Soil Carbon Dynamics in the Upper Mersey Estuarine Floodplain, Northwest England: Implications for Soil Carbon Sequestration

Osim Ogar Enya

Ecosystems and Environment Research Centre
School of Science, Engineering and Environment
University of Salford, Manchester
M5 4WT

Submitted in Partial Fulfilment of the Requirements of the Degree of Doctor of Philosophy

July, 2019

Contents

List of Figures

List of Tables

Acknowledgements

I must confess that, this is the most difficult section for me to write because the Almighty God has used many people to get me to where I am today, and particularly for the completion of this thesis. Firstly, all glory and adoration go to God Almighty for his divine provision and guidance during this period and beyond.

Special thanks go to my supervisors Prof. Chuxia Lin and Prof. Philip James for their show of love, inspiration and constructive criticism throughout the period of this research. I am very grateful to you guys for always leaving your office doors open so I can come in at any time.

Thanks to academic and support staff of the University of Salford for their contributions to my research. To my fellow researchers in Peel 334 and 335, I am very grateful for your useful comments and friendship. Special thanks go to Natalie Heaney and Rebecca Morgan for final proof reading. To my friends and benefactors, I deeply appreciate your support and words of encouragement.

Several organisations felt that I was studying a worthy topic and they sought my advice and made finances and resources available for this research. For this reason, I am grateful to The Cross River State Government and Niger Delta Development Commission, Nigeria for their financial assistance. I am also grateful to Mr. Paul Oldfield and the team at Mersey Gateway Crossings Board. Thank you for granting me the permission to use the research area and assisting me in the field throughout recent years.

Finally, to my dear wife Thelma Enya, my cheerful son Mark Enya and my gorgeous daughter Marvelous-Enara Enya, I owe you a lot for your love, patience and understanding during my absence from home in the cause of this work. To my mum, dad and siblings, I will always be grateful for your support and prayers throughout this research.

Abstract

There have been several studies on soil carbon dynamics in estuarine floodplains. However, little attempt has been made to examine the effects of heavy metal contamination on these processes. This represents a knowledge gap that needs to be filled to understand better soil carbon decomposition in contaminated estuarine floodplains and the implications for carbon sequestration. The current work aims to close this knowledge gap.

Field and laboratory investigations were conducted to collect data. These include (a) a soil survey to characterise heavy metal contamination in the study area, (b) seasonal monitoring of key parameters (soil organic carbon, bulk density, plant biomass etc.) at three selected sites with different land uses/land covers, and (c) a laboratory experiment to evaluate the impacts of heavy metals on soil carbon content, characterisation of humic substances, adsorption capacity, and microbial activities. The historically contaminated Upper Mersey Estuarine floodplain was selected as the study site.

The results indicated that the study area has elevated concentration of heavy metals with arsenic, chromium, copper, lead, and zinc being the major contaminants. Seasonal variations in organic carbon content and bulk density showed that, grazing locations stored more organic carbon and recorded high bulk density compared to non-grazing locations. The presence of heavy metals inhibited the activities of soil microbes, impeded decomposition of organic matter, resulting in incomplete carbon mineralisation and enhanced soil carbon storage. The functional group composition of the soil humic substances was also affected.

The findings obtained from this study have implications for understanding the role of soil carbon in limiting heavy metal mobilisation and the importance of microbial activity in soil carbon budgets, the management of saltmarsh under grazing regimes, national carbon budgets, and the design of future studies.

1. Introduction

1.1 Background and significance

The importance of soil to sequester carbon is gaining greater attention globally following the quest to mitigate climate change (Lal, 2004, Barreto et al., 2009; Ostle, Levy, Evans, & Smith, 2009; Stewart, Paustian, Conant, Plante, & Six, 2009; Powlson, Whitmore & Goulding, 2011; Burden, Garbutt, Evans, Jones, & Cooper, 2013; Mishra, Zakerinia, Yeh, Teter & Morrison, 2014). Soil organic carbon storage in a given soil is determined by the balance between the input of organic matter into the soil and the loss of soil organic matter through decomposition and erosion (Andrews, Samways & Shimmield, 2008; González=Alcaraz et al., 2012; He et al., 2013). Soil respiration (the mineralisation of organic carbon to carbon dioxide) is the most important processes affecting the carbon balance of a terrestrial ecosystem (Davidson et al., 2002). This is in both root and microbial respiration and can be measured using chamber-based measurement (Anthony et al., 1995; Davidson et al., 2002; Pumpanen et al., 2004). The balance between soil carbon storage and soil respiration determines whether the soil is a sink of carbon or a source of atmospheric carbon dioxide (Whiting & Chanton, 2001; Kayranli, Scholz, Mustafa & Hedmark, 2010). The factors affecting these processes are temporally and spatially variable (Bruland, Grunwald, Osborne, Reddy & Newman, 2006; Reddy & Delaune, 2008). Therefore, understanding the dynamics of soil organic matter is of great importance in terms of evaluating carbon sequestration potential of soils (Yang, Singh & Sitaula, 2004).

Saltmarsh ecosystems act as an important carbon sink. According to Reddy & Delaune (2008), soil carbon is the primary driver for all biogeochemical processes in wetlands. In addition to acting as a carbon sink, saltmarshes provide a wide array of benefits to coastal populations, including shoreline protection, immobilisation of pollutants (Burden et al., 2013), fishery support, water quality improvement, and wildlife habitat provision, (Wilson, Lamb, Leng, Gonzalez, & Huddart, 2005a, 2005b; Lamparter, Bachmann, Goebel, & Woche, 2009; Hopkinson, Cai, & Hu, 2012; Chen, Chen, & Ye, 2015; Hansen & Reiss, 2015). However, French (1997) and Martinez, Maun, & Psuty, 2004 reported that estuarine floodplains are at risk and in decline across the world. This is due to urban and industrial development, coastal

erosion, a rise in sea level, agriculture and tourism (Olsen et al., 2011). Boorman & Hazelden (2017) state that saltmarsh losses are associated with rising sea levels and climate change while French (1997) estimates that 25% loss of saltmarsh globally was due to agricultural or industrial use. Cooper et al. (2001) and Baily & Pearson (2001) estimated that approximately 15% of the UK's saltmarsh land has been lost because of land reclamation for agricultural production and industrial development over the past 70 years. In the UK, there are now approximately 47,000 hectares of saltmarsh (Burd, 1989), with thirty-four natural reference saltmarshes (Mossman, Davy, & Grant, 2012). They include vegetated inter-tidal habitats which are classified primarily by the frequency of tidal inundation (Cooper, Cooper & Burd, 2001; Baily & Pearson, 2001).

Saltmarsh soils are largely contaminated by organic and inorganic pollutants (Fox et al., 2001). Several factors have been reported to influenced metals concentration in estuarine floodplains (Salomons, De Rooij, Kerdijk & Bril, 1987; Du Laing, Rinklebe, Vandecasteele, Meers & Tack, 2009; Violante, Cozzolino, Perelomov, Caporale & Pigna, 2010; Salomons & Förstner, 2012). These include cation exchange capacity, clay or organic matter contents, topography, pH, salinity, and plant species. According to Du Laing et al. (2009), iron and manganese hydroxides are the main carriers for cadmium, zinc, and nickel under oxic conditions, while soil organic matter fraction is most important for copper. A report from Du Laing et al. (2008) suggested that mobility and availability of metals in estuarine floodplains are significantly reduced due to the formation of metal sulphide precipitates under anoxic conditions. Also, plants can affect the metal mobility in floodplain soils by oxidising their rhizosphere, taking up metals, excreting exudates and stimulating the activity of microbial symbionts in the rhizosphere (Chatterjee et al., 2013; Du Laing, Tack, & Rinklebe, 2013). According to Wang et al. (2007), polymerase chain reaction-denaturing gradient gel electrophoresis (PCR–DGGE) analysis shows that heavy metal pollution has a significant impact on bacterial and actinomycotic community structure. Landi, Renella, Moreno, Falchini & Nannipieri (2000) reported that heavy metal concentration may reduce the availability of substrate for soil respiration by forming complexes with the substrates or by killing microorganisms. Hence, the presence of these contaminants could affect soil carbon dynamics through their impacts on plant growth (organic matter input) and microbial

activities (decomposition of organic matter). The net effect of the presence of organic matter can either be a decrease or an increase in metal mobility (Du Laing et al., 2009).

The effects of soil properties on carbon dynamics have been extensively studied (A-lsheikh et al., 2005; Steinbeiss, Gleixner & Antonietti, 2009; Wiesenberg, Dorodnikov & Kuzyakov, 2010; Dorodnikov, Kuzyakov, Fangmeier & Wiesenberg, 2011; González-Alcaraz et al., 2012). Alsheikh et al. (2005) reported that small grains or fine soil particles contribute to the conservation of soil organic matter (SOM) and/or sequester soil organic matter-carbon and soil organic matter-nitrogen. According to Gao et al. (2014), carbon: nitrogen:phosphorus ratios increase at the same proportion in response to heavy rainfall. According to Fisk, Fahey & Groffman (2010), micro-arthropod abundance and microbial biomass carbon were significantly positively correlated, but neither was related to forest floor mass or to annual aboveground fine litter fall flux. Instead, a positive correlation with fine root biomass suggests that carbon supply from roots plays a key role in the fungal channel of the detrital food web of these forests (Fisk et al., 2010). According to Baldwin & Mitchell (2000), tidal impact in estuarine floodplains will lead to the release of carbon, nitrogen and phosphorus from leaf litter and soils. Several pathways of organic matter decomposition leading to soil carbon accumulation have been reported (Lamparter et al., 2009; Berhe, 2011; Chenu, Rumpel & Lehmann, 2015; Derenne & Quénéa, 2015; Gunina & Kuzyakov, 2014; Sandhu, Wratten & Cullen, 2010). Gunina & Kuzyakov (2014) discussed the pathways of litter carbon by the formation of aggregates and SOM density fractions as an implication of ^{13}C abundance. According to Lamparter et al. (2009), soil carbon mineralisation decreases with decreasing pH and increasing carbon/nitrogen ratio with the same significance. Therefore, looking at different soil properties becomes necessary to understand the variation that exists during soil carbon storage.

1.2 Research gap

Although there has been some research on soil carbon biogeochemistry in estuarine floodplains (Wilson et al., 2005a, 2005b; Andersson et al., 2008; Gao et al., 2014), in-depth research into soil carbon dynamics in contaminated coastal wetland soils is rare. This represents a knowledge gap that needs to be filled to develop appropriate strategies and methods for better management of such estuarine floodplains, especially from a carbon

sequestration perspective. Therefore, understanding carbon dynamics in saltmarshes following different land uses/land covers will provide fundamental knowledge that can be used to evaluate soil carbon status and predict future trends in soil carbon storage.

1.3 Research aim and objectives

The aim of this study is to assess the effects of heavy metal contamination during the decomposition processes of soil organic matter under different land uses/land covers. This will improve our understanding on how heavy metal contamination and soil organic matter influence the amount of soil carbon stored or lost over time. To achieve this goal, the following objectives are set:

1. To carry out a preliminary investigation to select appropriate sites for detailed monitoring.
2. To monitor the seasonal variation in soil carbon storage within the study site.
3. To evaluate the effects of heavy metal contamination on soil carbon status using a laboratory experiment.
4. To assess the soil microbial diversity through DNA next generation sequencing.

1.4 Thesis structure

This thesis will contain eight chapters, cited references and appendices. Chapter 1 contains a general introduction about soil carbon dynamics, estuarine floodplains, benefits derived from soil carbon storage, heavy metal contamination and implication of this to carbon sequestration as an option for mitigating global warming. Chapter 2 contains a critical evaluation of literatures relating to soil carbon dynamics under different land uses/land covers in a contaminated estuarine floodplain, and the implication for carbon sequestration. Chapter 3 is an outline of the research paradigm used, reasons for selecting the study area, field and laboratory experimental designs, the kind of data collection and laboratory analysis with appropriate procedures/protocols and technique used for data analysis. Chapter 4 contains a presentation of the data obtained on the heavy metal contamination status in soil-plant systems. There is also data presented on the bioaccumulation coefficient (BAC), biological concentration factor (BCF) and the translocation factor (TF) of heavy metals from the soil to plant roots and shoots. This will help to explain the phytoremediation potential of

the plant species and to see if the cattle grazing in the saltmarsh are free from heavy metal contamination or not. Chapter 5 contains data on the seasonal variation of soil organic carbon and carbon emission/flux measurement. This will help to explain whether the different land uses/land covers add to carbon storage and to determine if the Upper Mersey Estuary is a sink or a source of carbon. Chapter 6 is a report of the laboratory incubation data to evaluate the effects of heavy metal contamination on soil carbon storage. Chapter 7 contains data on the microbial diversity results from 16S next generation sequencing (NGS), to examine the effect of long–term contamination within the Upper Mersey Estuary. Chapter 8 is the general discussion drawing together the strands of this investigation, implications of the research and recommendations for future research.

2. Literature Review

2.1 Soil carbon dynamics

Soil carbon dynamics can be defined as the variation of carbon within an ecosystem (Sandhu, Wratten & Cullen, 2010; Powlson et al., 2011; Harrison-Kirk et al., 2014). Harrison-Kirk et al. (2014) suggested that soil organic matter content and aggregate stability also influence the dynamics of both carbon and nitrogen content because of different responses to dry/wet cycles. The dynamic carbon mineralisation in soil depends on chemical properties like pH and carbon: nitrogen ratio and physical properties such as aggregate distribution under different moisture conditions (Plante & Parton, 2007; Teixeira et al., 2011; Hopkinson et al., 2012; Vaccari et al., 2012; Kulawardhana et al., 2015). According to Lu & Cheng (2009), elevated temperature and precipitation influenced the soil carbon dynamics, and significantly increased the greenhouse gas emissions from the soil. Understanding the different turnover rates from plant and animal residues, microbial biodiversity, and SOM will also help in the monitoring of carbon, nitrogen, sulphur and phosphorous dynamics in the soil system (Plante & Parton, 2007).

