, Table 4.15 and Table 4.18), therefore the biochar surface is likely to be protonated. In contrast to earlier findings, Shen, Zhang, Jin et al. (2017) found RH700 biochar to have a point of zero charge of 7.50, over 1 unit less than in the present study. This could be attributed to inter-lab differences and different methodologies for point of zero charge determination. It is encouraging, however, that findings by Shen, Zhang, Jin et al. (2017) and in the current study, the point of zero charge values would support the protonation of the biochar surface. Due to the experimental pH, biochar point of zero charge and biochar pH (11.17), it is likely cadmium, lead and zinc would not be immobilised via electrostatic attraction. Based on the available literature, a possible speculated sorption mechanism for the removal would be via surface precipitation mechanisms (Inyang et al., 2012; Shen, Zhang, Jin, Alessi et al. 2018).

Sorption kinetics for the three metals revealed a good level of fit to both pseudo-first-order and pseudo-second-order mechanisms (Table 4.14, Table 4.17 and Table 4.20). Cadmium and lead followed pseudo-second-order kinetics. This implies that chemisorption may be the rate-limiting step involved in biochar immobilisation of cadmium and lead. The results are in good agreement with recent studies and may suggest precipitation or inner-sphere complexation rather than electrostatic exchange (Kolodynska et al., 2012). Zinc sorption showed a good level of agreement with both models. The modelled Qe value was closer to that of the experimental Qe value and therefore, zinc sorption by RH700 may be better described by pseudo-first-order kinetics suggesting physisorption mechanisms.

In summary, biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems was found to the order of lead (100 %) > zinc (83 %) > cadmium (79 %). The greater efficacy of rice husk biochar to retain lead may be explained by the smaller ionic radius and greater ionic potential compared to zinc and cadmium, meaning a strong affinity for adsorption via electrostatic attraction. Importantly, further data collection is required to determine the sorption mechanisms as due to the limitations of the batch study methodology employed the modelling might not be totally valid (see section 3.6.5). This should incorporate post-sorption analysis of the biochar materials or desorption studies to support the inferred sorption mechanisms.

5.3.2 Retention of metals by RH700 in the presence of LMWOAs

Once cadmium, lead and zinc dynamics in the control were observed, it was important to understand the metal-LMWOAs dynamics without the presence of biochar. In the current study, soluble cadmium and lead maintained the theoretical initial concentration (1 mmol L^{-1}) in citric and malic acid systems so were unaffected by LMWOAs (Table 4.13, Table 4.16 and Table 4.19). However, these acids significantly diminished zinc concentrations ($p < 0.05$). This may be explained by the formation of relatively insoluble zinc-citrate and zinc-malate complexes (Gadd, 1999). All the metals studied showed decreased concentrations in oxalic acid, with 50 % cadmium, around 80 % of zinc and nearly 100 % of soluble lead removed. This suggests the formation of insoluble metal-oxalate complexes as in Equation 5.1, where Me^{2+} refers to metals, specifically cadmium, lead and zinc:

$$Me^{2+}(aq) + 2C_2O_4^{2-} \rightarrow Me(C_2O_4)_2^{2-}(s)$$

Equation 5.1 Formation of insoluble metal-oxalate complexes

Insoluble complex formation reduces available metals in solution. Gadd (1999) compiled the solubility of cadmium, lead and zinc-oxalate amongst others, noting a decreasing order of solubility following cadmium-oxalate > zinc-oxalate > lead-oxalate. This may rationalise the order and amount of cadmium, zinc and lead removed from solution observed in the current study.

Concerning whether LMWOAs inhibited the biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems, the results found that soluble cadmium and lead was always greater in the control as opposed to the LMWOA-containing systems. Therefore, the results indicate that LMWOAs impeded the biochar-driven removal of cadmium and lead from solutions. There are two possible explanations for this result. The current study found the solution pH to be less than the point of zero charge suggesting protonation in the presence of LMWOAs. Cationic metallic species would undergo electrostatic repulsion from the active functional groups. This finding corroborates the ideas of Alozie et al. (2018) who found positive retention of anionic species in the presence of LMWOAs. Given the pH of the solutions was acidic (Table 4.12, Table 4.15 and Table 4.18), it is likely this was the primary factor to impede biochar-driven immobilisation of cadmium and lead. The results for zinc were less clear, possibly due to the reasons previously discussed in the no-added biochar systems.

A second reason for the inhibited cadmium and zinc retention could be the competitive effects between cation and dissolved organic matter (DOM) (including high-molecular-weight organic acids) (Lian et al., 2015; Pignatello et al., 2006; Sun et al., 2015; Wang et al., 2013; Zhou, Wang, Zhang, & Qiu, 2015). For instance, Deepatana and Valix (2006) found reduced steric hindrance and crowding reduced nickel and cobalt-organic acid complexes on purolite. It is plausible that DOM, such as LMWOAs and the associated metal-ligand complexes may block the pores on the surface of the biochar; leading to competition for active sites between free metals and metal-ligand complexes. This would inhibit biochar-borne driven immobilisation of cadmium, lead and zinc. To confirm the interference of DOM on the biochar-driven immobilisation of trace elements, further work is required.

Previous studies have indicated that pH is a major determining factor for metal solubility in soil-systems, tending to exhibit increased solubility with a decreased pH (Chuan, Shu, & Liu, 1994; Krishnamurti, Cieslinski, Huang & Van Rees, 1997; Martinez & Motto, 2000; J. Yang et al., 2006). For instance, it would be expected that with the more acidic pH, a stronger correlation between pH and metal concentration would be observed compared to the water systems. In the current study, a strong negative linear relationship existed between pH and the trace elements in the water systems (Table 4.21). However, in the LMWOA-containing systems, there was generally a lack of correlation. As shown in Table 4.13, Table 4.16 and Table 4.19, the sorption of cationic metals in the presence of LMWOAs was inhibited. As previously discussed, the addition of un-buffered LMWOAs to give an extremely low pH ~ 2 at the concentration used that likely inhibited adsorption. This may have masked any possible correlation between pH and metal concentration. Hence, the data from this study is only applicable to extremely low pH systems which are rarely encountered in bulk soils, where biochar-trace element dynamics likely adhere to normal pH-solubility relationships as previously reported (Chuan, et al., 1994; Krishnamurti et al., 1997; Martinez & Motto, 2000; J. Yang et al., 2006).

To conclude this study found that LMWOAs inhibited the biochar-driven immobilisation of cadmium and lead in aqueous systems under the experimental conditions. This may be attributed to competition from LMWOAs and LMWOA-metal complexes or protonation of the biochar surface repelling cationic metal adsorption. The reduced efficacy of biochar to immobilise cadmium and zinc in the presence of LMWOAs may increase the availability of these elements in soil pore waters. In turn, this would pose an increased chance of plant uptake and a risk to ecological receptors.

5.4 Discussion for hypothesis 2

The present study was designed to investigate whether LMWOAs will facilitate the biochar-driven immobilisation of nitrate and phosphate in aqueous systems. The first step determined the release of biochar-borne nutrients over the experimental time. Rice husk biochar released nitrate and phosphate into the solution. It was found 0.01 mmol L^{-1} of nitrate and 0.05 mmol L^{-1} of phosphate were released (Table 4.22). These findings further support the idea of biochar materials being nutrient-rich as proposed in Figure 4.5 and corroborate with previous findings (Jeffery et al., 2011; Limwikran et al., 2018) (see section 5.2.5).

Concerning nitrate-biochar interactions in water, the results of this study indicate that biochar was unable to remove nitrate in water. Although these results differ from some published studies (Chintala et al., 2013; Hagemann et al., 2017; Kanthle et al., 2016; Libutti et al., 2016), they are consistent with others (Gai et al., 2014; Hollister et al., 2013; Yao et al., 2012) whereby, biochar materials are reportedly unable to remove nitrate from solution. For instance, Hollister et al. (2013) found that corn stover and oak wood biochar prepared at 350 ^{o}C and 500 ^{o}C were unable to nitrate from solution. Yao et al. (2012) found from thirteen biochar materials, those produced at higher temperatures (600 ^{o}C) showed a slight reduction in soluble-nitrate from solution (0.12 to 3.70 %). However, the remaining nine biochar materials showed no effect and even leached nitrate into solution.

The results of the current study may be explained in part by the pH of the solutions and characteristics of the biochar materials. At the experiment end, the pH of the water system containing biochar was 9.23 for nitrate (Table 4.23) and the point of zero charge of the rice husk biochar was 8.59 (Table 4.2). Therefore, it is probable that under the experimental conditions, the biochar was negatively charged explaining why the adsorption of anions was unlikely. This corroborates with the findings by Ohe, Nagae, Nakamura, and

Baba (2003) who claimed optimum pH for nitrate adsorption onto different carbonaceous materials to be between pH 2 to 4. It also relates to the findings for hypothesis 1.