2.1.1 Factors affecting soil carbon dynamics

Several factors are linked to the dynamics of soil carbon (Chirinda, Elsgaard, Thomsen, Heckrath & Olesen, 2014). These include topography, types of season, tide inundation, heavy metal contamination, soil organic matter stabilisation with heavy metals, microbial biodiversity and management practices, as shown in Figure 2 (Granberg & Selck, 2007; Olsen et al., 2011; Spencer & Harvey, 2012; Chirinda et al., 2014; Edmondson, Davies, McCormack, Gaston, & Leake, 2014; Garrard & Beaumont, 2014; Maillard et al., 2015; Wiesmeier et al., 2015). Lal (2005) and Wiesmeier, et al. (2013) reported that both soil carbon accumulation and turnover rates of soil organic carbon are influenced by factors such as climate, topography, soil type, and land use/land cover, leading to large spatial variability of SOC stocks at both regional and local scales. Increasing production of forest biomass per se may not necessarily increase the soil organic carbon stocks (Lal, 2005).

2.1.1.1 Topographic position

Topographic position or elevation is an important parameter in trying to quantify and predict carbon budget in coastal wetlands (Spencer & Harvey, 2012; Chirinda et al., 2014; Kulawardhana et al., 2015). According to Kulawardhana et al. (2015), carbon concentrations and bulk densities showed significant and abrupt change at a depth of 10–15 cm. Neumann-Cosel, Zimmermann, Hall, van Breugel, & Elsenbeer (2011) reported that soil carbon stocks in the top 10 cm did not change with young forest development. Topography and associated texture variation can affect decomposition rates as well as soil nutrient transformations. Using a digital elevation model (DEM), Chaplot, Bernoux, Walter, Curmi, & Herpin (2001) showed that topographical attributes explained up to 75% of the profile organic carbon stock variability. Thus, a large amount of organic carbon accumulates in hydric valley bottom soils.

According to Hook & Burke (2000), topographic position and soil texture each explained much of the landscape-scale variation of carbon and nitrogen pools and vegetation structure. Most lowland plots were enriched in silt, clay, carbon, and nitrogen relative to adjacent upland plots, and topographic position affected most pools significantly. Carbon concentrations in plant material were not significantly different among the three topographic positions studied, resulting in higher carbon/nitrogen ratios in valley plots (Luizão, et al., 2004). Local topography (plateau, slope and valley) clearly was an influential factor in the nutrient distribution along the central Amazonian forest. Lower rates of nitrogen cycling processes in the valley are probably related to its sandy soil texture and seasonal flooding (Luizão et al., 2004). For temperate forests, it is well known that nitrogen mineralisation may be highly variable within a forest ecosystem (Hill & Shackleton, 1989), and that nitrogen transformation rates vary between different soil types within the same watershed (Cole, Compton, Van Miegroet, & Homann, 1991).

2.1.1.2 Seasonal variation

Many studies have reported the influence of seasonal types on soil carbon sequestration (Andrews et al., 2008; Granberg & Selck, 2007; Lu & Cheng, 2009; Ostle et al., 2009; Sandhu et al., 2010; Spencer & Harvey, 2012; Stockmann et al., 2013; Zhang, Ricketts, Kremen, Carney, & Swinton, 2007). Attention has been drawn to C turnover rates, dissolved organic carbon (DOC), carbon dioxide emission (CO_2), soil organic carbon and total organic

carbon (TOC). Dissoloved organicn carbon production increases under a warmer and drier moisture regime (Chaplot & Cooper, 2015; Hoggart et al., 2015; Lorenz, Lal, & Jiménez, 2009).

Microbial decomposition rate varies from season to season. During the winter periods, the decomposition rate is assumed to be insignificant in the annual cycling of carbon and nutrients (Aber, Nadelhoffer, Steudler & Melillo, 1989). Grogan & Chapin III (1999) and Thomas et al. (2014) reported that, litter decomposition and soil respiration occur over winter in both arctic and boreal ecosystems. Winter fluxes of carbon dioxide are substantial in annual carbon budgets and likely influence both the magnitude and direction of annual carbon fluxes. Other researchers have it that carbon dioxide released during the summer comes primarily from root respiration and decay of plant biomass in the surface organic horizon (Mikan, Schimel & Doyle, 2002), but during the winter it appears that microbial respiration may be driven by soluble material remaining in water films or deeper in the profile or into the mineral soils (Clein & Schimel, 1995). Thus, models of carbon dynamics developed for summer activity would be fundamentally flawed in modelling winter activity (Segoli et al., 2013). Several factors are considered when considering the seasonal variation of carbon dynamics. These include litter mass and nitrogen loss, microbial activities and temperature, and dissolved organic carbon fractions may also be more important than non–dissolved ones in supplying carbon to microbes even within the organic horizons (Clein & Schimel 1995). According to Coxson & Parkinson (1987), about 55% of overall winter soil respiratory activity can occur in soils at temperatures in the range of -4 and +4 °C within the top 8 cm of the soil profile, with the remaining respiratory activity largely occurring at temperatures up to 15 °C.

2.1.1.4 Land use/land cover

The effects of land use/land cover on soil organic carbon dynamics have been extensively studied (Mendoza-Vega, Karltun & Olsson, 2003; Lal, 2005; Yadav & Malanson, 2008; Ordóñez et al., 2008; Ostle et al., 2009; Robson, Baptist, Clément & Lavorel, 2010; Munoz-Rojas, De la Rosa, Zavala, Jordan & Anaya-Romero, 2011; Negrin et al., 2011; Vaccari et al., 2012; Wiesmeier et al., 2013a; Wiesmeier et al., 2013b; Lavelle et al., 2014; Wiesmeier et al., 2015). Walker & Desanker (2004) reported that soil carbon stock decreases with depth within different land uses/land covers. Carbon emissions from deforestation and degradation account for about 20% of global anthropogenic emissions (Mollicone et al., 2007). Anikwe

(2010) and Vaccari et al. (2012) reported that, the single biggest loss of soil carbon in the form of physically protected soil organic matter results from management such as tillage practice or land use change (such as conversion of forestry to grassland).

Neumann-Cosel et al. (2011) reported that soil carbon stock from the Panama region is not affected by the land-use transition from pasture to young secondary regrowth. Thus, an increase of soil carbon storage might be possible over a longer period. Within the cattle grazing saltmarsh in temperate regions, Olsen et al. (2011) reported that grazing had little effect on the rates of mineralisation of ^{14}C used as a respiratory substrate, but a larger proportion of ^{14}C was partitioned into microbial biomass and immobilised in long- and medium-term storage pools in the grazed treatment. Grazing slowed down the turnover of the microbial biomass, which resulted in longer turnover times for both leaf litter and root exudates. Grazing may therefore affect the longevity of carbon in the soil and alter carbon storage and utilisation pathways in the microbial community. Saltmarshes differ from other terrestrial systems since they are inundated by tides that saturate the soil and limit oxygen penetration.

The effects of land use and cover on carbon budget cannot be over emphasised. Recent studies report conflicting results concerning soil carbon trends as well as multiple confounding factors (e.g. soil type, topography and land-use history) affecting these trends. Neumann-Cosel et al. (2011) measured organic carbon stocks in the mineral soil up to 20 cm depth at 24 active pastures, 5–8 years old, and 12–15 years old secondary forest sites on former pastures. Their data indicated that soil carbon stock was higher in older forests than at the younger sites. According to Edmondson et al. (2014), the land cover did not significantly affect SOC concentrations in non-domestic greenspace, but values beneath trees were higher than under both pasture and arable land, whereas concentrations under the shrub and herbaceous land covers were only higher than arable fields. Munoz-Rojas et al. (2011) demonstrated the importance of land-cover change for carbon sequestration in vegetation from Mediterranean areas, highlighting possible directions for management policies to mitigate climate change and promote land conservation.

2.1.1.5 Environmental factors

Temperature, soil pH and soil moisture condition affect the amount of carbon stored in the soil and sediment (Anderson & Domsch, 1993). Seasonal changes in temperature play a significant role in controlling the rates of biogeochemical processes regulating organic matter decomposition, including enzyme activities, dead organic matter production, carbon dioxide and methane emissions (Reddy & Delaune, 2008). Some studies using varieties of plant matter and freshwater systems, show seasonal variations in decomposition rates with faster breakdown during warmer periods (Webster & Benfield, 1986). Detrital matter from Typha sp. was found to be less sensitive (1.5 times higher during summer months than winter months) to seasonal temperature than that from Sagittaria (5.5 times higher during summer months than winter months) (Reddy & Delaune, 2008).

Temperature is one of the key regulators influencing biogeochemical processes in wetlands, by influencing the growth, activity, and survival of organisms (Reddy & Delaune, 2008). Chemical and enzymatic reactions regulating organic matter decomposition proceed at a faster rate as the temperature is increased (Reddy & Delaune, 2008). Thus, soil microbial activity and organic matter decomposition are enhanced by an increase in temperature. For each microbial community, there is a maximum temperature above which growth is inhibited, a minimum temperature below which growth no longer occurs, and an optimal temperature range in which growth is most rapid (Reddy & Delaune, 2008). This meaning that above certain temperatures, proteins, nucleic acids and other cellular components may be irreversibly denatured. This may lead to a collapse in the plasma membrane and thermal lysis of cells may occur. However, temperature response to biogeochemical processes is often expressed in terms of Q_{10} function (Reddy & Delaune, 2008), where Q_{10} is the change in the decay constant associated with a 10 °C change in temperature. It is reported as a coefficient.

Decomposition rate increases with temperature at 0 °C with a Q_{10} coefficient as high as 8, and the temperature sensitivity decreases with increasing temperature, as indicted by the Q_{10} coefficient decreasing to 4.5 at 10 °C and 2.5 at 20 °C (Kirschbaum, 1995; Davidson, Duncan, Littlejohn, Ure & Garden, 1998). The Q_{10} coefficient for peatlands and bogs were reported to be in the range of 1.8–6.1 (Lafleur, Moore, Roulet, & Frolking, 2005). Earlier studies have shown a prolonged lag phase in carbon dioxide production at low temperatures

(7 °C) in soils amended with plant matter (Pal, Broadbent & Mikkelsen, 1975). Reddy & Delaune (2008) reported that when the temperature was increased to 22 °C, the decomposition rate increased by a Q_{10} coefficient of 12 during the first 2 days, and a further increase in temperature to 37 °C resulted in a Q_{10} value of 1.5. The Q_{10} coefficient were highest during early stages of decomposition and decreased sharply and remained at a constant value for the remaining 4-month decomposition period (Pal et al., 1975). Surface soil temperatures were found to be better predictors of ecosystem respiration than temperatures in deeper soil depths (Lafleur et al., 2005). Temperature sensitivity of organic matter decomposition is much greater at a lower temperature and decreases with an increase in temperature (Reddy & Delaune, 2008). Results from Fang, Smith, Moncrieff, & Smith (2005) indicated that that temperature sensitivity for resistant organic matter does not differ significantly from that of labile pools, and that both types of soil organic matter will therefore respond similarly to global warming.

It is also suggested that temperature sensitivity is much greater for organic matter decomposition than for net primary productivity. This has important implications for organic matter storage in the ecosystem (Kirschbaum, 1995). Decomposition of labile soil carbon is more sensitive to temperature than slowly degradable or resistant soil organic carbon. High levels of labile organic carbon pools are present in soils at low temperatures, whereas at higher temperatures relatively high levels of more recalcitrant organic matter are maintained (Dalias, Anderson, Bottner & Coûteaux, 2001). Thus, recalcitrant soil organic materials mineralise more efficiently at higher temperatures (Bol, Bolger, Cully & Little, 2003).

2.2 Soil organic matter distribution

Soil organic matter can be defined as all organic materials found in soil that are part of or have been part of living organisms. It is a continuum of materials at various stages of transformation due to both abiotic and biotic processes. Several studies have reported the importance of soil organic matter, linking it with ecosystem services (Wu, Chen, Wang & Wang, 2006; von Luetzow et al., 2007; Scheiter & Higgins, 2009; Erfanzadeh, Bahrami, Motamedi & Pétillon, 2014; Harrison-Kirk et al., 2014; Wiesmeier et al., 2015). According to Erfanzadeh et al. (2014), soil organic matter had a spatial variation which was probably affected by the plant species. Soil organic matter is essential for soil physical fertility,

increasing water retention, retaining organic pollutants and heavy metals, protecting and reducing water quality, aggregating mineral particles, thus reducing soil erosion. Soil organic matter consists of various functional pools that are stabilised by specific mechanisms and have certain turnover rates (von Luetzow et al., 2007; Wiesmeier et al., 2014). Depending on the authors, the pools are termed as active, intermediate or slow, and passive or inert (McGill, 1996; Smith et al., 1997).

The chemical constituents of soil organic matter are grouped into humic substances, phenolic substances and non-humic substances (Stevenson, 1994). Humic substances consist of heterogeneous mixtures of high-molecular-weight aromatic structures that result from secondary synthesis reactions. Non-humic substances are carbohydrates, proteins and fats, while phenolic substances are lignins and tannins. The relative proportion of the various constituents within the humic, phenolic and non-humic substances varies with the type and source of detrital matter, the degree of decomposition, and the age of the material (Stevenson, 1994). Humic substances, or humin, are not soluble in alkali or acid (Stevenson, 1994).

Humic materials are colloidal in nature and exhibit a very large surface area and negative charge due to exposed -COOH and -OH groups, which have H^+ ion available for exchange with metals. Thus, they have a higher capacity to form complexes with metals and hold water than clay. Insoluble high-molecular-weight humic acids are very effective in immobilising most trace and toxic metals (Reddy & Delaune, 2008). Humic acids can also reduce certain oxidised metal species in such a way that they make it easier for the metal to be fixed to the humic matter and make it unavailable for further mobilisation or plant uptake (Stevenson, 1982). According to Boulton & Boon (1991), tannins and lignins play an important role in the decomposition of labile plant constituents by complexing with proteins, exhibiting antibiotic activity, and forming an association with cellulose and hemicellulose. In wetland ecosystems where aerobic and anaerobic interfaces play a major role in the soil and water column and accumulation of organic matter, primary productivity often exceeds the rate of decomposition processes, resulting in net accumulation of organic matter (Stevenson, 1994). The net accumulation of organic matter is regulated by the activity of various decomposers,

including benthic invertebrates, fungi and bacteria. In general, decomposition may be viewed as a three-step process:

- Breakdown of particulate organic matter by fragmentation by grazers;
- Hydrolytic activity of extracellular enzymes involved in the conversion of particulate organic matter (polymers such as polysaccharides);
- Microbial catabolic activities (conversion of monomers into carbon dioxide and methane) (Stevenson, 1994).

According to Reddy & Delaune (2008), decomposition processes of soil organic matter in wetlands is different from that in upland ecosystems. There is rapid decomposition of biomass in upland soils due to the predominance of aerobic conditions, while the decomposition rate is slower in wetland soils due to the predominance of anaerobic conditions (Reddy & Delaune, 2008). This results in a moderately decomposable organic matter accumulating along with lignin and other recalcitrant fractions (Stevenson 1994; Reddy & Delaune, 2008). Thus, the accumulation of organic matter in wetlands is typically characterised by a stratified build-up of partially decomposed plant remains, with a low degree of humification (Reddy & Delaune, 2008). The biodegradability of organic matter decreases with depth and low soil temperature, as the material accreted deep into the soil ages and undergoes humification compared to the new material accumulating in the surface layers (Reddy & Delaune, 2008).

2.2.1 Transformation of soil organic matter

According to Leifeld, Franko & Schulz (2006), an absorbance of infrared bands representing aliphatic C-H functional groups is a potential indicator of soil organic matter transformations related to changes in its labile fractions. By quantifying relative changes in functional groups, a Fourier-transform infrared (FTIR) spectrometer can be used to help explain soil organic matter transformations and stabilisation (Margenot, Calderón, Bowles, Parikh & Jackson, 2015). In an organically managed systems, mineralisation of soil organic matter is crucial for meeting crop nutrient demand. According to Margenot et al. (2015), relating the functional group composition of soil organic matter to labile fractions can provide insight into the degree to which the chemistry of soil organic matter can influence its lability. Long-term experiments have shown that organic management can increase labile carbon in the short term and total soil carbon in the longer term (Marriott & Wander, 2006). This is

because labile soil organic matter responds more rapidly to management than total soil organic carbon (Marriott & Wander, 2006).

The degree to which soil organic matter compositional changes are associated with these increases in soil organic matter is largely unknown and may offer insight into observed increases in labile soil organic matter. The differences in soil organic matter functional groups characterised by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) have been implicated in stabilisation of carbon fractions under different input treatments (Verchot, Dutaur, Shepherd & Albrecht, 2011). According to Wickings, Reed & Cleveland (2012), during the decomposition of soil organic matter, decomposer community characteristics regulate changes in litter chemistry, which could influence the functionality of litter-derived soil organic matter and the turnover and stabilisation of soil carbon. Therefore, an on-farm research across any landscape provides an opportunity to examine fields under different management practices and with variation in soil organic matter quantity, allowing determination of relationships between soil organic matter functional group composition with differences in labile soil organic matter.

2.3 Soil carbon sequestration

Carbon sequestration is gaining wide attention with the quest to reduce greenhouse gas (GHG) emissions (Brevik & Homburg, 2004; Barreto et al., 2009; Anikwe, 2010; Burden et al., 2013; Beaumont, Jones, Garbutt, Hansom & Toberman, 2014). The term soil carbon sequestration is often associated with sustainable management of our soil resources (Barreto et al., 2009). Soil carbon sequestration is considered an important ecosystem service and the enhancement of above-and below-ground carbon stocks have become a recognised forest management strategy. According to Neumann-Cosel et al. (2011), there are growing opportunities for such strategies to become economically profitable because of the increasing implementation of clean development mechanism (CDM) projects.

In a study to examine the potential for restored saltmarshes to sequester carbon, Burden et al. (2013) found that saltmarsh can provide a modest, but sustained, sink for atmospheric carbon dioxide. Recently, there have been increasing amounts of attention paid to the potential for saltmarsh ecosystems to sequester carbon (Burden et al., 2013; Chen et

al., 2015; Hansen & Reiss, 2015). Thus, restoration of coastal wetlands such as saltmarshes may contribute more to carbon sequestration, and therefore to climate regulation, than peatlands (Burden et al., 2013). According to Hopkinson et al. (2012), climate change including global warming, human engineering of river systems, continued agricultural expansion, and sea level rise will also negatively impact carbon sequestration of coastal vegetated wetlands.

2.4 Effects of chemistry and contamination

Reports on how heavy metal contamination affects the distribution of soil organic carbon dynamics are not fully documented (Hopkinson et al., 2012; Maillard et al., 2015; Mukwaturi & Lin, 2015). However, Mukwaturi & Lin (2015) reported that reductive dissolution of iron and manganese compounds was markedly enhanced by organic matter. Assessing stabilisation of soil organic matter with heavy metals will invariably provide an answer to whether heavy metal mobilisation affects the stability of soil organic matter (Kumpiene, Lagerkvist & Maurice, 2008; Kumpiene, 2010). Zhang et al. (2014) reported that under anoxic conditions, acid-volatile sulphides mainly reduce the solubility and toxicity of metals, while organic matters, iron and manganese oxides, clay or silt can stabilise heavy metals in elevated oxidative-reductive potential (ORP). Other researchers have investigated the safety level of heavy metals when they enter the body through inhalation of dust, oral ingestion of contaminated soil, and consumption of food plants harvested from contaminated soil (Hawley 1985; Dudka & Miller, 1999; Pendergrass & Butcher, 2006). Some of the organisations which deal with these safety levels are:

- European Commission (EC)
- International Agency for Research on Cancer (IARC)
- International Programme on Chemical Safety (IPCS)
- US Environmental Protection (USEPA)
- US Agency for Toxic Substance and Disease Registry (ATSDR)
- World Health Organization (WHO).

The chemical properties of metal pollutants also influence toxicity. The species of metal or metal speciation determine the behaviour in aquatic and wetland environments. Valence, the formation of oxyanions, sorption to the particulate or sediments, complexation with organic matter, precipitation, and interaction with microorganisms are processed

governing the availability or toxicity of heavy metals in wetlands (Reddy & Delaune, 2008). Soils generally contain a low concentration of toxic metals from natural sources whereas anthropogenic factors increase metal content in wetland soils, resulting in potential ecological risks. However, elevated total concentrations of metals do not necessarily result in problem releases to water or excessive bioavailability (Reddy & Delaune, 2008). The amount of organic matter and clay minerals, the soil acidity (pH), and the sediment oxidation–reduction status (Eh) of soils are very important physicochemical properties influencing the mobility of toxic metals (Reddy & Delaune, 2008). According to Bryan & Langston (1992), concentration and bioavailability of metals in estuarine sediments depend on the following processes:

- Mobilisation of metals to the interstitial water and their chemical speciation.
- Transformation (e.g. methylation) of metals including arsenic, mercury, lead, and tin.
- The control exerted by major sediment components (e.g. oxides of iron and organics) to which metals are preferentially bound.
- Competition between sediment metals (e.g. copper and silver; zinc and cadmium) for uptake sites in organisms.
- The influence of bioturbation, salinity, redox or pH on these processes.

2.4.1 Inorganic contaminants

There have been several reports on the amount of heavy metals being added to soil globally due to various anthropogenic activities, raising serious concerns for environmental health (Babich & Stotzky 1985; Davis, Ruby & Bergstorm, 1994; Vig, Megharaj, Sethunathan & Naidu, 2003; Wang et al., 2007; Wang, Li, Guo, & Ji, 2017). According to Babich & Stotzky (1985), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) are toxic heavy metals and have effects on nitrification and denitrification in soil while the ecotoxicological assessment of heavy metals and the impact on soil organisms was reported by Giller, Witter & McGrath (1998), Obbard (2001), and Giller, Witter & McGrath (2009). According to Panagos, Van Liedekerke, Yigini & Montanarella (2013), 34.8% and 30.8% of heavy metals have been reported to be affecting soil and groundwater, respectively, in Europe due to several factors, with municipal and industrial waste disposal topping the list. Introduction of heavy metals in the soil could have effects on soil carbon status due to their interaction with soil organic carbon.

According to the National River Authority (NRA) (1995), the rise in the importance of zinc as an anticorrosion agent in the 20th century and the output of arsenic as a by-product of smelting copper concentrates from south-west England and the Americas in the early 1900s are matched by increased levels in the sediments. Jemmett (1991) also reported the saltmarsh here to be contaminated with elevated concentrations of the heavy metalscadmium, copper, lead, and zinc comparison with a saltmarsh at Foryd Bay on the Menai Strait in North Wales. According to Jemmett (1991), saltmarshes on the northern bank of the estuary near Hale Head had undergone continuous, but variable rate, accretion since 1911. A decline in metal concentrations was observed, in line with reducing inputs; remobilisation of previously consolidated saltmarsh sediments was considered responsible for significant perturbations in the overall reduction trend (NRA, 1995; Harland, Taylor, & Wither, 2000).

2.4.2 Heavy metal and organic matter complex formation

One major characteristic of heavy metals is the formation of complexes with organic matter due to the binding action of soil organic matter and the speciation of the trace element. For example, monovalent cations (K^+, Na^+, etc.) are held by cations exchange through the formation of the salt with carboxyl groups (R-COO-K^+ and R-COO-Na^+) whereas multivalent cations (Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{3+}, etc.) form coordinate linkages with organic matter (Stevenson 1994). Other researches have reported positive correlation of heavy metals with soil organic matter (Stevenson 1994; Reddy & Delaune, 2008). Generally speaking, the level of significance of correlation depends on the type of extractant used, soil depth, the concentration of the heavy metal, and sampling periods. In surface and sludge amended soils, nickel may be increasingly bound to organic matter, a part of which forms easily soluble chelates (Kabata-Pendias & Mukherjee, 2007).

In the presence of fulvic and humic acids, these complexes are much more mobile and may be more important than the hydrated divalent cation in soil solution chemistry (ATSDR, 2008). Studies have reported that cadmium concentration in soil is found in the topsoil, most likely due to the combination of the sources of pollution and the higher near-surface levels of organic matter to which it readily binds (Alloway, 1995; ATSDR, 2008).

Soluble and insoluble complexes with organic matter can also be important, although cadmium forms less-stable complexes with humic and fulvic acids than those formed by copper and lead (Alloway, 1995; Kabata-Pendias & Mukherjee, 2007). Surface adsorption processes rather than precipitation appear to control the distribution of cadmium between soil solution and soil-bound forms at the concentrations relevant to most polluted soils (Alloway, 1995). There are several general chemical forms of metals in soils and these differ in their mobility and bioavailability (Gambrell & Patrick, 1991; Wuana & Okieimen, 2011). A listing of some of the common chemical forms of metals ranging from most available to least available is as follows: (a) readily available: dissolved and exchangeable forms; (b) potentially available: metal carbonates, metal oxides and metal hydroxides, metals adsorbed on or occluded with iron and manganese oxides, metals strongly adsorbed or chelated with insoluble high-molecular-weight humic materials and metals precipitated as sulphide; and (c) unavailable: metals within the crystalline lattice structure of clay and other residual minerals.

Metals dissolved in pore water are the most mobile and bioavailable. Adsorbed (exchangeable) metals are also bioavailable due to equilibrium between exchangeable and dissolved metals. Both dissolved and exchangeable metals are readily mobilised and bioavailable. At the opposite extreme are metals bound with the crystalline lattice structures of clay and other residual minerals. Metals in this form are essentially permanently immobilised and thus unavailable. Only under a long period of mineral weathering, would residual metals become mobile and bioavailable. Between these two extremes are potentially available metals. In metal-contaminated soils, excess metals become primarily associated with these potentially available forms rather than the readily available soluble and exchangeable forms (Feijtel et al., 1988). By contrast, in uncontaminated soils or sediments, only background levels of metals exist in these forms.

2.5 Cycles of soil

Cycles of soil show the various interactions that exist between soil biota, soil and atmosphere. This effect is seen in the ability of the soils to provide food and fibre for humans (Stevenson & Cole, 1999). Different cycles, like carbon, nitrogen, phosphorus, sulphur, and the micronutrient cations (boron, copper, iron, manganese, molybdenum, and zinc) are of

importance in soil as a medium of plant growth, for natural use of organic and inorganic fertilisers, for disposal of waste in soil, and prevention of soil-derived pollution of air and water (Stevenson & Cole, 1999). As plant biomass undergoes microbial transformation or decay in the soil, it releases nitrogen, phosphorus, sulphur and micronutrient cations. According to Stevenson & Cole (1999), the carbon cycle acts as a driving force for other cycles.

2.5.1 Carbon cycle

The carbon cycle results from the interaction or transformation of plant detritus and animal materials by microorganisms within the soil to release carbon dioxide (CO_2) to the atmosphere while nitrogen is made available as ammonium (NH_4^+) and nitrate (NO_3^-), nitrite (NO_2^-), phosphorus, sulphur and various micronutrients appear in inorganic forms required by higher plants (Stevenson & Cole, 1999). According to Stevenson & Cole (1999), during the cycling process of carbon, some nutrients are assimilated by microorganisms and the formed part of microbial biomass. Mineralisation occurs when carbon, nitrogen, phosphorus, and sulphur are converted to inorganic minerals while the reverse is immobilisation (Figure 2.1).

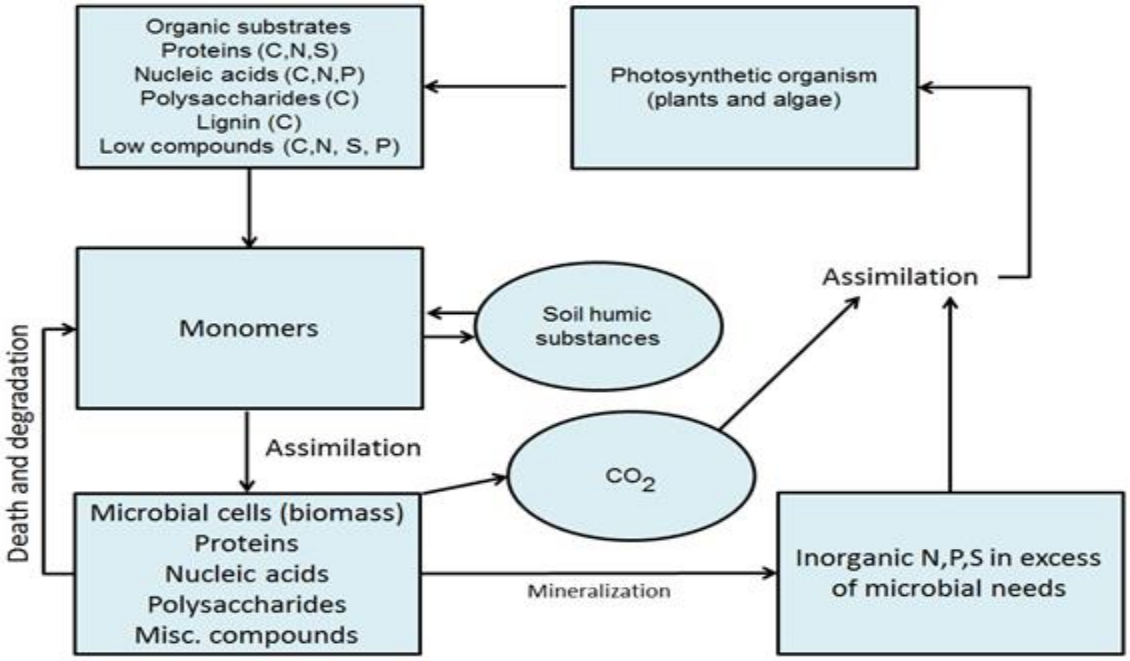


Figure 2. 1 Compartments of the global carbon cycle and interchanges between them adapted from Stevenson & Cole (1999).