Moving to the experimental evidence on biochar-nitrate dynamics in the presence of LMWOAs, the results of this study showed that biochar significantly enabled the biochar to adsorb the soluble nitrate and how different solutions had variable effects on retention to the order of malic acid > citric acid > oxalic acid. The amount of nitrate being adsorbed by biochar increased over time. Within the first 24 h of the experiment, 63, 55 and 60 per cent of the added nitrate were removed for the citric acid treatment, malic acid treatment and oxalic acid treatment, respectively (Table 4.24). Removal of nitrate continued for the citric and malic acid treatments and almost all of the added nitrate was removed by 120 h. The addition of LMWOAs likely allowed for the protonation of the biochar surface as previously suggested. In response, this could create favourable conditions for anion adsorption such as nitrate onto the biochar surfaces, as shown in Equation 5.2:

$$[\text{Biochar}]^+ + \text{NO}_3^- \rightarrow [\text{Biochar}]^+\text{-NO}_3^-$$

Equation 5.2 The adsorption of nitrate to the biochar surface following protonation of the active sites

A second possible explanation for the immobilisation of nitrate ions in LMWOAs may be through a bridging mechanism between nitrates and biochar-borne divalent ions. Few papers have shown the effect of coexisting ions with nitrates, and those that have used cations and coexisting model contaminants. For instance, Han et al. (2013) showed how Cd^{2+} facilitated the sorption of sulfamethoxazole to biochar using a bridging mechanism. The results of this study suggest that when nitrates occur in the presence of cations such as calcium (Ca^{2+}) or magnesium (Mg^{2+}) that similar facilitation may occur, enhancing nitrate adsorption. Indeed, Behrends, Miecznik, and Kaatze (2002) observed favourable interactions with divalent Ca^{2+} compared to monovalent Na^+. The results in section 4.2.9 of this study revealed rice husk biochar-borne LMWOA-extractable calcium (64.73 mg kg^{-1}) and magnesium (137.06 mg kg^{-1}). Based on the release of biochar-borne calcium, the mechanism shown in Equation 5.3 could also be speculated. Here, coexisting calcium is facilitating the adsorption of anionic nitrate by the bridging mechanisms formally described. It is plausible that the resulting biochar-$CaNO_3$ complex would then undergo cation exchange with other biochar-borne cations or experience deprotonation, especially as the pH increases with distance away from the soil-root interface to greater than the point of zero charge of the rice husk biochar (8.59) (Chintala et al., 2013).

$$[\text{Biochar}]\text{-}\ [\text{Ca}]^{2+} + [\text{NO}_3^-] \rightarrow [\text{Biochar}]^- \text{-}[\text{CaNO}_3]^+$$

Equation 5.3 The release of biochar-borne Ca^{2+} facilitating the adsorption of nitrate onto the biochar surface

In the oxalic acid treatment, no further removal of nitrate was found after 24 h compared to the other systems, indicating that a saturation status was achieved by 24 h. This may be due to the competition of oxalate ions with the nitrate for the sorption sites of the biochar surfaces. The lower acid disassociation constants (pKa) of oxalic acid compared to the other LMWOAs used (Table 5.5) (Strobel, 2001) would support the higher amount of extractable biochar-borne elements as found by Vause et al. (2018). If this is the case in the current study, the greater amount of oxalic extractable biochar-borne elements will compete for more for active sites explaining the retardation of facilitated nitrate adsorption in oxalic acid-containing systems.

Table 5.5 Acid disassociation constants (pKa) for the model LMWOAs used in the current study as reported by Strobel (2001)

Model low-molecular-weight organic acid	Reported pKa constant
Citric	3.13
Malic	3.46
Oxalic	1.25

Another important finding from this experiment was although oxalic acid enabled the retention of soluble-nitrate onto the surface of the biochar materials, the amount of immobilisation was much lower than that of the other acids as indicated by a lower k_2 value compared to the other LMWOAs. A lower k_2 value is indicative of kinetically slower adsorption (Ahmad et al., 2013; Idrees et al., 2016; Plazinski, Rudzinski, & Plazinska, 2009). The lower pKa value of oxalic acid compared to the other LMWOAs used (Table 5.5), causes the formation of more free organic ligands (oxalate ions) (Jiang et al., 2017). Therefore, the lower nitrate retention observed in oxalic acid-containing systems may be caused by nitrate-oxalate competition for active sites on the biochar surface. To the author's knowledge, at the time of writing, no papers have examined biochar-nitrate-oxalate relationships. However, in a similar vein, Su and Puls (2004) found that the retention of nitrate ions to zerovalent iron due to competition was lessened by competition from oxalate and citrate ions. This further supports the idea of nitrate-oxalate competition for active sites on the biochar, as found in the current study. More work to elucidate the exact mechanism responsible for the weaker capacity of oxalic acid, relative to the other two LMWOAs, to enhance nitrate removal from the aqueous solutions needs to be further explored. Furthermore, the inferences from the kinetic modelling must be applied within the limitations outlined in section 3.6.5 and further work such as post-sorption FTIR or XRD analysis of the biochar materials would support these findings.

Turning now to the experimental evidence on biochar-phosphate dynamics in solution, rice husk biochar showed no ability to immobilise phosphate. While, these results disagree with some published studies (Chen, Chen, & Lv, 2011; Takaya, Fletcher, Singh, Anyikude, & Ross, 2016), they are consistent with a great deal of the previous work. For example, Yao et al. (2011) found no ability to remove phosphate from solution when using raw sugar beet tailing biochar produced at 600 ^{o}C. Similarly, Hale et al. (2013) found no phosphate adsorption to either cacao shell or corn cob biochar. Others found either no or limited removal of phosphates using a diverse range of biochar feedstock and production temperatures (Hollister et al., 2013; Yao et al., 2012). What is clear is the inconsistency in biochar efficacy for phosphate immobilisation within the field.

The phosphate ions were not electrostatically attracted to the active sites on the surface of the biochar materials under the experimental conditions. During the experiment, the pH of the control systems with biochar increased from 7.11 to 9.24 (Table 4.26). The majority of the experiment was spent at pH > the point of zero charge (Table 4.2). Therefore, the net charge of the biochar materials was negative. This finding is in agreement with Wang, Shen, Shen and Li (2016) who found with decreasing pH, phosphate adsorption increased. This result suggests that the phosphate ions displayed a low affinity for the protonated biochar surface.

Phosphate-biochar solution dynamics in the presence of LMWOAs were different from nitrate-biochar dynamics in the reflective systems. The addition of LMWOAs did not result in adsorption of phosphate but increased the concentration of phosphate in the solution over time, ranging from 26 % to 36 %, for the different LMWOAs (Figure 4.6). At 120 h, the order of extractable phosphate decreased following oxalic acid > citric acid > malic acid-containing systems (Table 4.27). This corroborates with the pKa values of LMWOAs which follow oxalic > citric acid > malic acid and as a result, the extracting power is greater for oxalic acid (Table 5.5). RH700 biochar was found to leach 1.14 mmol L^{-1} of soluble phosphate into solution (Table 4.27). It is widely reported that the release of biochar-borne phosphate can occur (Cantrell et al., 2012; Yao et al., 2011; Yao et al., 2012). The increase in solution-borne phosphate was probably derived from the dissolution of insoluble biochar-borne phosphate minerals such as calcium phosphate (Uchimiya &

Hiradate, 2014). The results confirm the biochar had a low affinity for the protonated biochar surfaces compared to nitrate.

5.5 Discussion for hypothesis 3

5.5.1 Metal systems

When applied to metal systems, hypothesis 3 stated that biochar would reduce the LMWOA-driven solubilisation of cadmium and zinc in soils. In the water system without biochar, there was evidence of soil attenuation of cadmium. A small amount of zinc was mobilised in the control and tended to fluctuate declining overtime likely caused by cation exchange between soil-borne cations and zinc (Alozie et al., 2018). Biochar-driven immobilisation of soil-borne cadmium and variable immobilisation of zinc was found in the controls. These results agree with the findings of other studies, in which cadmium and zinc were immobilised by biochar (Beesley & Marmiroli, 2011; Melo et al., 2016; Mohamed et al., 2015). Initially, biochar showed some efficacy for zinc immobilisation (Figure 4.9) which aligns with past work (Heaney et al., 2018). However, at 120 h biochar-driven mobilisation of soil-borne zinc occurred. During this time, the pH of the overlying water layer increased from 6.30 to 6.74 (Table 4.30). After a pH of 6.5, zinc solubility typically decreases (Rengel, 2015). This was not found in the current study. Zinc is easily exchangeable within the soil matrix (Harter, 1983). Park et al. (2015) found zinc could be easily exchanged with other cations while bound to the biochar surface. It is likely that between 72 h and 120 h the weakly retained zinc on the biochar surface was under attack by either biochar-borne or soil-borne cations such Pb^{2+} which would liberate zinc into solution. An explanation for the possible biochar-driven immobilisation again may be down to electrostatic attractions. The pH at 120 h was 6.89 and 6.74, which is unlikely to favour cationic adsorption (Table 4.30). Immobilisation may have been via precipitation or inner-sphere complexation (Kołodyńska et al., 2012).