The carbon cycle shows the flow of carbon starting from the photosynthetic organism (plant and algae) undergoing breakdown either by the death of the photosynthetic organism or consumption which leads to an organic substrate like proteins, nucleic acids, etc. They are further broken down to smaller units. The death and degradation of monomers through assimilation results in a further breakdown of proteins, nucleic acids, etc. through the process of mineralisation to inorganic nitrogen, phosphorus, sulphur and carbon dioxide (Figure 3). Assimilation of inorganic carbon, nitrogen, phosphorus, and sulphur by green plants revolves the cycle around. During monomer degradation, soil humic substances are formed (Stevenson & Cole, 1999). Humic substances represent the most active fraction of humus, consisting of a series of highly acidic, yellow-to-black humic acid and fulvic acid (Stevenson & Cole, 1999).

2.6 Microbial biodiversity

Significant spatial variability has been reported relating microbial biodiversity with soil carbon dynamics (Prescott, 2005; Fisk et al., 2010; Huang, Hendrix, Fahey, Bohlen, & Groffman, 2010; Olsen et al., 2011). Fisk et al. (2010) reported a positive correlation with fine root biomass, suggesting that carbon supply from roots plays a key role in the fungal channel. Increased microbial biomass and soil respiration were observed in grazed saltmarsh by Olsen et al. (2011) as a factor that influences the variation in soil carbon storage. Allison, Wallenstein, & Bradford (2010) show how microbial enzyme activities affect carbon storage in Figure 2.2. The decomposition of organic matter is the primary ecological role of heterotrophic microflora in soils (Reddy & Delaune, 2008). Microbial decomposers derive their energy and carbon for growth from detrital and soil organic matter and facilitate recycling of energy and carbon within and outside the wetland ecosystem. Reddy & Delaune (2008) shows how soil microbes exert a significant influence on ecosystem energy flow in the form of feedback, because mineralisation of organically bound nutrients is a regulator of nutrient availability for both primary production and decomposition.

During the heterotrophic breakdown of particulate organic carbon (POC) and dissolved organic carbon (DOC), a portion of labile organic compounds hydrolysed by enzymes is assimilated into microbial biomass (Reddy & Delaune, 2008). Under aerobic conditions, approximately 50% of the monomers formed can be assimilated into cell biomass, whereas the remainder is oxidised to carbon dioxide (Reddy & Delaune, 2008; Allison et al., 2010). Due

to the competition for available nutrients among microbes and macrophytes, nutrients may be held tightly within the microbial biomass (a small fraction of plant detritus and soil organic matter) because of efficient recycling of re-mineralised organic compounds. Thus, environmental perturbations such as alterations in redox conditions may result in microbial mortality, resulting in a significant remineralisation of nutrients (Reddy & Delaune, 2008).

Soil microbial communities contain the highest level of prokaryotic diversity of any environment, and metagenomic approaches involving the extraction of DNA from soil can improve our access to these communities (Delmont, Robe, Clark, Simonet, & Vogel, 2011). According to Delmont et al. (2011), most analyses of soil biodiversity and function assume that the DNA extracted represents the microbial community in the soil, but subsequent interpretations are limited by the DNA recovered from the soil. Unfortunately, extraction methods do not provide a uniform and unbiased subsample of metagenomic DNA, and therefore, accurate species distributions cannot be determined (Delmont et al., 2011). Moreover, any bias will propagate errors in estimations of overall microbial diversity and may exclude some microbial classes from study and exploitation (Delmont et al., 2011).

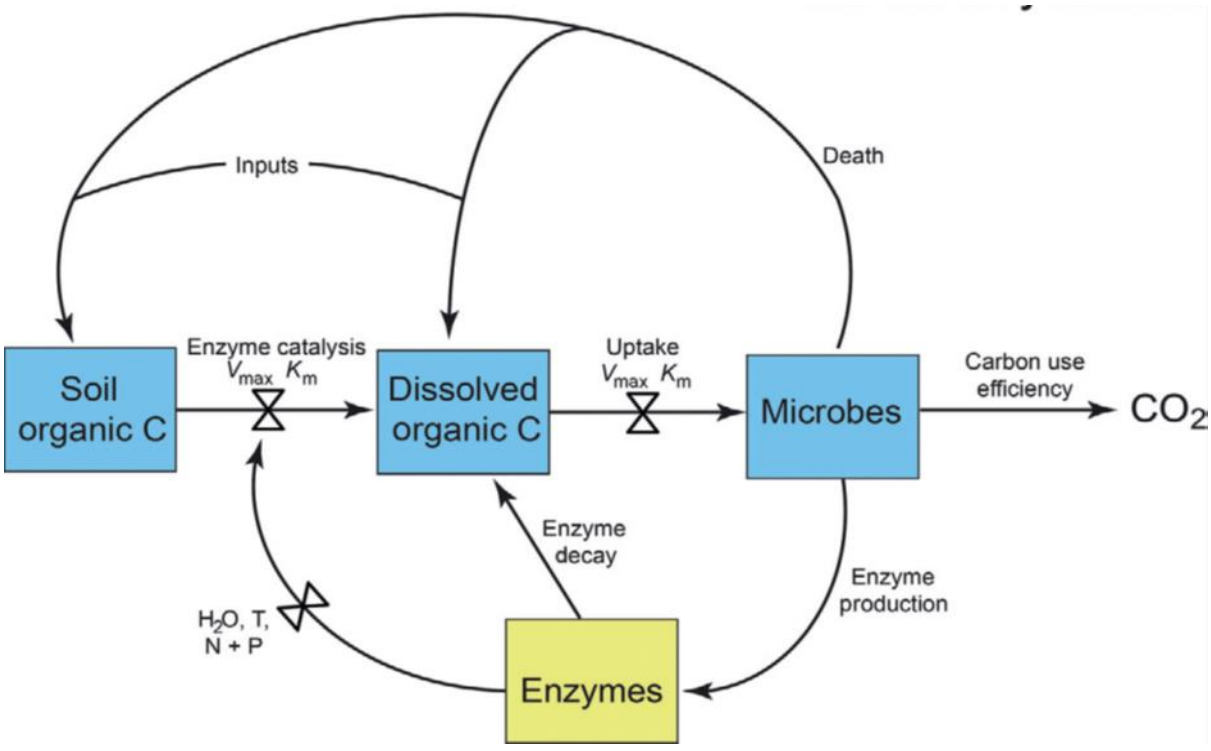


Figure 2. 2 Simplified flow diagram for microbe growth and enzyme kinetic models (adapted from Allison et al., 2010).

Increased attention has been given to DNA extraction methods for most of the microbial diversity studies conducted in complex ecosystems, such as soil (Hugenholtz, Goebel, & Pace, 1998; Martin-Laurent et al., 2001; Sagova-Mareckova et al., 2008; Edet et al., 2017). This is because there has been biased essentially due to the unculturability of many microorganisms and the lack of sensitivity of traditional microbiological methods (Martin-Laurent et al., 2001). Tsai and Olson (1991) investigated new methods to improve extraction, purification, amplification, and quantification of DNA from soils. This new molecular biology method provides more understanding of the composition, richness, and structure of microbial communities (Hill et al., 2000). The impact of three different soil DNA extraction methods on bacterial diversity was evaluated using PCR-based 16S ribosomal DNA analysis by Martin-Laurent et al. (2001). Generally speaking, the results of molecular analysis of microbial communities rely both on the extraction of DNAs representative of the indigenous bacterial community composition, and factors related to PCR, such as the choice of primers, the concentration of amplified DNA, errors in the PCR, or even the method chosen for analysis.

Nucleotides, RNA, single-stranded DNA (ssDNA), and double-stranded DNA (dsDNA) all absorb at 260 nm. Thus, they contribute to the total absorbance of the sample. Therefore, to ensure accurate results, nucleic acid samples will require purification prior to measurement (Wilfinger, Mackey & Chomczynski, 1997). The ratio of absorbance at 260 nm and 280 nm (260/280) is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as ‘pure’ for DNA; a ratio of ~2.0 is generally accepted as ‘pure’ for RNA (Maniatis, 1989). If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm (Wilfinger et al., 1997). Thus, change in sample acidity, the wavelength accuracy of the spectrophotometers and nucleotide mix in the samples affects the 260/280 ratios.

2.7 Types of Estuarine floodplains

Estuarine floodplains can be defined as places of transition between land, sea and fresh water. According to a Natural Environment Research Council (NERC) (1975) report, an estuary is a partially enclosed body of water, open to saline water from the sea and receiving fresh water from rivers. Estuaries all over the world are known for their potential to act either as a source of carbon, thereby contributing to global carbon emission, or as a carbon sink,

hence mitigating global warming mitigation. Redox potential, nitrification, immobilisation and mineralisation are all biogeochemical processes that influence soil carbon storage in wetland ecosystems (Stevenson, 1994; Stevenson and Cole, 1999; Reddy & Delaune, 2008). According to Davidson et al. (1991) estuaries are among the most fertile and productive ecosystems in the world because of continual input, trapping and recycling of sediments and nutrients; thus, supporting large numbers of animals, such as invertebrates, fish and birds.

Estuaries are a major component of Britain's coastal zone. Over 155 estuaries have been identified around the whole coastline (Davidson et al., 1991). Estuaries are grouped into nine types based on their geomorphology and topography: bar-built, barrier beach, coastal plain, complex, embayment, fjard, fjord, linear shore and ria (Davidson et al., 1991). The most extensive of these are bar-built estuaries (47 sites) where sedimentation has kept pace with rising sea levels, and coastal plain estuaries (35 sites) with wide sandy mouths. Fjards (20 sites) and fjords (6 sites) are both glacial features, fjards are shallower inlets, found mostly within the west and north Scotland. There are 15 rias (narrow drowned river valleys) in south–west England and south Wales, and 10 estuaries are of complex origin. There are 13 embayments, often with rivers discharging into them, 7 shallow linear shores, found at the outer parts of the Greater Thames estuaries and 2 barrier beach systems (the North Norfolk Coast and Lindisfarne) (Davidson et al., 1991). The 9,320 km of estuarine shoreline is almost half (48%) of the longest estimate of the British shoreline. According to Davidson et al. (1991), British estuaries are greater in number, size and diversity of form than any other estuaries in Europe. The estuaries comprise 28% of the entire estuarine area of the Atlantic and North Sea Coastal states. British estuaries freeze less often than estuaries of The Gulf Stream, bring warm water, and their tidal flats are exposed more consistently due to the large tidal ranges in Britain (Davidson et al., 1991).

2.7.1 Vegetation types within estuarine floodplains

Saltmarshes, couch-grass (*Elytrigia repens*) and the reed bed (*Phragmites australis*) are the most dominant plant species in many estuaries (Davidson et al., 1991; Saltonstall, 2002; Skelcher, 2003; Boon et al., 2015; Imentai, Thevs, Schmidt, Nurtazin, & Salmurzauli, 2015). According to Davidson et al. (1991) saltmarshes can be defined as vegetation developed in a series of characteristic zones on fine sediments on the upper shore in sheltered

parts of estuaries. Saltmarshes are found to dominate in areas where there is an abundance of sediment. Saltmarshes larger than 0.5 ha are found in over 135 estuaries. This makes up over 95% of the British saltmarsh resource and almost 14% of the total inter-tidal area of estuaries (Davidson et al., 1991). The largest saltmarshes are found in the Greater Thames estuaries of Essex and Kent, and Liverpool Bay. Saltmarshes are used extensively for livestock grazing, and provide space for scientific interest, as well as having other conservation values for different types of estuarine wildlife.

There have been many studies on the benefits and harmful effects of *Phragmites australis* grown mostly in wetlands (Cronk & Fuller, 1995; Lissner & Schierup, 1997; van der Putten, 1997; Chambers, Meyerson & Saltonstall, 1999; Windham & Lathrop, 1999; Batty, Baker, Wheeler & Curtis, 2000; Mauchamp, Blanch & Grillas, 2001). The advance of *Phragmites australis* beds into tidal wetlands of North America may have been facilitated by widespread coastal changes since European settlement, including disturbance of hydrologic cycles and nutrient regimes (Chambers et al., 1999). Ecosystem services, such as support of higher trophic levels, enhancement of water quality and sediment stabilisation are associated with any tidal wetland dominated by *Phragmites australis* (reed bed) However, *Phragmites australis* has the potential to decrease plant diversity in areas where it has spread and as such is a nuisance species in North America, due to this form of biological pollution (Cronk and Fuller, 1995). In Europe, scientific efforts are being designed to understand and reverse *Phragmites australis* decline.

According to van der Putten (1997), increased eutrophication, changed water table management, temperature, reduced genetic variation and their interactions may contribute to reed die–back. *Phragmites australis* in comparison to neighbouring estuaries grasses, were found to have nearly 10 times the live aboveground biomass during the growing season. They also had lower soil salinity at the surface, a lower water level, less pronounced microtopographic relief, and higher redox potentials (Windham & Lathrop, 1999). Lissner & Schierup's (1997) salinity study along the eastern and western coasts of Jutland, Denmark, reported that *Phragmites australis* (reed bed) have adapted to saline conditions by adjusting the level of osmotically active solutes in their leaves. Wetland plant species, including *Phragmites australis* and *Typha latifolia* (common bulrush), are used to remove potentially

toxic metals from contaminated drainage (Batty et al., 2000). According to Mauchamp et al. (2001), the *Phragmites australis* level of tolerance to submergence increases with age. However, very little is known about the *Phragmites australis* contribution to soil carbon storage within the estuarine floodplain. However, the reports on die-back and high aboveground biomass may suggest increased soil organic matter (van der Putten, 1997).

The different literature reviewed shows that estuaries are very useful ecosystems to mankind with benefits such as means of transportation, fisheries, potential to store soil organic, and global warming mitigations. On the other hand, estuaries around the world have suffered organic and inorganic contamination since the beginning of the Industrial Era. The literature review also looks at the concepts of soil carbon dynamics and soil carbon sequestration and the factors affecting them such as topography, types of season, tide inundation, heavy metal contamination, soil organic matter stabilisation with heavy metals, microbial biodiversity, environmental factors, anthropogenic factors, interactions that exist between soil biota, soil and atmosphere, and management practices.

3. Materials and Methods

3.1 Introduction to the chapter

This chapter is a general overview of the research flow charts, fieldworks, laboratory incubation and analyses carried out to actualise the desired research objectives detailed in Chapter 1. This chapter also tries to justify the reasons for the selection of the Upper Mersey Estuary, north-west England as the study area.

3.2 Selection of study area

The study site was selected to collect data that were needed to address the aim and objectives of the study. Estuarine floodplain benefits cannot be over emphasised even though the threats are enormous. According to McLusky (1987), estuaries has been very useful to mankind from the beginning of time; thus, becoming places for human settlement, markets and industrial centres (Kron, 2013). Several benefits have been listed in Chapter 1 (Section 1.1) of this thesis. Although, there are still some dis-benefits such as poor underground water quality, especially in estuaries that have links with organic and inorganic pollutants.

The selection of the study area was based on the following considerations:

- Should be located within an area with potential to store soil organic carbon
- An estuarine floodplain with a known history of environmental contamination
- An estuarine floodplain with diverse land uses/land covers
- In close proximity to the university
- Physically accessible and safe for field work

Globally estuarine floodplains are known for their potential to store a large amount of soil organic carbon (Mitra, Wassmann, & Vlek, 2005; Zedler & Kercher, 2005; Bernal & Mitsch, 2012; Mitsch et al., 2013). Also, the history of contamination of organic and inorganic pollutant of these habitats is common to others (Bia et al., 2011). However, some of the estuarine floodplains are more contaminated compared to others due to the different impact from the industrial revolution and other anthropogenic factors (Heim et al., 2004; Heim & Schwarzbauer, 2013; Bigus, Tobiszewski, & Namieśnik, 2014; Dsikowitzky & Schwarzbauer, 2014). The estuarine floodplains are also known for diverse land use/land cover types. In

terms of the proximity of the research to the University of Salford, Dee, Mersey and Ribble estuarine floodplains are the closest and, hence, possible alternative.

The Mersey estuarine floodplain was chosen because of the access through 'living laboratory' as part of the Mersey Gateway Project to collaborate with other researchers. The first site visit was undertaken during February 2015. A series of walkovers across the site was conducted and a decision was made on the feasibility of the site for research given the listed considerations. Observations from the first visit included evidence of cattle grazing and historical evidence or confirmed previous chemical industries located within the study area. Before any work took place, consent from the landowner, Mersey Gateway Crossing Board was sought and granted. They Mersey Gateway Crossing Board also provide field vehicle, protective clothes and accompanying staff during field sampling which makes the field sampling safe.

As the River Mersey is similar to other esturaties in terms of it containing stored soil organic carbon, having a history of environmental contamination and being surrounded by diverse land uses/land covers, the findings of the research reported here are transferable to other estuarine floodplains around the globe.

3.3 The Upper Mersey Estuary

3.3.1 Study site description

According to Davidson et al. (1991) and Dyer (2002), the Mersey Estuary is classified as a ria. The estuary was formed by drowning of the river valley and a rise in sea level during the last marine transgression (Davidson et al., 1991). The tidal periods within the Upper Mersey Estuary have been described in Chapter 2. According to Pye & Blott (2014), an estuary which has a wide mouth, narrows and becomes shallower towards the head is likely to be flood dominated, especially if it has a large tidal range, while an estuary which has a narrow mouth and widens and/or becomes deeper towards the head is more likely to display ebb dominance, especially if it has a relatively small tidal range. Wide-mouthed estuaries are influenced to a greater degree by wave processes than estuaries with a narrow mouth (Pye & Blott, 2014). The Upper Mersey Estuary shares the same menace to varying degrees by the

embanking, land claim, dredging, sea wall breaching and managed realignment found in UK estuaries.

The Mersey Estuary is generally divided into four main sections (NRA 1995) (see Figure 3.1): the Upper Mersey Estuary, between the tidal limit at Howley Weir, Warrington (occasionally overtopped during high tides) and Runcorn Gap; the Inner Estuary, between Runcorn Gap and Otterspool (on the northeast bank); the Narrows, between Otterspool and Egremont (on the SW bank); and the Outer Estuary, seawards of Egremont and taking in part of Liverpool Bay. The distance from Howley Weir to the opening into Liverpool Bay is approximately 47 km and in the Inner Estuary a maximum width of approximately 5 km is attained. With a mean maximum tidal range of almost 9 m, the estuary is macrotidal. In terms of the mixing of fresh and saline waters, the estuary is considered partially mixed (Dyer, 2002).

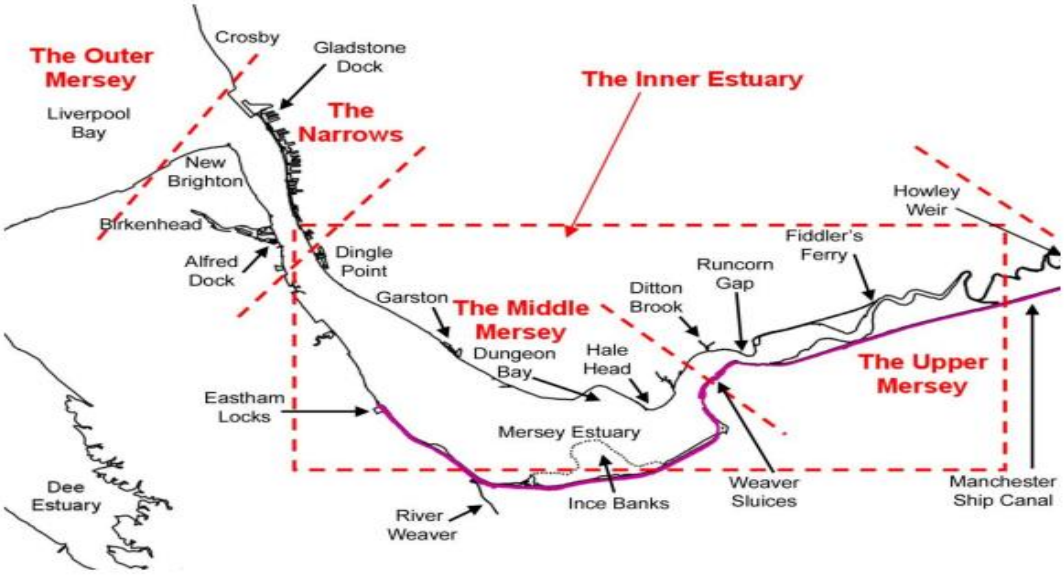


Figure 3. 1 The Mersey Estuary (adapted from Mersey Gateway Project Delivery Phase Chapter 4 page 4.9).

The Mersey River drains into Liverpool Bay in the south-east of the eastern Irish Sea. It covers an area of around 5,000 km^2, including Merseyside, Greater Manchester, Cheshire, Derbyshire and Lancashire, with the port of Liverpool lying on its north-eastern bank, near the estuary mouth. Along or adjacent to the banks of the estuary, major manufacturing

centres are found at Liverpool, Birkenhead, Bromborough, Ellesmere Port, Stanlow, Runcorn, Widnes and Warrington (Ridgway & Shimmield, 2002). The Mersey catchment has a population of over 5 million people and more than 830,000 people live within 1 km of the estuary shoreline (NRA, 1995). Further inland, the industrial centres of south-east Lancashire, including Manchester, Rochdale, Bury, Bolton, Stockport and St Helens, are drained by rivers which feed into the Mersey (NRA, 1995; Jones, 2000).

The geology of the study area indicates that the site is underlain by sandstone bedrock from the Upper Mottled Sandstone and Pebble Beds while the British Geological Survey (BGS) drift geology map shows glacial till (formerly known as boulder clay) overlying the bedrock (Griffiths, Shand & Ingram, 2003; Mersey Gateway project, 2011). The glacial till is described as comprising clay interbedded with discontinuous horizons of sand or sand and gravel (Ridgway et al., 2012). This is shown to form an extensive sheet, which varies in thickness from the north to the south side of the river. The glacial deposits are shown as being overlain by marine and estuarine alluvium on the BGS drift geology map. Alluvium is shown beneath Widnes Warth saltmarsh (Ridgway et al., 2012). The general topography of the Mersey Basin reflects its underlying geology (Ridgway et al., 2012). The Mersey valley is eroded in relatively low-lying Triassic marls and sandstones. Most of the area, except Liverpool and some parts of the Wirral, is covered by glacial till and postglacial alluvial deposits, which serve to mantle the underlying geology and produce a smooth or gently undulating topography. The Wirral peninsula has a generally smooth topography, with low hills in the north and west, reaching 65 m at Bidston Hill and 110 m at Poll Hill (Ridgway et al., 2012). On the Liverpool side of the Mersey, the land rises smoothly to about 50 m above sea level (Wedd, Smith, Simmons, & Wray, 1923).

Geomorphology can be defined as the study of the land formation, its processes, form, and sediments at the surface of the Earth (and sometimes on other planets). According to Dyer (2002), estuaries in the UK originated from glaciated valleys, drowned river valleys and drowned coastal plains. The present-day estuary geomorphology probably reflects the influence of the pre-Pleistocene bedrock geology and the Pleistocene deposits (Ridgway & Shimmield, 2002). Davidson et al. (1991) reported that coastlines are transient in geological time, and the precise physical location of estuaries and other coastal landforms depends

strictly on the sea level and it changes over time. Thus, the estuaries we have today are a result of glaciation – the process in which lands are covered by glaciers (a huge mass of ice that moves slowly over land). According to Buck (1993), the Mersey Estuary is classified as a coastal plain geomorphological type, whereas Dyer (2002) said it is a ria (a coastal inlet formed by the partial submergence of an unglaciated river valley) without spits, because of the geological constraint of the narrow mouth between rocky shores. The estuary's unusual banana-shaped profile does not conform to the typical funnel shape common to estuaries in England and Wales (e.g. Severn, Thames). Therefore, it is unlikely that its present course is a purely a result of marine incursion into incised valleys. The saltmarsh in the Upper Mersey Estuary becomes inundated when the high tide is at or above the 10-metre prediction. From the community knowledge perspective, the saltmarsh is unlikely to have been totally covered with water at 9.3 m and 8.7 m tides unless there was a mixture of strong winds and atmospheric pressure (MGET, 2017 report).

The Upper Mersey Estuary, the study area, stretches from the Runcorn Gap in Halton (British National Grid SJ 511 835) to Howley Weir in Warrington (SJ 616 876). The Upper Mersey Estuary comprises 1,655 hectares of mud flats, salt marsh, open water, and reed bed. It is a narrow, meandering channel that widens from less than 100 m near Warrington to just over 1 km near Widnes before being constricted to 250 m at the Runcorn Gap, by a north-south trending sandstone ridge. For much of this 12 km distance, the Upper Mersey Estuary is constrained to the south by the Manchester Ship Canal (MSC) and to the north by the St Helens Canal. Extensive sand and mud banks are exposed at low water. A disposal ground for material dredged from the MSC is located on both sides of the river, near the confluence with Sankey. Within the study area, there are significant areas of managed, homogeneous saltmarsh found on the northern and southern banks. Figure 3.2 shows the land cover within the study area and are include saltmarshes, rough grassland and horticulture, natural grassland, broadleaved woodland, *Phragmites australis* and brownfields– formed because of the demolition of factories associated with the late 20th century. Figure 3.3 shows the soil types across the Mersey estuarine floodplain, indicating that the study area is classified as gleysol according the World Reference Base system (2014). The land cover and soil types in the present study are marked in yellow and red colours in the Figures 3.2 and 3.3 respectively.

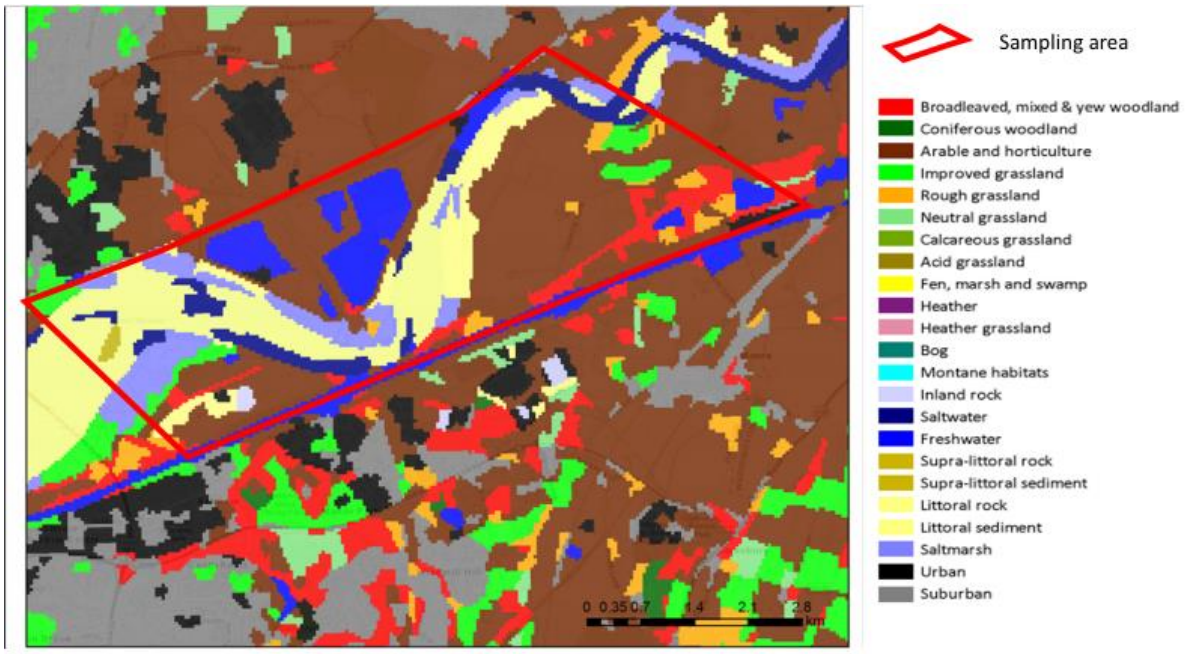


Figure 3. 2 Showing the different land use/land cover types within the study area