LMWOA-mobilisation of cadmium and zinc was observed. In cadmium soils, availability followed a decreasing order of citric acid > malic acid > oxalic acid. In zinc soils availability followed a similar decreasing order of oxalic acid > malic acid > citric acid. Typically, larger amounts of LMWOA-mobilised cations would correlate with lower pKa (Table 5.5). In the current study, this pattern was not observed; oxalic acid-driven mobilisation of cadmium and zinc was limited compared to the other acids. It may be this can be explained by the formation of cadmium and zinc-citrate, -malate and -oxalate complexes with varied solubility as previously suggested. This would corroborate with past work, which found citrate to increase cadmium solubility more than malate and oxalate (Schwab et al., 2008).

Biochar inhibited the LMWOA-driven mobilisation of soil-borne cadmium and zinc. Cadmium solubility and transport in soils are thought to be mostly pH-dependent. At low pH, cadmium usual exists in the more mobile forms: Cd^{2+}, $CdSO_4$ or $CdCl^+$, compared to $CdHCO_3^+$, $CdCO_3$ or $CdSO_4$ that exist at more alkaline pH (Shahid, Dumat, Khalid, Niazi, & Antunes, 2017). Therefore, at higher pH, there is reduced soluble cadmium for biochar immobilisation. The low, yet positive soluble cadmium concentration and the protonated biochar surface under the experimental pH (Table 4.2) suggest electrostatic repulsion were not primary factors in governing biochar sorption. Biochar-borne alkaline components and the high ash content of rice husk biochar are likely to contribute to the inhibition of LMWOA-driven mobilisation of soil-borne cadmium via acid neutralisation effects. For instance, Table 4.1 showed that RH700 had a high alkaline pH of 11.17, which would effectively neutralise LMWOAs (Alozie et al., 2018). In response, less cadmium would be mobilised by acid-driven dissolution. Besides, the results in Table 4.7, Table 4.8

and Table 4.9 also suggest LMWOA-mobilised biochar-borne cations from rice husk materials. It is possible cations such as Ca^{2+}, Mg^{2+} and Cu^{2+} could compete with cadmium for sorption to organic ligands, reducing the complexation-driven mobilisation of soil-borne cadmium. Earlier work supporting this theory has described competition between Ca^{2+} and Cd^{2+} for the binding sites of fulvic acids (Cao, Xue, & Sigg, 2006).

Concerning zinc, biochar limited the oxalic acid-driven immobilisation of zinc more so than in the presence of citric and malic acids. The similarities between the unamended and biochar-amended citric, and malic acid systems suggest that biochar-facilitated acid neutralisation assisted zinc reduction more so than biochar retention. However, the discrepancy between the two systems in the presence of oxalic acid indicates that biochar had a more significant effect at limiting LMWOA-driven mobilisation of soil-borne zinc. Again, the stronger pKa value of oxalic acid compared to other organic acids (Table 5.5) relates to a higher number of anions in solution (Jiang et al., 2017). As the surface of the biochar materials is still likely protonated owing to the pH < the point of zero charge in the overlying solutions (Table 4.30), it is possible the higher number of oxalate ions allow for retention onto the biochar surface. This would then facilitate the adsorption of Zn^{2+} via a metal bridging mechanism (Guppy et al., 2005).

To conclude, this study found that in soil, rice husk biochar inhibited the LMWOA-driven mobilisation of soil-borne cadmium and zinc primarily via acid neutralisation. The initial inhibition of biochar-driven immobilisation observed for malic acid was likely caused by a weaker pKa value compared to the other acids. The reader is reminded to interpret these in line with the method limitations outlined in section 3.6.5 and 3.7.2. For instance, anaerobic conditions may affect the release of soil-borne trace elements into solution affecting biochar-soil-trace element dynamics in the presence of LMWOAs. Future should consider the use of column studies with aerated feed solutions to avoid anaerobic conditions (CETaqua, 2012).

5.5.2 Nutrient systems

When applied to nutrients, hypotheses 3 stated that LMWOAs will enhance the biochar-borne driven immobilisation of nitrate and phosphate in soils. To test this, a batch incubation experiment was conducted in spiked soils. Firstly, for unamended nitrate spiked soils, relatively little of the nitrate was retained by the soil colloids. This is probably due to the weak affinity of nitrate to be retained by the soil and the solubility of nitrate (Barber, 1995; Dickinson & Murphy, 2008; Heaney et al., 2018). However, in the LMWOA-containing systems, an overall decrease of soluble nitrate of 33.12 % in citric, 20.93 % in malic and 11.23 % in oxalic acid-containing systems was found. Possibly, the LMWOAs protonated the soil colloids to some extent allowing for the retention of the nitrate ions (Karickhoff & Bailey, 1976). The dynamics between LMWOAs and nitrates in biochar-amended systems are not as clearly defined. Biochar did not enhance the immobilisation of the added nitrate in the presence of LMWOAs but, to the opposite, reduced the immobilisation rate of the added nitrate. This can be explained by the liming effects of biochar, which increased soil pH (Table 4.31) and consequently weakened the protonation of the soil colloids.

Phosphate is relatively insoluble and is highly adsorbed by aluminium and iron oxides and organic matter within soils (Jones, 1998; Pratiwi, Hillary, Fukuda, & Shinogi, 2016; Ström, Owen, Godbold, & Jones, 2002). In the soil without added LMWOAs, the addition of biochar enhanced the immobilisation of the added phosphate. Possibly, this was a result of the formation of practically insoluble calcium phosphate by using the added biochar as a soluble source of calcium (Uchimiya & Hiradate, 2014). In the presence of LMWOAs this enhanced effect was reduced (malic and oxalic acid treatments) or not observed (citric acid treatment) although the protonation of the biochar surface is expected to be favourable for anion adsorption. This observation supports the conclusion drawn from the hypothesis 2 experiment (Table 4.27 and section 5.4), that phosphate had a low affinity for the protonated biochar surfaces. The reduced amount of immobilised phosphate in the presence of malic and oxalic acid treatments can be attributed to the competition of organic ligands with phosphate for the available calcium in the soil systems (Hinsinger, 2001). Whereas, the results for citric acid are possibly related to citrate being the most effective LMWOA in the study for altering the availability of phosphorus in soils (Duputel et al., 2013; Hu, He, Li, & Liu, 2001; Jones, 1998; Oburger, Jones, & Wenzel, 2011).

5.6 Discussion for hypotheses 4a to 4d

Biochar phytotoxicity research typically addresses the effect on one or two plant species (Gascó et al., 2016). The first hypothesis was that biochar and LMWOA-activated biochar would increase the germination percentage of lettuce (*Lactuca sativa*), white mustard (*Sinapis alba*), pea (*Pisum sativum*), radish (*Raphanus sativus*) and tomato (*Solanum lycopersicum*) seeds. All species except tomato germinated. Importantly, neither biochar nor LMWOA-activated biochar affected the germination percentage of different plant species. The result of this study is in accord with others (Gascó et al., 2016; Van Zwieten et al., 2010). The findings imply that the release of biochar-borne soluble elements into the soils does not pose a toxic effect under the experimental conditions. It also implies that either biochar was unable to improve germination conditions in the soils studied during the short period of growth for these select plants. Solaiman, Murphy and Abbott (2012) found feedstock and dose-dependent variations in germination efficacy for agricultural plant species. It may be possible that at the low application rate studied (1 %) biochar was unable to enhance or impede germination.

However, the tomato seeds did not germinate in either of the biochar-treated soils. Tomato germination is pH-sensitive and is favoured in acidic soils (Gascó et al., 2016). In the current study, the mean pH of the soils used was 7.92 (Table 3.5). Likely, the tomato germination was unsuccessful as the soil was not acidic. Other reasons may be that the ambient temperature of the greenhouse during the experiment (15 ^{o}C) may have been too low for germination within fourteen days. Ideally, controlled temperature conditions or a growth period in a warmer season would remove the uncertainty of unsuccessful germination. Lastly, research has shown a decreased germination rate of tomato species when exposed to rare earth elements (Thomas, Carpenter, Boutin, & Allison, 2014). The complete profile of the contaminated wastes at Moston Brook that made up 50 % of the contaminated soil is relatively unknown (section 3.4.4). Despite knowledge of the commonly studied heavy metals existing at the site, the exact nature of the landfill is unclear (Mukwaturi & Lin, 2015). These factors were likely contributory to the unsuccessful tomato germination.

Hypothesis 4b was that biochar and LMWOA-activated biochar would increase the total biomass of different edible plant species. Studies usually report increased biomass following biochar application (Nie et al., 2018; Rue et al., 2019). This is thought to be because of increased amounts of nutrient and micronutrients within the rhizosphere and residency time within the rhizosphere (Kameyama et al., 2012; Nie et al., 2018; Rue et al., 2019). The findings of this study found variable effects on the total biomass of the studied species. Increased mustard and pea biomass were observed following the application of normal biochar, whereas lettuce and radish biomass tended to have less biomass following biochar application. For LMWOA-activated biochar, either a decreased biomass was observed (lettuce and radish) or no significant effect was found (mustard and pea). The reasons for decreased biomass may be specific to root and shoot responses to biochar application, so are discussed separately.

Hypothesis 4c theorised that biochar and LMWOA-activated biochar would increase the shoot height of different edible plant species. The results found biochar and LMWOA-activated biochar in soils did not affect the shoot height of lettuce, mustard and radish. However, the effect of biochar on pea shoots was variable. The growth data showed no effect on shoot height (Table 4.33). An inhibitory effect of biochar on shoot height has been previously reported (Gascó et al., 2016; Intani, Latif, Islam, & Müller, 2018; Prapagdee et al., 2014) likely caused by the different soil properties and nutrient differences leading to different biochar efficacy on early plant growth response.

In a similar vein, hypothesis 4d theorised that biochar and LMWOA-activated biochar would increase the root length of different edible plant species. Increased root lengths have often been reported following biochar application (Brennan et al., 2014; Prapagdee et al., 2014). These are usually attributed to improved soil conditions following biochar application (Lehmann & Joseph, 2009). In the current study, an adverse effect was found. Pea roots in contact with LMWOA-activated biochar reduced by 37 %. No other systems were affected by biochar application. Earlier work has reported a decrease in root length (Jones & Quilliam, 2014; Zheng et al., 2012; Zheng et al., 2011). Jones and Quilliam (2014) suggested two reasons for decreased root growth. Firstly, inhibition due to biochar-borne toxicants. This may apply to the current findings, based on the LMWOA-solubilised elements found in of this work (Table 4.7, Table 4.8 and Table 4.9).

The authors also proposed that the improved nutrient status following biochar application reduces the plants' allocation to root production. Likewise, the current study found rice husk biochar to contain soluble nutrients (Figure 4.5). Previous work has also suggested decreased root lengths to be associated with increased phosphorus solubility accredited to the dissolution of biochar-borne phosphorus (Zheng et al., 2012; Zheng et al., 2011). Biochar activation with LMWOAs may have increased the amount of phosphorus organo-mineral phases within the biochar matrix which may become labile via reductive dissolution with biochar-borne iron (III) hydro(oxides) or via LMWOA-driven dissolution (Joseph et al., 2015; Kammann et al., 2015).

In conclusion, there were mixed results obtained in terms of the effects of either biochar or LMWOA-activated biochar on the different plant growth parameters. It is important to note that plant growth in complex soil systems are affected by various factors such as soil type and plant traits (Cortois, Schröder-Georgi, Weigelt, van der Putten, & De Deyn, 2016; Marschner, Crowley, & Yang, 2004). Therefore, the generalisability of the plant growth results is subject to limitation. Notably, a short growth period of fourteen days to one month may not be enough to extrapolate the plant growth response data for these species following biochar application which should be considered when applying this data. The efficacy of biochar to improve crop production is likely to be case-sensitive, i.e. plant, biochar and soil condition specific. As such, the efficacy of biochar to improve vegetation growth should not be overestimated in practice.

5.7 Discussion for hypotheses 5a and 5b

5.7.1 Growth parameters in pea plants after one month

Prior studies that have noted the changes in plant growth parameters when grown in biochar-amended soils as previously outlined in section 5.6 and earlier in Chapter 2. Peas grown in activated biochar-amended soils had larger biomass overall, and a taller shoot height whereas normal biochar had an insignificant effect (Table 4.35). Previous studies have reported increased biomass and shoot heights with biochar application (Nie et al., 2018; Rue et al., 2019) but not relating to LMWOA-activated biochar. It is plausible these findings further support the idea of improved nutrient availability and rhizospheric residency via the LMWOA-driven dissolution of biochar-borne trace elements nutrients during the activation stage and during the experiment (Jeffery et al., 2011; Kameyama et al., 2012; Lehmann & Joseph, 2009; Liu et al., 2017; Vause et al., 2018). Root systems were not affected by the presence of either biochar (Table 4.35). The limitations of this experiment, as previously stated in section 3.8.4, noted a short growing time was a fundamental issue with this experiment which may explain the lack of positive growth parameters for the normal biochar treated soils. However, further studies would be needed to clarify the complex soil-plant-biochar interactions. It must also be noted, different UKBRC biochar types may warrant further research to compare the differences in pea growth response.

5.7.2 Photosynthetic pigment production in pea plants

A further physiological process which may be affected by biochar application is the production of photosynthetic pigments (e.g. chlorophyll a and chlorophyll b) in the pea leaves. Several reports have suggested that biochar may inhibit chlorophyll production on various plant species (Akhtar et al., 2015; Asai et al., 2009). Opposing this field of thought, some have found that biochar improves chlorophyll production (Akhtar et al., 2014; Batool et al., 2015; Yousseff, et al., 2018). The current study has been unable to prove that biochar or LMWOA-activated biochar affects either chlorophyll a or chlorophyll b content in pea leaves (Table 4.36). Although these results differ from some published studies, they are consistent with those of Ventura et al. (2012). Reasons for the neutral effect of rice husk biochar on photosynthetic pigment production may be attributed to several factors. While temperature and light intensity are chlorophyll productions main dependent factors (Gottschalk and Kaul, 1980), the arrangement of treatment trays in the greenhouse ensured equal opportunities to both temperature and light throughout the growth period. Variations in chlorophyll content related to biochar dose rate have been reported (Waqas et al., 2018; Ngulube et al., 2018). Finally, the wide-ranging characteristics of biochar, as reported in section 4.2 of this thesis could affect growth rates of different plant species and the rate of chlorophyll production in plant foliage. Further research inclusive of a greater range of biochar feedstock and application rates and a longer growth period may confirm the associations between biochar and chlorophyll contents in pea leaves found in previous work (Akhtar et al., 2014; Batool et al., 2015; Yousseff, et al., 2018).

5.7.3 Trace element uptake by pea in biochar and LMWOA-activated biochar-amended soils

The final hypotheses were that (a) biochar would reduce trace element uptake by a selected plant species and (b) LMWOA-activated biochar would not affect trace element uptake by a selected plant species. The current study quantified the trace element uptake in pea tissues by determining the root and shoot concentrations. Uptake of trace elements by pea plants has been previously reported (Lozano-Rodriguez, Hernandez, Bonay and Carpena-Ruiz, 1997; Hattab et al., 2009; Shtangeeva et al., 2018). Concerning root trace element concentrations, the application of biochar reduced aluminium, iron, manganese, copper, and nickel uptake by a pea plants grown over one month (Figure 4.16 and Figure 4.17). When grown in LMWOA-activated biochar treated soils, aluminium, iron, manganese, cobalt, copper, lead and nickel uptake by pea plants were reduced (Figure 4.16 and Figure 4.17). Lower concentrations were found for cobalt, copper, lead and nickel suggesting these were affected by the process of biochar activation with LMWOAs. The application of either biochar significantly reduced the root accumulation of copper, with activated biochar having a greater effect ($p < 0.05$). Reduced bioavailability of these elements following biochar application usually leads to decreased root concentrations (Yousaf et al., 2016; Rehman et al., 2016). However, this does not fit with the calcium chloride extractions of the post-experimental soils (Table 4.34).

Dissolved organic carbon (DOC) can increase the solubility of elements within soils (Rékási & Filep, 2015). Earlier work has found a relationship between biochar application and enhanced lead concentrations attributed to increased DOC (Beesley & Dickinson, 2011; Zheng et al., 2012). Sun et al. (2016) measured the changes in DOC following the LMWOA activation of biochar materials and found increased DOC release. It is possible that the LMWOA-solubilisation of biochar-borne DOC enhanced the release of lead from the soil, making it more available and more accumulated in root tissue. In the same vein, copper was more affected by the application of LMWOA-activated than normal biochar. The results are, therefore, are likely explained by a strong affinity with organic ligands and the solubility of the formed organic-complexes (Ndung'u, Franks, Bruland, & Flegal, 2003; Rékási & Filep, 2015).