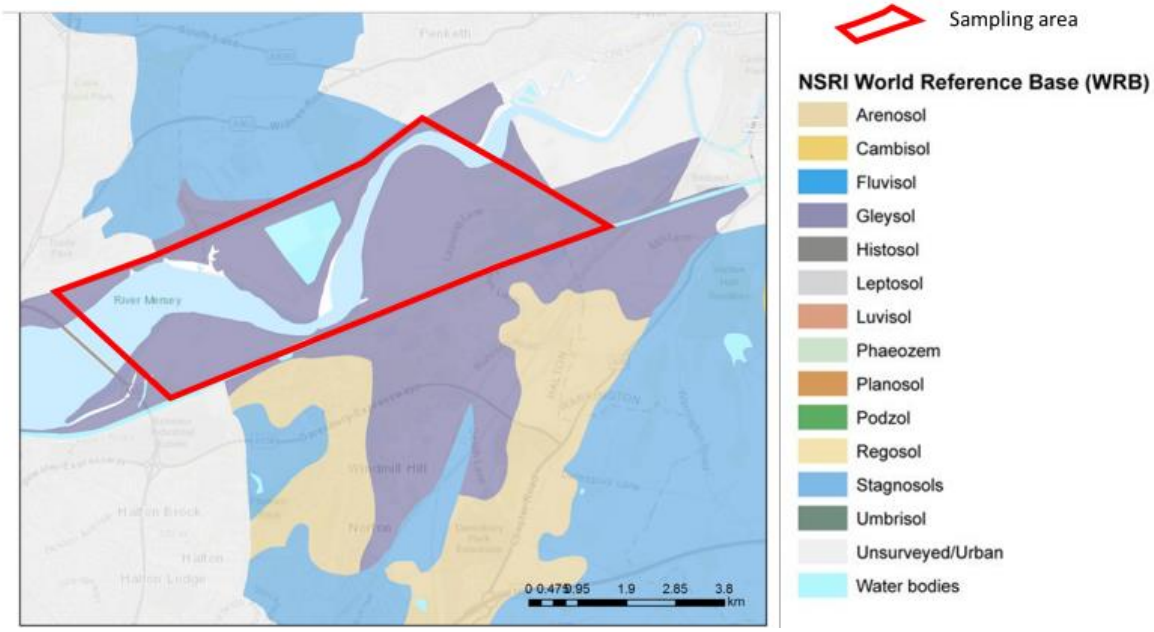


Figure 3. 3 Showing the different soil types within the study area

(Sources for Figures 3.2 and 3.3): Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, UKSO: NERC, James Hutton Institute, Cranfield University NSRI, Agri-Food and Biosciences Institute, Forest Research, Forestry Commission and Natural Resources Wales and Ordnance Survey)

Recently, there have been serious efforts to conserve wildlife in the estuary and provide areas for recreation (Mersey Gateway Project), but considerable quantities of organic and inorganic contaminants are still present in the estuarine sediments (Jones, 2000). The Mersey Gateway Environmental Trust (MGET) started saltmarsh management after the purchase of Widnes Warth saltmarsh by Halton Borough Council from a private landowner in 2011. To put the saltmarsh into a favourable condition through restoration and management and research collaboration through the 'Living Laboratory' cattle grazing was intorduced (MGET, 2011 report). This will habitat management intervention will increase both habitat and species diversity, especially breeding and wintering birds. This is because in an ungrazed condition, the saltmarsh was unable to sustain a regular breeding population of redshank, skylark, meadow pipit and reed bunting, along with an increase in wintering species such as teal, dunlin and other wading birds, as the grass sward is too high during the breeding season (MGET, 2011 report). The grazing is carried out with conservation breeds of cattle (Belted Galloway and English longhorn) that are suitable for a saltmarsh habitat rehabilitation. The grazing density used is between 1 and 1.25 cows per hectare, in line with generally accepted conservation grazing guidelines. However, the death of four cows was reported in the third year of grazing due to salt poisoning, from drinking water from the estuary, or maybe because of high lead poisioning (MGET, 2012 report). If the deaths were caused by lead, this leads to serious concerns in the case of the animal being on the table as food. However, the strategy adopted was based on decontamination; where any cattle to be sent for slaughter for human consumption should have a period of at least 16 weeks away from this grazing to allow decontamination. This suggests there are no major obstacles to the biodiversity management plan using cattle grazing as the main management tool, provided safeguards are met should the cattle end up in the food chain in the future (MGET, 2012 report).

3.3.2 Contamination history of Mersey Estuary

The Mersey Estuary is arguably one of the most contaminated (both inorganic and organic pollutants) estuarine systems in Europe (NRA, 1995; Collings, Johnson, & Leah, 1996; Jones, 2000; Fox et al., 2001; Vane et al., 2009; Hurley, Rothwell, & Woodward, 2017). The history of contaminaton dates back to the beginning of the Industrial Revolution in the early 18th century. The level of contamination was probably at its worst in the mid-1960s, when sewage effluent was combined with a complex mixture of inorganic and organic chemicals

originating from factories in the Mersey catchment and along the estuary shores (Fox et al., 2001). The origin/sources of the contamination level of the Mersey Estuary have been a topic of considerable debate over the past 40 years (Ridgway & Shimmield, 2002). Figure 3.4 shows how historical contamination of major chemicals entered the Mersey and Irwell estuarine floodplains.

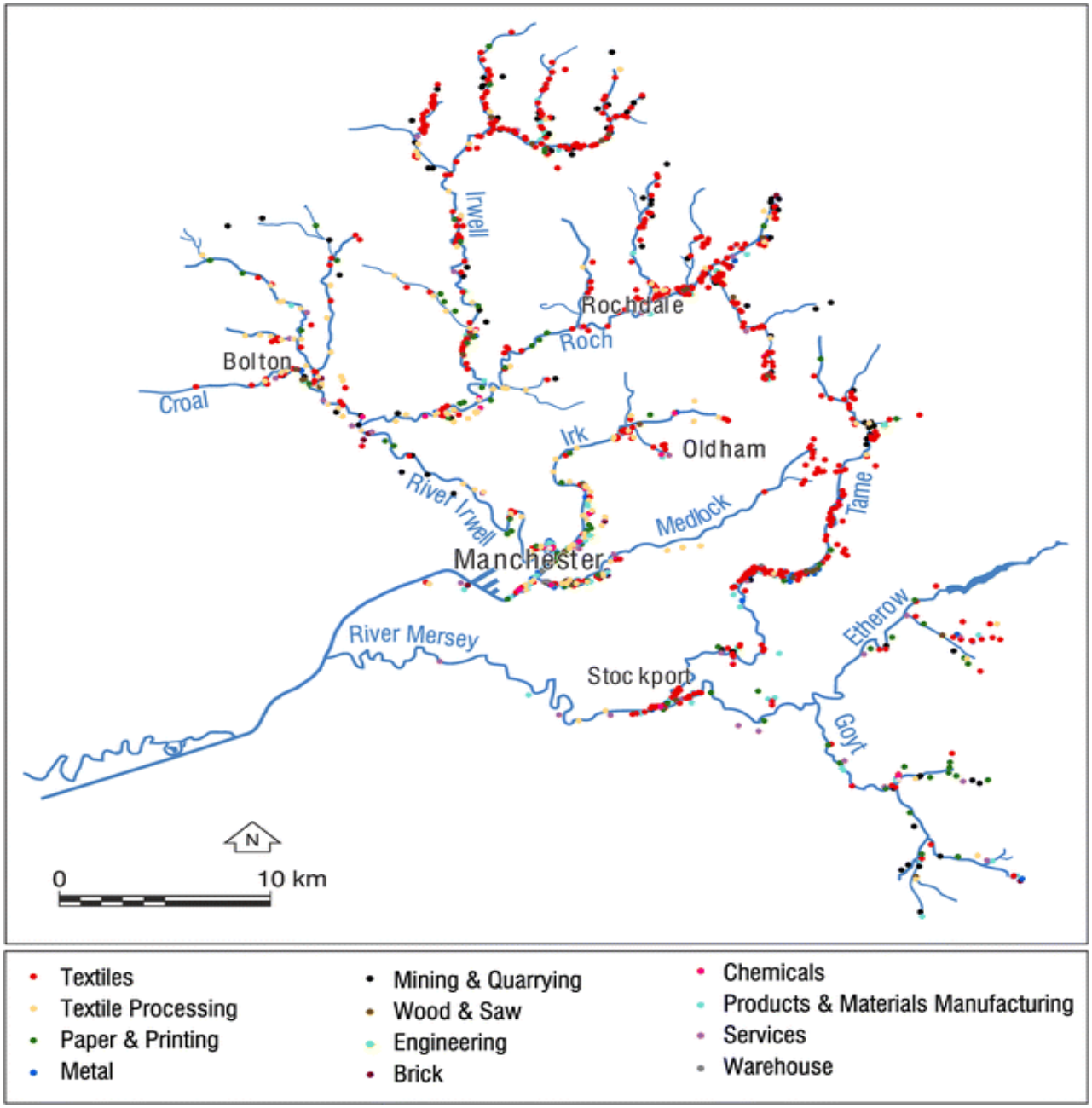


Figure 3. 4 Map of historical points as an indicator of possible contamination sources within a 1km corridor of river channels during the peak in Manchester Industrial Revolution in 1890 (adapted from Hurley, 2017)

Major changes due to the Industrial Revolution begin as far back as 1700. During the Industrial Revolution, industrial activities such as textiles, paper and printing, chemicals, metal, mining and quarrying, products and materials manufacturing from Manchester, Bolton, Rochdale, Oldham, and Stockport (see Figure 3.4). The waste products from these industries drained to river Mersey and its tributaries (Hurley et al., 2017). Many research findings show that several chemical industries, for dying, bleaching and printing, and the gas industry were linked alongside the textile industry (Ridgway & Shimmield, 2002 and Jones, 2006). There has been a series of reports of early chemical industries for the production of ammonia, chlorine, sodium carbonate, caustic soda, and the smelting of copper (Fox et al., 1999 and Fox et al., 2001). Fox et al. (1999) described Widnes Warth as the dirtiest, ugliest town in England. There was a serious situation leading to vegetation being lost, mounds of chemical waste covering the landscape, and noxious seepings polluting the rivers and brooks.

At Widnes, an alkali industry which was developed in the middle of the 19th century used the Leblanc process and chemical waste was dumped on the salt marshes beside the estuary (Fox et al., 1999). A major chemical works was also established at Weston Point, Runcorn, starting in 1885, and in 1897 the Castner Keller works, using the electrolysis process, was opened beside the Manchester Ship Canal (Bennett, Curtis, & Fairhurst, 1995). Widnes also had a copper industry based on the extraction of copper from the pyrites used at the Leblanc works, whilst Warrington was a base for iron-founding, wire-working, brewing and tanning, all contributing pollutants to the estuarine system (Porter, 1973). Although, the Environment Agency (EA) and the National Monitoring Programme (NMP) carry out monitoring work on estuarine contamination in England and Wales, the overall distribution of contaminants, thickness of contaminated sediment and relationships to the sedimentary regime and naturally varying background concentrations are inadequately documented (Ridgway & Shimmield, 2002). A systematic survey approach, such as that described here for the Mersey, provides a way to remedy these deficiencies and aid the authorities concerned in compliance with international agreements, such as the OSPAR Action Plan 1998–2003 with regard to Hazardous Substances (OSPAR, 1998) and the EU Water Framework Directive, which sets out standards for water quality and aquatic habitats (Ridgway & Shimmield, 2002).

A study of heavy metals in surface sediments of the Mersey Estuary, monitored over a period of 25 years, has shown that metal concentrations are strongly correlated with organic matter and particle size (Harland et al., 2000). This results in distribution patterns which reflect sediment characteristics and dynamics rather than the position of input sources (Harland et al., 2000). As part of their efforts to examine the contamination history of the Mersey estuary, Fox, Johnson, Jones, Leah, & Copplestone (1999) determined dichlorodiphenyltrichloroethane (DDT) and a series of heavy metals and radionuclides in 3 cm slices of 1 m cores of saltmarsh sediments from Widnes Warth and Ince Banks. The contamination histories of the two marshes appeared different with core depth alone but using the event dating technique the similar lengths of the core at the two sites were shown to represent different periods of sedimentation in which contamination histories were compatible (Fox et al., 1999). The Widnes Warth core covers a 120-year time span, whilst that from Ince Bank represents a little over the last 50 years of sedimentation (Fox et al., 1999). NRA (1995) linked contamination profiles for selected metals in the Widnes Warth to specific industrial activities. For example, copper smelting in NW England in 1870 and the use of mercury cathodes in the production of chlorine from 1897 are clearly shown in the gradual rise in concentrations of these elements after the late 19th century. Figure 3.5 shows how heavy metal from urban and industries that entered Mersey estuarine floodplains. According to Deepali & Gangwar (2010) textiles production is the major sources of chromium, copper, lead, and zinc while Calace et al. (2005) reported that paper manufacturing is the major sources of arsenic, chromium, copper, lead, and zinc.

Industry/source	Arsenic	Chromium	Copper	Lead	Zinc	References
Textiles		X	X	X	X	Deepali and Gangwar (2010)
Coal mining	X	X	X		X	Querol, Fernández-Turiel, and López-Soler (1995); West, Stewart, Duxbury, and Johnston (1999)
Dyeing		X	X	X	X	Ademoroti, Ukponmwan, and Omode (1992); West et al. (1999)
Bleaching					X	Ademoroti et al. (1992)
Tanning		X		X	X	Möller, Müller, Abdullah, Abdelgawad, and Utermann (2005); West et al. (1999)
Printing		X	X		X	West et al. (1999); Alloway and Ayres (1997)
Paper	X	X	X	X	X	Calace et al. (2005); West et al. (1999)
Brick			X	X		West et al. (1999)
Wood preservatives	X	X	X		X	West et al. (1999)
Engineering works			X	X	X	West et al. (1999)
Road deposited sediment (RDS)			X	X	X	West et al. (1999)
Pesticide use	X		X	X	X	West et al. (1999)
Sewage		X	X	X	X	West et al. (1999)

Figure 3. 5 Sources of metaloid and metals from urban and industrial sources (adapted from Hurley, 2017) X= Heavy metals associated with the different sources of contamination.

An environmental impact assessment (EIA) carried out by The Mersey Gateway Project (2011) during its delivery phase, shows that concentrations of heavy metals (arsenic, lead, chromium, copper, and zinc) along with hydrocarbons exceeds the water quality standards in

the study area. Concentrations of polycyclic aromatic hydrocarbons (PAHs) including naphthalene that exceeded the water quality standards were also obtained from the ground on the Wigg Island Landfill, and alluvium on the saltmarshes (Mersey Gateway Project, 2011). Therefore, further investigation within the study area will be important in order to examine the recent concentration of heavy metal and how the relate to the present research objectives detailed in Chapter 1 (Section 1.3).