An important observation is the soil pH. Soil pH is a driving factor of trace element solubility in soils (Nworie et al., 2019). Notably, the application of biochar did not affect arsenic, chromium, cadmium, cobalt, lead and zinc in pea roots grown for one month. Also, LMWOA-activated biochar did not affect arsenic, chromium, cadmium and zinc uptake by pea roots. This is likely because of the lower bioavailable and plant exchangeable concentrations found in the soils pre- and post-experiment (Table 3.5 and Table 4.34). As found in section 4.3.4 of this thesis, the behaviour between LMWOAs and zinc is variable, although extractable zinc decreased.

As well as root concentrations of trace elements, the changes in shoot tissue concentrations following biochar soil amendment were analysed. Shoot concentrations of aluminium, iron, manganese, arsenic, cadmium, copper, nickel, zinc, chromium and cobalt were not affected by biochar application. However, shoot-borne aluminium, lead and nickel reduced in the presence of biochar (for lead) and LMWOA-activated biochar application (aluminium, lead and nickel). As well as a similar pattern in reduced root concentrations, aluminium and lead have well-documented associations with silicon (Adrees et al., 2015; Pontigo et al., 2017; Zheng et al., 2012). The biochar materials used in this study are high in ash content, which is rich in silicates (Shen, Zhang, McMillan, et al. 2017). It could be that the formation of aluminium and lead complexes played a role in decreasing shoot concentrations. The formation of insoluble silicon-metal complexes in the root tissues can decrease translocation and increase the deposition of silicon-complexes into cell walls and vacuoles (Adrees et al., 2015; Zheng et al., 2012). This would result in low translocation to aerial biomass. However, the ash contents of biochar reportedly decrease with LMWOA treatment (Liu et al., 2017; H. Zheng et al., 2019). Although not measured in this study, if the ash content of the LMWOA-activated biochar was less then this would not explain the lower lead accumulated in peas grown in the LMWOA-activated treated soils. Based on the findings in section 4.3.3 (Table 4.16), it is also possible that the LMWOAs complexed with lead forming insoluble lead-LMWOA complexes reducing lead availability in soils (Gadd, 1999).

Although the study has successfully demonstrated that biochar and LMWOA-activated biochar can influence plant uptake of certain trace elements, it has particular limitations in terms of the generalisability of these results. Other factors may be responsible for the plant uptake results found, such as the availability of trace elements (Antoniadis et al., 2017; Khan et al., 2015). As the results were varied for both biochars used, biochar usage to reduce plant uptake of trace elements must be considered on a case-by-case basis to avoid the overestimation of biochar efficacy in remediation practices. Furthermore, a longer growth period or the use of different biochar materials may yield different results to those found in this thesis.

5.7.4 Bioaccumulation and translocation factors

To help quantify the pea shoot and root concentrations, metal contents of the pea plants (root and shoot) and soil values from this study (Table 3.5) were used to calculate the bioaccumulation factor of each element for pea seedlings. This refers to the ability of a plant to accumulate trace elements in its tissue compared to the amount present in the soil (Nworie et al., 2019). Equation 5.4 shows the calculation for peas' bioaccumulation factor where $C_{PLANT\ ROOT}$ is the concentration of the target element in the plant root (mg kg^{-1}) and C_{SOIL} is the total amount of target element in the soil (mg kg^{-1}). In addition to bioaccumulation, translocation can quantify plant trace element uptake. Translocation factor refers to the plants' ability to move metals from the root biomass into the aerial parts of the plant tissues (Nworie et al., 2019). Equation 5.5 shows the calculation for pea translocation factor where $C_{PLANT\ SHOOT}$ is the concentration of the target element in the plant shoot (mg kg^{-1}) and $C_{PLANT\ ROOT}$ is the concentration of the target element in the plant root (mg kg^{-1}).

$$BF = \frac{c_{PLANT\ ROOT}}{c_{SOIL}}$$

Equation 5.4 Determination of the root bioaccumulation factor

$$TF = \frac{c_{PLANT\ SHOOT}}{c_{PLANT\ ROOT}}$$

Equation 5.5 Determination of the plant translocation factor

The bioaccumulation factors are presented in Table 5.6. Generally, there was variation between the elements and different treatments but values tended to decrease in bioaccumulation factor through the treatments (Table 5.6). For aluminium, arsenic, cobalt, copper, iron, manganese and nickel the bioaccumulation factor in plant tissues was less than 1. This suggests that these elements have low phytoavailability to pea plants under the experimental conditions due to a lack of bioaccumulation in the root tissues compared to the total concentration in soils (Nworie et al., 2019). These results are in accord with Eid et al. (2019) who found the bioaccumulation of all the metals in the present study, in pea plants grown in sewage sludge, to be less than 1. However, this research found the bioaccumulation factors greater than 1 for cadmium (7.08 to 10.22), chromium, (8.13 to 11.47), lead (1.03) and zinc (4.71 to 6.30) respectively. These values were extremely high indicative of a strong phytoextraction capacity of pea plants when comparing root tissues concentration to soil values. This suggests pea may be tolerant of the concentrations found within the soils (Table 3.5).

The translocation factor values for all studied elements were < 1 showing low levels of translocation between the plant roots to the aerial biomass. However, there was variation between the elements and different treatments with values ranging from 0.01 to 0.64. Notably, there was an elevated translocation of manganese in peas grown in the unamended soils (0.46), biochar-amended soil (0.62) and activated biochar-amended soils (0.64). The translocation of zinc also seemed elevated for all three treatments showing elevated translocation factors (0.34, 0.44 and 0.46), compared to the other elements. These values are somewhat similar to those of Eid et al. (2019) who reported a manganese and zinc translocation factor of 0.42 and 0.29 respectively, for peas grown in soil with sewage sludge amendment. These results suggest that pea plants had a slightly enhanced capacity to translocate manganese and zinc (to a lesser extent) compared to other elements under the experimental conditions. Similar variable translocation factors for different trace elements between pea roots and shoots have been previously reported (Sharma, Sharma, &

Mehrotra, 2010). However, further work is required to generalise the bioaccumulation and translocation of trace elements in pea plants following biochar and LMWOA-activated biochar amendment. As suggested previously, a longer growth time may affect the observed bioaccumulation and translocation factors, so should be considered in future work.

Table 5.6 Mean bioaccumulation and translocation factors for pea plants

Element	**Bioaccumulation factor**[1]		
	0% biochar	**1% biochar**	**1% activated biochar**
Aluminium	0.13	0.09	0.07
Arsenic	0.09	0.07	0.07
Cadmium	**10.22**	**7.08**	**8.51**
Cobalt	0.18	0.14	0.11
Copper	0.04	0.03	0.02
Chromium	**11.47**	**9.36**	**8.13**
Iron	0.24	0.14	0.10
Manganese	0.25	0.17	0.15
Nickel	0.46	0.29	0.23
Lead	**1.03**	0.84	0.56
Zinc	**6.30**	**4.90**	**4.71**
	Translocation factor		
Aluminium	0.03	0.04	0.04
Arsenic	0.09	0.28	0.19
Cadmium	0.01	0.02	0.02
Cobalt	0.08	0.10	0.12
Copper	0.19	0.28	0.33
Chromium	0.07	0.09	0.09
Iron	0.07	0.12	0.15
Manganese	0.46	0.62	0.64
Nickel	0.15	0.21	0.24
Lead	0.04	0.04	0.04
Zinc	0.34	0.44	0.46

[1] Values in bold indicate a BF > 1.

CHAPTER 6

Research summary

6.1 Overview

The research reported in this thesis was aimed to examine the effect of biochar on trace element and nutrient solubility in the presence of LMWOAs. In the preceding chapters, a framework for the study was provided (Chapter 2) and the methods for each experiment have been specified (Chapter 3). Then, a detailed characterisation of UKBRC biochar materials was completed (Chapter 4, section 4.2). Then, trace element (Chapter 4, section 4.3 and section 4.5) and nutrient (Chapter 4, section 4.4) dynamics in aqueous and soil systems in the presence of LMWOAs were explored. Finally, the efficacy of biochar and LMWOA-activated biochar on plant growth and metal uptake was examined (Chapter 4, section 4.6 and section 4.7). In the previous chapter (Chapter 5), a critical discussion of the key findings was completed. Here, a research summary is provided to explore (a) whether the research aim was achieved, (b) if the research contribution was novel and, (c) give recommendations for future research.