3.4 Overview of data collection and analysis

The general overview of the data collection and laboratory analysis is shown in the research flow charts (Figures 3.6 and 3.7). Full details of the different methods and laboratory procedures are presented in different experimental chapters (Chapters 4, 5, 6, and 7). The research flow charts were in three phases. Phase one was to prove the research concept and is reported on in Chapter 4 where the sampling locations are identified and possible sources of variability such topographic position, seasonality are reported, and the kind of samples to be collected and the different laboratory analyses conducted anre described. Here, nine sampling locations were selected covering 1,653 hectares of lands across the lowland and the upland of the estuarine floodplain. Soil and plant samples were collected and analysed for physical and chemical properties (see Figure 3.6). Phase two of the research, presented in Chapter 5, was to expand on the research and reduced the sources of variability due to topographic position. Here, more seasonal monitoring was carried out compared to phase one: phase three of the study was more detailed in terms of seasonal monitoring, soil and plant samples collected, and laboratory analysis conducted. The methods for phase three are detailed in chapters 5, 6, and 7 of this thesis.

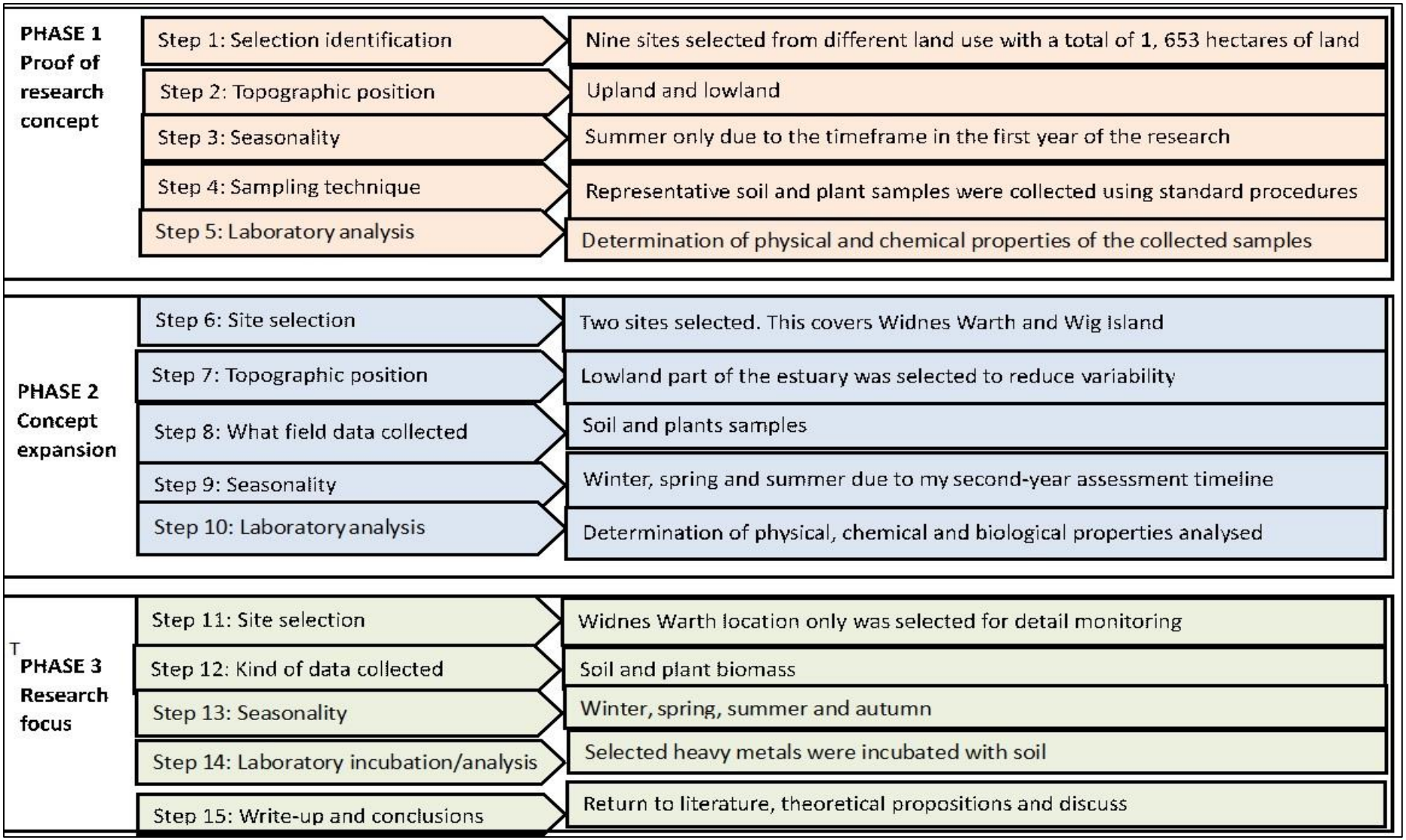


Figure 3. 6 Research flow chart for different phases.

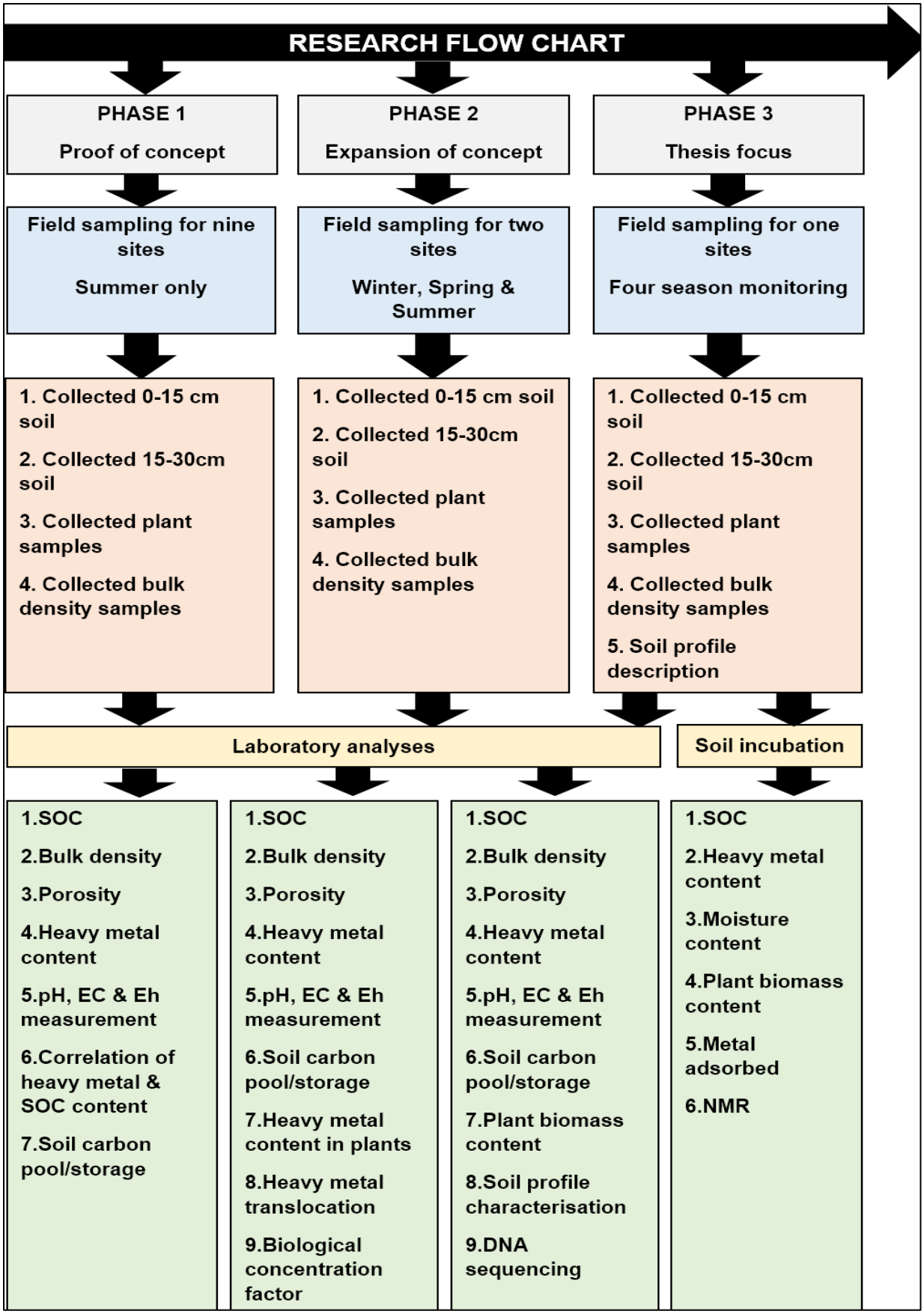


Figure 3. 7 Field and laboratory flow chart showing different sampling depths and soil parameters analysed following different research phases

3.5 Laboratory experimental design

Soils from the study area were incubated in the laboratory with selected heavy metals (arsenic, chromium, copper, lead, zinc, and control). The reasons for the selection of these metals have been outlined in Section 3.3.2. Arsenic, cadmium, chromium, copper, lead, and zinc are toxic heavy metals and have effects on nitrification and denitrification activities in soil (Babich & Stotzky, 1985). The incubation was important to be able to constrain things to ensure that the level of heavy metal contamination is controlled. This will help to address one of the research objectives: To evaluate the effects of heavy metal contamination on soil organic carbon status. Full details of the procedures are presented in Chapter 6.

The period of incubation was 200 days at about 20 °C. Data collection was carried out at three intervals (60, 120, and 200 days) and data collected are shown in Figures 3.6. Incubation periods of 130 days at 20 °C to monitor soil respiration rates were reported by Rawlins, Bull, Ineson & Evershed (2007). De Nobili, Contin, Mahieu, Randall & Brookes (2008) reported 215-day soil laboratory incubation at 25 °C for the assessment of chemical and biochemical stabilisation of organic carbon in soils. The choice for incubation periods and temperature depends on the research objectives.

Laboratory procedures and statistical tests are detailed in different experimental chapters. Physical, chemical and biological properties were carried out using different laboratory protocols. The laboratory analysis was carried out at every phase and season of the research (see Figures 3.6 and 3.7) using the University of Salford laboratory in which different soil parameters were analysed. However, some analysis was sent to external laboratories to compare with the results obtained at Salford University. The DNA sequencing was done at Macrogen laboratory, South Korea. All statistical tests were completed in triplicate and analysed using one-way analysis of variance (ANOVA), Pearson's correlation, multivariate analysis, and Duncan's multiple range test (DMRT). Full details of the laboratory analysis and statistical tests are discussed in Chapters 4, 5, 6, and 7. Laboratory safety measures stated on the risk assessment submitted to the University of Salford were strictly adhered to.

4. Heavy Metal Contamination Status in Soil-plant System in the Upper Mersey Estuarine Floodplain

4.1 Introduction to the chapter

Despite the series of debates and background information (fully documented in Chapters 2 and 3) on the contamination level of heavy metal pollution within the Mersey Estuary, it was identified that there is a strong need to investigate the most recent heavy metal contamination status, especially in the soil-plant system. This is because estuarine floodplains all over the world are known for their rapid changes in geomorphological properties particularly those that are characterised by deposition of materials from sea level rise (Davidson et al., 1991 and Ridgway et al., 2012). According to Gwynne (2004), short-term spatial differences exist in heavy metal sorption and mobility in sediments. This may be due to changes in the physical environment with regard to factors like water level, tidal inundations and the sedimentation rates (Gwynne, 2004). According to Williams, Bubb & Lester (1994), saltmarshes have the potential to act as very efficient sinks for metal contaminants although metal concentrations in halophytes do not generally reflect environmental contamination levels. Bryan, Langston & Hummerstone (1980) and Bryan & Langston (1992) reported higher concentrations of mercury and manganese and suggested that Mersey bird kill (in 1979, 1980, 1981) was attributable to alkyl-lead pollution from industry.

The objective of the chapter is linked to the phase one of the present research (see Section 3.4), to identify potential sites with elevated concentration of heavy metals to conduct detailed monitoring in line with the overall aim and objectives of the study. This will be necessary to see if there are any effects of heavy metal contamination on soil organic carbon, heavy metal concentration, and transfer and accumulation of heavy metals from soil to roots and shoots.

4.2 Material and methods

This section will be looking at field sampling, sample preparation, and laboratory analyses carried out to achieve the aim and objectives. An overview of the methods is

presented in this sub-section. Different sampling depths have been reported by several scientists. Kalbitz & Wennrich (1998) reported 25 cm depth sampling in their study on mobilisation of heavy metals and arsenic in polluted wetland soils. Imperato et al. (2003) used 30 cm depth sampling to assess the spatial distribution of heavy metals in urban soils. The sampling depth adopted was within 0–15 cm and 15–30 cm to compare the distribution of heavy metals at different depths. Sequential extraction procedures have been reported by several authors (Tessier, Campbell & Bisson, 1979; Davidson et al., 1998; Ptistišek, Milačič, & Veber, 2001; Gwynne, 2004) to measure heavy metal concentration. Tessier et al. (1979) used five stages or fractions to partition desired trace metals while Davidson et al. (1998) and Ptistišek et al. (2001) used the three-stage procedure recommended by Community Bureau of Reference (Van der Eijk, 1977). The purpose of the different steps is to extract the trace metals found to be existing in the soil solution, carbonates, exchangeable metals, iron/manganese oxyhydroxides, organic matter and sulphides, and residual form. This will help to determine heavy metal bioavailability and mobilisation. Alvarez, Mochón, Sánchez & Rodríguez (2000) and Sneddon, Hardaway, Bobbadi & Reddy (2006) used acid digestion involving the use of oxidising agents (hydrogen peroxide) and mineral acids to affect the dissolution of a sample. Hydrofluoric acid (HF) is for dissolving silicates. The heavy metal analysis in the present study was carried out using heavy metal fractionation involving four different procedures to identify different fractions of heavy metals:

- Fraction one (F1) is H_2O extractable - identifying the soluble fractions.
- Fraction two (F2) is 1 M HNO_3 extractable - identifying carbonates, iron/manganese oxides and oxidizable fractions.
- Fraction three (F3) is 1 M NH_4Cl extractable - identifying soluble plus exchangeable fractions.
- Fraction four (F4) is HF- HNO_3-H_2O_2 microwave digestion - identifying residual fractions.