6.2 Research conclusions

The first hypothesis of this research was that LMWOAs will inhibit the biochar-driven immobilisation of cadmium, lead and zinc in aqueous systems which was tested using a batch sorption method. The main findings were that rice husk biochar could successfully immobilise cadmium, lead and zinc in water (Table 4.13, Table 4.16 and Table 4.19). The biochar favourably retained lead removing it entirely from solution. LMWOAs impeded biochar-driven removal of cadmium and lead from solutions whereas biochar the trend for zinc was unclear. The findings in the control were caused by electrostatic attraction or a surface precipitation mechanism. The inhibition of removal happened because of either competition from metal-organic acid complexes or LMWOA-protonation of the biochar surface. These findings were in accord with our previous findings (Alozie et al., 2018). As such, hypothesis 1 can be partially accepted; **LMWOAs inhibited the biochar-driven immobilisation of cadmium and lead in aqueous systems** under the experimental conditions. For zinc, the trend was unclear therefore hypothesis 1 must be partially rejected; **LMWOAs did not affect the biochar-driven immobilisation of zinc in aqueous systems** under the experimental conditions. Further work is required to confirm biochar-zinc-LMWOA dynamics.

The second hypothesis of this research stated that LMWOAs will enhance the biochar-driven immobilisation of nitrate and phosphate in aqueous systems. The first significant finding was that LMWOAs protonated the biochar surface and facilitated the adsorption of nitrate (Table 4.24). Without LMWOAs, biochar was unable to remove soluble nitrate. Nitrate adsorption by rice husk biochar in the presence of LMWOAs tended to follow pseudo-second-order kinetics (Table 4.25). However, nitrate adsorption appeared to be kinetically slower in the presence of oxalic acid, suggestive of competition between nitrate and oxalate ions for the positively charged active sites on the biochar surface. This would also align with the findings from hypotheses 1. Therefore, hypothesis 2 can be in part, accepted; **LMWOAs enhanced the biochar-driven immobilisation of nitrate** under the experimental conditions. A second significant finding was the inability of biochar to remove phosphate from the solution. On the contrary, LMWOAs increased the amount of soluble phosphate caused by the dissolution of insoluble biochar-borne minerals, increasing concentrations in solution (Table 4.27 and Figure 4.6). The lack of positive retention by the protonated biochar suggested phosphate had a weak affinity to the biochar active sites. Therefore, the hypothesis regarding phosphate in solution can be rejected; LMWOAs **increased the biochar-driven immobilisation of phosphate in aqueous systems** under the experimental conditions.

Hypothesis 3 stated that biochar would reduce the LMWOA-driven solubilisation of trace elements and nutrients in soils. The rice husk biochar inhibited the LMWOA-driven solubilisation of both cadmium and zinc (Figure 4.8 and Figure 4.10). This was primarily attributed to the LMWOAs, acid neutralisation of the LMWOAs, or the protonation of the biochar surfaces being unfavourable for cation retention under the experimental pH conditions (Table 4.29 and Table 4.30). Therefore, in part, hypothesis 3 can be accepted; **biochar reduced the LMWOA-driven solubilisation of cadmium and zinc in soils** under the experimental conditions.

For nitrate in soils, nitrate was immobilised over time but to a lesser extent than observed in the no-added biochar systems (Figure 4.11 and Figure 4.12). The LMWOAs protonated the soil colloids and enhanced the retention of soluble nitrate. Furthermore, the addition of biochar reduced the immobilisation rate observed for the LMWOA systems. The liming effect of biochar raised the pH, therefore, weakening the protonation of the soil colloids and the attenuation of nitrate (Table 4.31). Therefore, hypothesis 3 for nitrate must be rejected; **biochar did not affect the LMWOA-driven solubilisation of nitrate in soils** under the experimental conditions.

Regarding phosphate in soil systems, biochar enhanced phosphate immobilisation due to the formation of insoluble phosphate complexes (Figure 4.14) but reduced the amount of immobilised phosphate due to competition between organic ligands and phosphate for biochar-borne calcium in soil systems (Table 4.7 and Figure 4.14). Therefore, the hypothesis can be accepted, **biochar reduces the LMWOA-driven solubilisation of phosphate** under the experimental conditions via the precipitation of the practically insoluble phosphate minerals.

Hypothesis 4a stated that biochar and LMWOA-activated biochar will increase the germination percentage of different edible plant species. As per the findings in Table 4.33, biochar and LMWOA-activated biochar unanimously showed no effect on germination percentage. Therefore, hypothesis 4a can be rejected; **biochar and LMWOA-activated biochar did not affect the germination percentage of lettuce, mustard, radish or pea** under the experimental conditions.

Hypothesis 4b stated that biochar and LMWOA-activated biochar will increase the fresh biomass of different edible plant species. The different plant species showed varying responses to either biochar type. For instance, the lettuce had lower biomass in the presence of either biochar, but the normal biochar had a greater diminishing effect on total biomass. Based on these results, hypothesis 4b can be accepted; **biochar and LMWOA-activated biochar increased the fresh biomass of lettuce** under the experimental conditions. Mustard plants had significantly larger biomass when grown in normal biochar amended soil, Therefore hypothesis 4b can be partially accepted; **biochar increased the fresh biomass of mustard** under the experimental conditions. When mustard was grown in the presence of LMWOA-activated biochar, no significant difference in fresh biomass was observed compared to the control. So, hypothesis 4b must also be partially rejected; **LMWOA-activated biochar did not affect the fresh biomass of mustard** under the experimental conditions. For radish, a negative effect on fresh biomass was observed for plants grown in both biochar treated soils. As such, hypothesis 4b must be rejected; **biochar**

and LMWOA-activated biochar decreased the fresh biomass of radish under the experimental conditions. Lastly, for pea plants grown in either biochar treated soils, only normal biochar affected fresh biomass. Significantly larger pea biomass was recorded for plants grown in normal biochar treated soils. So, hypothesis 4b must be partially accepted; **biochar increased the fresh biomass of pea** under the experimental conditions. Also, hypothesis 4b must be partially rejected; **LMWOA-activated biochar did not affect the fresh biomass of pea** under the experimental conditions.

Hypothesis 4c stated that biochar and LMWOA-activated biochar will increase the shoot height of different edible plant species. Neither biochar material affected the shoot heights of any of the study plant species. Therefore, hypothesis 4c can be rejected; **biochar and LMWOA-activated biochar did not affect the shoot heights of lettuce, mustard, radish or pea** under the experimental conditions.

Hypothesis 4d stated that biochar and LMWOA-activated biochar will increase the root length of different edible plant species. For lettuce, mustard and radish root lengths, no significant difference was observed in any of the systems. So, hypothesis 4d must be rejected; **biochar and LMWOA-activated biochar did not affect the root lengths of lettuce, mustard, or radish** under the experimental conditions. For pea plants, the root lengths were much more developed than the other plants (Table 4.33). However, roots grown in normal biochar were unaffected compared to the control plants. Therefore, hypothesis 4d must be rejected; **biochar did not affect the root lengths of pea** under the experimental conditions. In addition, the pea roots grown in LMWOA-activated biochar treated soils had significantly shorter root lengths compared to the control. So, **LMWOA-activated biochar decreased the shoot lengths of pea** under the experimental conditions. In conclusion, the results concerning hypothesis 4 varied with plant species and biochar type, as such generalisation is difficult. The results were attributed to the complexity of the different soil systems and plant-specific responses to trace elements.

For hypotheses 5a, it was stated biochar would reduce trace element uptake by a selected plant species. The root concentrations of aluminium, iron and nickel reduced in the presence of biochar. So, hypothesis 5a for could be partially accepted; **biochar reduced aluminium, iron, manganese, copper, and nickel uptake by pea plants** under the experimental conditions. For, arsenic, chromium, cadmium, cobalt, lead and zinc, normal biochar did not affect the root accumulation. Therefore, hypothesis 5a must be partially rejected; **biochar did not affect arsenic, chromium, cadmium, cobalt, lead and zinc uptake by pea plants** under the experimental conditions. Interestingly the results found that biochar also decreased lead concentrations in pea shoots (Figure 4.17). This was attributed to possible to the formation of insoluble silicon-metal complexes limiting translocation to aerial foliage (Table 5.6) or low soil availability (Table 4.34).

Hypothesis 5b stated that LMWOA-activated biochar will have no effect on heavy trace element uptake by a selected plant species. However the findings in Figure 4.16 and Figure 4.17 Show the pea root concentrations of aluminium, iron, manganese, cobalt, copper, lead, and nickel were significantly reduced in LMWOA-activated biochar amended soils. Therefore, hypothesis 5b must be partially rejected; **LMWOA-activated biochar reduced aluminium, iron, manganese, cobalt, copper, lead and nickel uptake by pea plants** under the experimental conditions. Also, **LMWOA-activated biochar did not affect arsenic, chromium, cadmium and zinc uptake by pea plants** under the experimental conditions. As such, hypothesis 5b was accepted for these elements. LMWOA-activated biochar also had a limited effect on pea shoot concentrations of trace elements (Figure 4.18 and Figure 4.19). Lead reduced in the presence of normal biochar whilst aluminium, lead and nickel shoot concentrations reduced in the LMWOA-activated biochar treated soils. This may have been caused by lead forming insoluble lead-LMWOA complexes in soils or the formation of insoluble silicon-metal complexes and low soil availability as previously outlined.

6.3 Research limitations and recommendations for future work

The findings in this report, summarised in section 6.2, are subject to certain limitations. The major limitations identified have been summarised in Table 6.1. The batch sorption technique applied for hypothesis 1, 2 and 3 is one of the major limitations. The method employed was affected by the removal of 10 mL aliquots at each interval (1 h, 24 h, 72 h and 120 h) with no liquid replenishment and no quantification of removed biochar which may have affected the solid: solution ratio to some extent, overestimating the amount of retained adsorbate. Future work should consider replenishing the liquid to maintain the solid: solution ratio and incorporate the quantification of the removed biochar per interval. The batch study was also carried out in a closed system, therefore anaerobic conditions may have formed. This introduces a further shortcoming to the applied methodology as such conditions could increase the reductive dissolution of iron and manganese oxides and their associated compounds. These may affect biochar-LMWOA dynamics in the batch reactors. It would be preferential to consider column studies owing to their easy operation and continuous flow-through (Limousin et al., 2007) which would aid dissolved oxygen in the systems and kinetic modelling. Furthermore, the inclusion of an aerated feed could further reduce anaerobic conditions if a column study was selected (CETaqua, 2012). Also relating to the batch sorption studies, the use of kinetic models was applied to help elucidate the sorption mechanisms. However, the limitations of the method employed for the batch studies introduce a weakness to the method so the results should be construed with care.

Post-sorption analysis using FTIR, SEM or desorption studies would help elucidate the sorption mechanisms further and should be considered in future work.

A further major limitation of mention is the pH of the systems. Often for experiments used for hypothesis 1, 2 and 3, the pH of the systems was very acidic as no buffer was applied. This may have had a significant effect on the solubility of trace elements which are usually more soluble at acidic pH (Chuan et al., 1994; Krishnamurti et al., 1997; Martinez & Motto, 2000; J. Yang et al., 2006). Therefore the results in these systems should be interpreted with care. The use of a buffer to increase the pH reflective of a pH in more commonly encountered soils (p >=5) would be effective. A third major limitation is the concentration of the LMWOAs used. The three LMWOAs selected (citric, malic and oxalic acid), were chosen for their prevalence in the rhizosphere (Dinh, et al., 2017). The concentrations used are on the upper limit of reported concentrations within the rhizosphere but have been utilised in previous works to represent the soil-plant interface (Qin et al., 2018). Also under stress conditions, the amount of LWMOAs exuded is likely to increase (Jones, 1998). However, the results found in this study may overestimate the LMWOA-activation effect and the interference with biochar dynamics when concentrations of LMWOAs are less than described in this study. Likewise, this study only used three model acids at set concentrations. Differential plant-specific exudations in terms of type and concentration are expected which may affect the generalisation of these results. To avoid overestimation of the results in this study further work should incorporate the use of LMWOAs over a range of concentrations.

The XRF method applied to study the biochar total metal content in this research was also identified as a major limitation to the findings. The high organic matter content of the biochar materials and calibration with a standard of lower organic matter likely affected the readings to some extent. Furthermore, short scanning time and the use of larger particle biochar may have further affected the validity of the biochar total metal contents. This also affected the LMWOA-extraction release rates calculated in Table 4.10. Ideally, a longer scanning time with smaller particle biochar should be used alongside acid digestion of biochar materials followed by analysis by ICP-OES.

The greenhouse studies were limited by several factors. If the work was to be repeated, a range of different plant types should be studied to examine the efficacy of biochar for other vegetable plant species. Also, as previously mentioned, the plant-specific LMWOAs released may have different effects on trace element solubility and subsequent plant uptake. The growth length for the uptake study was 1 month. This was due to lack of time. It is likely that with a longer growth period, a more exacerbated effect may be seen with regards to biochar efficacy to reduce plant trace element uptake. However, further work would be required to confirm this assumption.

This research has thrown up many questions in need of further investigation. Further research needs to examine more closely the relationship between biochar materials and LMWOAs. For instance, this research has used only one model biochar from the UKBRC standard set and three model LMWOAs. Specifically, exploration of the effects of different standard biochar under the conditions of this study, or under a range of different organic acids and the associated factors (i.e. type, pH, environmental medium, concentration, co-existence with other root exudate compounds) would be worthwhile. Future trials should assess the standard biochar materials on plant uptake. Quantification of trace element uptakes by a host of different edible plant species across the UKBRC standard biochar set would much support future research directions. This should include the calculation of vegetable consumption rate as carried out by Weber et al., (2019) to calculate the associated risk of consumption of plants grown on biochar-amended soils.

Table 6.1 A summary of the sources of limitations found in the thesis with considerations to account for future work

Limitation	Considerations for future work
Batch sorption technique ➢ Solid: solution ratio affected ➢ No replenishment of liquid ➢ No quantification of removed biochar	The batch sorption technique would be greatly improved by (i) replenishment of liquid to maintain the solid/solution ratio throughout the experiment (ii) quantification of the removed biochar per interval (iii) or by shortening the length of the study to improve dissolved oxygen in the systems.
Anaerobic systems ➢ Closed batch system for over 120 hours	By selecting columns studies rather than a closed batch sorption system, the inclusion of continuous of aerated feed solution could be delivered through the column preventing anaerobic conditions
pH of systems ➢ Low pH without the use of a buffer	The pH of the systems was extremely acidic. Future work should use LMWOAs in a buffered system to that reflective of more frequently encountered pH (>= 5).
Concentration of LMWOAs ➢ Elevated concentration to reflect the upper limit of those found in stress conditions	Concentrations of LMWOAs more reflective of frequently encountered bulk soils should be considered over a range of concentrations.
Lack of post-sorption analysis	Post-sorption SEM, FTIR or desorption studies should be considered after batch experiments. This would allow for the sorption mechanisms to be more accurately defined rather than a dependence of kinetic modelling.
XRF analysis technique for biochar element analysis ➢ Large particle biochar used ➢ Short scanning time ➢ Calibration using a standard with different organic matter content	A standard of similar organic matter content should be used (e.g. coal fly ash). A longer scanning time, use of smaller biochar particles and post-analysis element dependent corrections should be considered to compensate for the dilution effect of a majority organic matrix. Where necessary, other methods laser-induced breakdown spectroscopy (LIBS) in addition to total digestion with acids (e.g. microwave assisted nitric acid digestion) and ICP-OES analysis.
Short growth period	A longer growing period may improve growth parameters and trace element uptake results
Only one biochar feedstock used	Further biochar from the UKBRC standard set should be used with LMWOAs
No LMWOAs were measured in the greenhouse study	LMWOA concentrations should be measured to help elucidate biochar-LMWOA dynamics in vegetated soils

6.4 Novel research contribution and research implications

This study systematically examined the interactive effects of biochar and LMWOAs on trace element and nutrient solubility. The results outlined above have provided new insights into the complication of biochar functioning upon the interaction with LMWOAs. The novelty of this research lies with the incorporation of LMWOAs in the biochar-trace element and nutrient studies. For instance, a LMWOA extraction procedure was used to demonstrate the release of potential elements of toxicity via LMWOA-driven dissolution. The work also included sorption studies to systematically examine the interactive effects of biochar and LMWOAs on trace element and nutrient solubility in aqueous and soil systems. Finally, this work included standardised biochar and LMWOA-activated biochar to examine the effects on vegetable plant growth and metal uptake which is novel at the time of writing.

The findings have implications for optimising biochar remediation of vegetated contaminated soils. One instance is if rhizospheric LMWOAs impede the biochar-borne driven immobilisation of cadmium and zinc, then the increased uptake of these elements by plant tissues is more likely. However, the current study found neither biochar nor LMWOA-amended biochar to affect the uptake of cadmium and zinc by pea plants. This shows the complex dynamics in biochar-amended soil-plant systems and highlights the requirement for further work. Furthermore, if biochar enhanced LMWOA-solubilisation of elements in biochar-amended multi-contaminated soils occurs, this might increase the uptake of toxic elements such as Al^{3+} ions by plant tissues, resulting in stress-induced toxicity (Muhammad, Zvobgo, & Guo-ping, 2018). As a result, the effect of biochar on trace element solubility may have been until now, underestimated given the lack of investigation involving LMWOAs. This shows the importance of identifying the main target elements before the biochar application. Furthermore, it is essential to understand the LMWOAs exuded in contaminated soils put forward for biochar application.

The enhanced retention of nitrates as found in aqueous systems implies that biochar can increase the rhizospheric residency of nitrates, which may decrease the need for fertiliser application (Kamenya et al., 2012). Furthermore, the dissolution of insoluble phosphate minerals suggests that biochar would be useful to improve nutrient-deficient soils. However, the conflicting results for soil systems imply that biochar reduced the natural attenuation of nitrates by rhizospheric soils. This may affect soil nutrient status, hence why further work would be beneficial to the field to understand biochar-nutrient dynamics in soils.

Lastly, the findings have implications for urban agriculture. Often at allotments, for example, concentrations of heavy metals can exceed the UK Soil Guidelines Values (SGV) (Weber et al., 2019; Guisti 2011; Prasad and Nazerth 2000). This may pose a risk to plants grown for consumption. This study systematically examined the effect of standard biochar (rice husk) on trace element uptake by pea plants. The reduced concentrations of certain trace elements in peas grown in biochar and LMWOA-activated biochar-amended soils suggest the application of this standardised biochar may be useful to limit uptake in leguminous vegetables.

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APPENDIX A1

Chemical information

The information regarding the reagents used in this research are shown in Table A1.1.

Table A1.1 Product information concerning the metal and nutrient salts, organic acids and other reagents used within this research.

Product and Purity	Linear formula	Molecular weight (g/mol)
Cadmium nitrate tetrahydrate	$Cd(NO_3)_2 \cdot 4H_2O$	308.49
Citric acid	$C_6H_8O_7 \cdot H_2O$	210.15
Lead nitrate	$Pb(NO_3)_2$	331.23
Malic acid	$C_4H_6O_5$	134.09
Oxalic acid	$C_2H_2O_4 \cdot 2H_2O$	126.04
Potassium Bromide	KBr	119.08
Sodium chloride	NaCl	58.44
Sodium hydroxide	NaOH	40.00
Sodium hexametaphosphate	$Na_6P_6O_{18}$	611.77
Sodium nitrate	$NaNO_3$	84.9947
Sodium dihydrogen phosphate	H_2NaO_4P	119.977
Zinc nitrate hexahydrate	$Zn(NO_3)_2 \cdot 6H_2O$	297.49

APPENDIX A2

Experimental adsorption capacity values

Adsorption capacity Qe (mmol g^{-1}) was referred to in Chapter 3 and Chapter 4. Values are calculated using the below equation where V is volume (L) and m is biochar mass (g). Results are shown in Table A2.1.

$$qe = \frac{Ci - Ce}{m}V$$

Equation A1.1 Calculation for the adsorption capacity values

Table A2.1 Experimental adsorption capacity (Qe mmol g^{-1}) values at 120 h.

Trace element/nutrient	**Adsorption capacity (Qe mmol g^{-1})**			
	Water	**Citric acid**	**Malic acid**	**Oxalic acid**
Cadmium	0.079	0.046	0.003	0.046
Lead	0.100	0.009	0.009	0.099
Zinc	0.083	0.031	0.030	0.081
Nitrate	0.000	0.096	0.097	0.061
Phosphate	-0.005	-0.032	-0.026	-0.036

APPENDIX A3

UKBRC standard biochar manufacturer provided properties

The UKBRC provided characterisation information for the biochar purchased in 2015 (Table A3.1 and Table A3.2). Although the manufacturers provided characterised biochar, it is important to examine the biochar materials before use. Inter-lab differences and different techniques may cause different values to those outlined in this study. The values have been included as many of the characteristics could not be determined at the University of Salford, for instance, the elemental percentages (wt %).

Table A3.1 Physicochemical properties of MSP-derived, OSR-derived and RH-derived biochar, as provided by the UKBRC, University of Edinburgh UK.

Property	MSP550	MSP700	OSR550	OSR700	RH550	RH700
Moisture (wt%)	1.83	2.53	2.61	3.63	1.54	1.49
Carbon total (wt%)	75.41	79.18	68.85	67.74	48.69	47.32
Hydrogen (wt%)	2.42	1.26	1.82	1.09	1.24	0.63
Oxygen (wt%)[1]	9.24	6.99	8.91	7.84	2.47	2.06
Hydrogen:Carbon (molar ratio)	0.38	0.19	0.32	0.19	0.28	0.16
Oxygen:Carbon (molar ratio)	0.09	0.07	0.10	0.09	0.04	0.03
Carbon total (wt%)	12.15	11.55	19.5	21.92	47.93	47.93
Hydrogen (wt%)	0.78	1.03	1.59	1.26	1.04	0.85
pH	9.77	9.72	9.78	10.41	9.71	9.81
Electrical Conductivity (dS/m)	0.82	1.91	2.27	3.11	0.48	0.69
Total Surface Area (m^2/g)	33.60	37.2	7.30	25.20	20.1	42.00
PAH (mg/kg dry wt)	0.53	0.12	0.54	< 0.11	0.21	0.34
Arsenic (mg/kg dry wt)	1.35	1.60	1.09	1.09	0.26	< LOD
Cadmium (mg/kg dry wt)	0.71	4.58	1.76	2.98	17.84	19.97
Chromium (mg/kg dry wt)	8.67	36.14	< LOD	4.36	4.99	< LOD
Cobalt (mg/kg dry wt)	2.69	3.91	3.06	3.17	2.94	4.59
Copper (mg/kg dry wt)	26.64	5.88	7.86	13.78	5.40	26.93
Lead (mg/kg dry wt)	< LOD[2]	< LOD	17.62	< LOD	< LOD	< LOD
Mercury (mg/kg dry wt)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Molybdenum (mg/kg dry wt)	7.90	22.63	1.29	1.68	0.64	0.67
Nickel (mg/kg dry wt)	4.95	30.40	2.49	3.27	3.00	2.71
Selenium (mg/kg dry wt)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Zinc (mg/kg dry wt)	63.40	44.53	7.22	8.80	23.58	36.17

[1] wt% by difference [2] < LOD; less than the limit of detection

Table A3.2 Physicochemical properties of SS-derived, SWP-derived and WSP-derived biochar, as provided by the UKBRC, University of Edinburgh UK.

Property	SS550	SS700	SWP550	SWP700	WSP550	WSP700
Moisture (wt%)	2.48	1.69	1.52	1.00	1.88	2.17
Carbon total (wt%)	29.53	29.55	85.52	90.21	68.26	69.04
Hydrogen (wt%)	1.33	0.83	2.77	1.83	2.10	1.18
Oxygen (wt%)[1]	6.50	2.75	10.36	6.02	6.92	5.30
hydrogen:Carbon (molar ratio)	0.54	0.34	0.39	0.24	0.37	0.20
Oxygen:Carbon (molar ratio)	0.17	0.07	0.09	0.05	0.08	0.06
Total Ash (wt%)	58.89	63.91	1.25	1.89	21.25	23.82
Total Nitrogen (wt%)	3.75	3.79	< 0.10	< 0.10	1.39	1.32
pH	8.17	9.60	7.91	8.44	9.94	10.03
Electrical Conductivity (dS/m)	280.80	113.40	0.09	0.16	1.7	1.52
Total Surface Area (m^2/g)	no data	no data	26.40	162.30	26.40	23.20
PAH (mg/kg dry wt)	3.76	1.40	4.39	0.18	0.4	< 0.11
Arsenic (mg/kg dry wt)	< 0.72	< 0.72	0.90	0.61	1.16	1.26
Cadmium (mg/kg dry wt)	11.69	12.36	3.48	8.16	3.15	1.27
Chromium (mg/kg dry wt)	275.69	292.72	34.57	123.35	<LOD	4.45
Cobalt (mg/kg dry wt)	11.58	12.68	1.04	4.37	1.54	1.58
Copper (mg/kg dry wt)	255.22	296.63	19.41	9.66	3.63	4.68
Lead (mg/kg dry wt)	201.19	195.97	< LOD[2]	< LOD	< LOD	< LOD
Mercury (mg/kg dry wt)	< 0.23	< 0.23	< LOD	< LOD	< LOD	< LOD
Molybdenum (mg/kg dry wt)	5.59	4.67	3.36	38.54	0.84	3.26
Nickel (mg/kg dry wt)	57.19	66.25	3.30	74.07	1.00	2.50
Selenium (mg/kg dry wt)	< 1.40	< 1.40	5.68	< LOD	< LOD	< LOD
Zinc (mg/kg dry wt)	835.69	896.21	25.71	99.60	10.50	12.03

[1] wt% by difference [2] < LOD; less than the limit of detection; no data; not provided by the UKBRC

APPENDIX A4

Growth experiment: further details

In section 3.4.4, it is reported that a previous growth experiment found obvious and severe signs of necrosis and stunted growth when plants were grown in soils from Moston Brook than had not been mixed with garden soil. In Figure A4.1, there are signs of purpling, necrosis and other toxicity indicators on a *Brassica juncea* seedling during one of the trial runs using the undiluted Moston Brook soil. For this reason, and for the Results of other preliminary studies where poor growth was found (results not shown), the soil was diluted with garden soil for future growth experiments for the thesis.

Figure A4.1 Signs of heavy metal toxicity on *Brassica juncea* seedlings during a preliminary study using undiluted Moston Brook soil