Microwave digestion in this research was measured using a little modification of Sandroni, Smith & Donovan (2003) procedures. One molar (1 M) nitric acid (HNO_3) extractable and ammonium chloride (NH_4Cl) extractable were measured according to Mukwaturi & Lin (2015) and Nworie, Qin & Lin (2017) procedures. The water extractable fractions were measured according to Nworie et al. (2017) procedures. Metal recovery was calculated using

the equation: % Recovery = (metal extracted by different fractions/metal extracted by total fraction) * 100. Other methods used for the determination of the heavy metal concentration included an X-ray Fluorescence analyser (XRF). This method determines the total concentration of heavy metal without looking at any form or fraction of heavy metal.

4.2.1 Field sampling

Fifty-four composite soil samples were collected at different depths in the ranges 0–15 cm and 15–30 cm (three replicates for the nine locations (shown in Figure 4.1) respectively) as detailed in phase one of the research (see Section 3.4). Twenty-seven plant samples collected were roots and shoots at each soil sampling point (three replicates for the nine locations) (see phase one in Figures 3.2 and 3.3). Nine sampling locations were selected from a recently designed land cover map for the Mersey Gateway Crossing Board 2015. Within the locations, different land use/land cover types (grazing saltmarsh, non-grazing saltmarsh, reed bed, broad–leaved woodland, natural grassland and rough grassland) were studied. The selection adopted was based on a stratified randomised design from a recently designed land cover map for the Mersey Gateway Crossing Board 2015, considering the considerations listed in the second paragraph in Section 3.2. The soil and plant samples were collected during summer 2015 sampling period (2nd July 2015, 8th July 2015, and 23rd July 2015). At each sampling time field data were collected for analyses such as:

- Soil organic carbon
- heavy metal concentration
- Soil pH, redox potential, electrical conductivity
- soil bulk density etc.

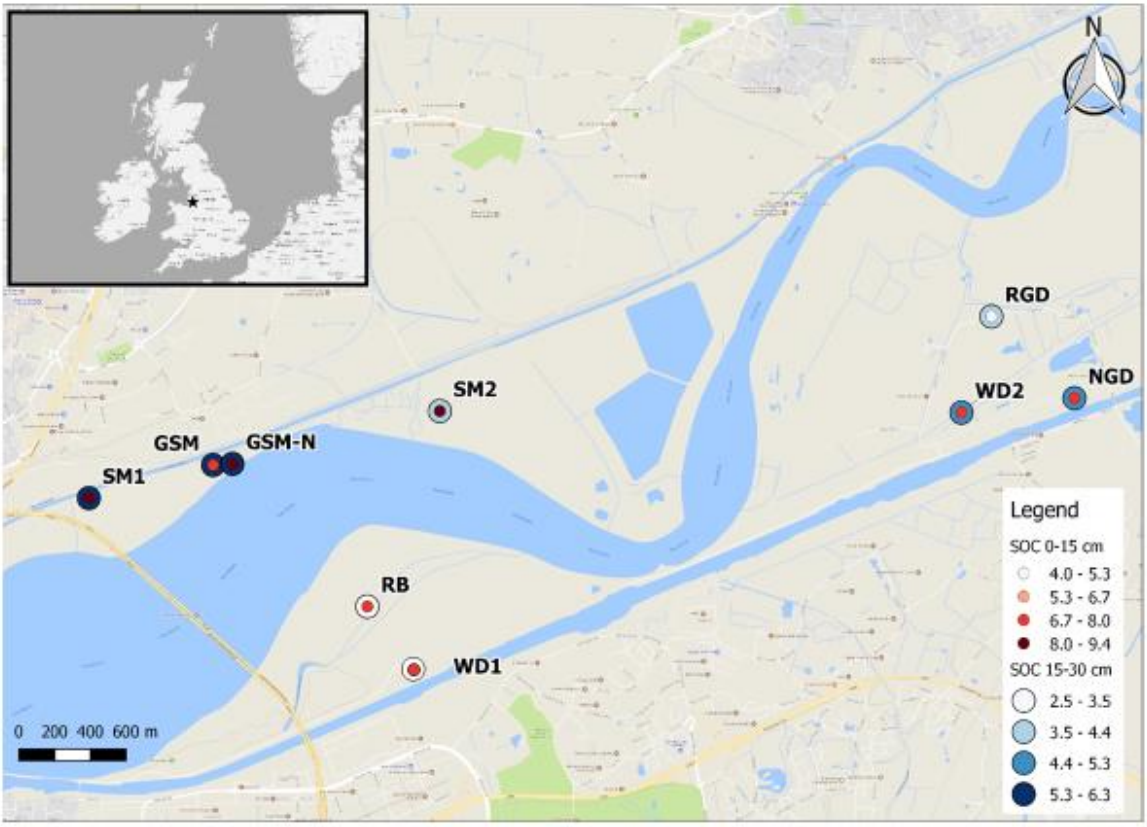


Figure 4. 1 Sampling Location Map showing mean values of % SOC distribution at 0-15 cm and 15-30 cm depths (SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD = Rough Grassland, % SOC= Percentage Soil Organic Carbon).

4.2.2 Sample preparation

Samples were manually and mechanically prepared. The soil and plants samples were sorted out from the field sampling bags into the paper bags, labelled and air dried on plastic tray and oven dried using paper bags. The samples were dried after a constant weight was obtained using a weighing balance. Soil samples were crushed manually using a mortar, sieved mechanically using a mechanical shaker and were passed through 63 µm and 2 mm sieves for laboratory analysis. Plants samples were oven dried at a maximum temperature of 70 °C and pulverised using an electrical blender. The samples were stored for laboratory analysis.

4.2.3 Laboratory analysis

4.2.3.1 Soil pH, redox potential (Eh), and electrical conductivity (EC)

Meters were used to measure soil pH, redox potential (Eh) and electrical conductivity (EC). pH and Eh were measured using an HI-2020 edge meter and EC was measured with a Mettler Toledo EC meter. The procedure involves the weighing of 5 g of 2 mm sieved soil into 150 ml bottles. Twenty-five millilitres of deionised water were added and shaken with a mechanical stirrer for 5 minutes before inserting the pH, Eh and EC probes.

4.2.3.2 Soil organic matter determination

Soil organic matter content was determined using the loss on ignition and Walkley-Black methods. The loss on ignition method was carried out as follows: crucibles were selected, washed and dried in an oven at 105 °C for 24 hours. The crucibles were placed in a desiccator to avoid absorption of atmospheric pressure. The empty weight was taken before placing back in the desiccator. Five grams of 2 mm sieved samples were measured into the crucibles and placed in the oven at 105 °C for another 24 hours to completely remove the water moisture from the soil. The samples were then transferred to the furnace at a temperature of 550 °C for 4 hours. The weights of the samples were taken and recorded.

4.2.3.3 Heavy metal determination by H_2O extraction

Five grams of each soil sample were weighed into 125 ml plastic bottles and 25 ml of deionised water were added and shaken for 1 hour using an electrical shaker. Fifteen millilitres of the supernatant solution were transferred into a 15 ml centrifuge tube and centrifuged for 5 minutes. The samples were stored at 4 °C in the fridge prior to inductively coupled plasma optical emission spectrometry (ICP-OES) measurement.

4.2.3.4 Heavy metal determination by 1 M HNO_3 extraction

Sixty-three millilitres of concentrated HNO_3 was diluted into 1 l of water; or 94.5 ml in 1.5 L water, and the solution was shaken for 1 minute. Twenty-five millilitres of the solution were added to 5 g of soil in 125 ml plastic bottles and was shaken for 1 hour using an electrical shaker. Fifteen millilitres of the supernatant solution were transferred into a 15 ml centrifuge tube and centrifuged for 5 minutes, filtered using Whatman paper (grade 93, pore size 125

mm) and then transferred into a clean centrifuge tube. The samples were stored at 4 °C in the fridge prior to ICP-OES measurement. Four times dilution (2.5 ml stock + 7.5 mL ICP deionised water) was carried out before introduction to the ICP machine.

4.2.3.5 Heavy metal determination by 1 M NH_4Cl extraction

About 54 g (53.491 g) of NH_4Cl was dissolved into 1 l of ICP grade water and the solution was shaken for 1 minute. Twenty-five millilitres of the solution were added to 5 g of soil in 125 mL plastic bottles and was shaken for 1 hour using an electrical shaker. Fifteen millilitres of the supernatant solution were transferred into a 15 ml centrifuge tube and centrifuged for 5 minutes, filtered using Whatman paper (grade 93, pore size 125 mm) and then transferred into a clean centrifuge tube. The samples were stored at 4 °C in the fridge prior to ICP-OES measurement. Four times dilution (2.5 ml stock + 7.5 ml ICP deionised water) was carried out before introduction to the ICP-OES machine.

4.2.3.6 Metals and metalloids in soils using microwave digestion

Six millilitres of concentrated HNO_3, 2 ml HF and 2 ml H_2O_2 were added to about 0.3 g of dry soil that passed through a 63µm sieve in microwave digestion tubes. Digestion lasted for 1 hour at a maximum temperature of 180 °C. The digest was filtered with Whatman filter paper (grade 93, pore size 125 mm) and diluted with deionised water to 25 ml and stored at 4 °C in the fridge prior to ICP-OES measurement. Four times dilution (2.5 ml stock + 7.5 ml ICP deionised water) was carried out before introduction to the ICP machine.

4.2.3.7 Heavy metals in plant tissues using microwave digestion

Microwave digestion by concentrated HNO_3 and H_2O_2, followed by ICP-OES measurement was carried out. Half a gram of pulverised plant samples was weighed into digestion tubes and 9 mL of NHO_3 and 1 ml of H_2O_2 were added, this was then digested in a microwave digester for about 1 hour at a maximum temperature of 180 °C. The digest was filtered with Whatman filter paper (grade 93, pore size 125 mm) and diluted with deionised water to 25 ml and stored prior to ICP-OES measurement. Four times dilution (2.5 ml stock + 7.5 mL ICP deionised water) was carried out before introduction to the ICP machine.

4.2.3.8 XRF method

Air-dried soil was prepared and passed through a 63 µm stainless steel sieve. Five grams of each subsample was weighed and transferred to the XRF sampling cup and covered with a thin cling film. The XRF was calibrated with standard certified material to ensure results accuracy. The samples were analysed in duplicate at 240 s (4 min).

4.3 Statistical analysis/metal uptake calculation

Statistical analyses were carried out using IBM SPSS 24.0 version to test for significant differences using multivariate analysis, one-way ANOVA and Pearson's correlation coefficient to test the interaction between the data. A Post hoc multiple comparison for observed means from the IBM SPSS 24.0 was carried out using Duncan Multiple Range Test to further separate the mean to test for significant differences using lower case letters (a, b, c, d etc.), were the letters indicates different degree of significance (at $P < 0.05$). For example, letter a is more significant compared to other letters. Heavy metals uptake from soil to roots and shoots was calculated using the following equations according to Malik, Husain & Nazir (2010);

- BCF= $[\text{Heavy metal}]_{root}$/ $[\text{Heavy metal}]_{soil}$.
- TF= $[\text{Heavy metal}]_{shoot}$/ $[\text{Heavy metal}]_{root}$.
- BAC= $[\text{Heavy metal}]_{shoot}$/ $[\text{Heavy metal}]_{soil}$.

Where, BAC= Bioaccumulation Coefficient; TF= Translocation Factor; and BCF= Biological Concentration Factor.

4.4 Results

4.4.1 Soil pH, redox potential (Eh) and electrical conductivity (EC)

The soil pH of the study area indicated that variation exists across sampling locations (Table 1). Locations GSM, GSM-N, SM2 and RB were neutral to slightly alkaline ranging from (pH 6.6 to 8.1) while WD2-upland of the estuary was slightly acidic (pH 5.8 to 6.3). Based on the Duncan Multiple Range Test at 0.05 level of confidence, GSM and GSM-N were significantly different compared to WD2. The Eh values across the sampling locations were negative except for the upland location. Based on the Duncan Multiple Range Test at 0.05 level of confidence, GSM and GSM-N were significantly different compared to SM1, WD2, NGD and RB locations. This may reflect an increase in the amount of soil organic matter and

the anaerobic condition or hydrologic fluctuation of the study site. The EC mean and standard error values indicated that locations SM1, GSM and GSM-N (lowland samples) were highest compared to locations WD1, WD2 and NGD (upland samples), see Table 4.1. Based on the Duncan Multiple Range Test at 0.05 level of confidence, there were significance different in GSM, GSM-N and SM1 compared to other locations. This may reflect the level of salinity within the sampling locations. Pearson's correlation coefficient (Table 4.2) indicated that EC has both positive and negative correlation with heavy metal concentration at different sampling locations. SM1 and SM2 were positively correlated with arsenic, chromium, copper, iron, manganese, lead, and zinc concentration compared to other sampling locations. NGD, RGD, WD1 and WD2 show a general significant negative correlation with arsenic, chromium, copper, iron, manganese, lead, and zinc concentration compared to other sampling locations. This may be due to the influence of tidal inundation experienced within the lowland sampling locations.

Table 4.1 Mean and SE of selected chemical properties at 0–15 cm and 15–30 cm depth

Sites	0–15 cm			15–30 cm		
	pH	Eh (mV)	EC (mS/cm)	pH	Eh (mV)	EC (mS/cm)
GSM	7.6±0.1a	-37.9±4.0b	5.6±1.2a	7.5±0.2ab	-29.1±12.2a	3.1±0.5ab
GSM-N	8.0±0.3a	-63.0±15.4b	3.4±1.2b	7.8±0.3ab	-51.4±16.0a	2.6±1.0b
NGD	7.1±0.9ab	-10.1±56.8ab	0.1±0.0c	7.5±0.8ab	-27.3±55.2a	0.1±0.0d
RB	7.2±0.6ab	-13.7±35.9ab	0.9±0.4c	7.8±0.3ab	-46.0±17.4a	0.8±0.3cd
RGD	6.9±0.1ab	8.9±7.5ab	0.1±0.0c	7.1±0.1ab	0.0±5.5a	0.1±0.0d
SM1	6.6±0.6ab	12.7±29.4ab	6.1±1.2a	7.1±0.3ab	-7.3±17.2a	4.1±0.7a
SM2	8.1±0.1a	-69.9±5.3b	2.0±0.5bc	8.0±0.1a	-64.7±6.8a	2.0±0.4bc
WD1	7.7±0.3a	-42.0±18.7b	0.2±0.1c	7.8±0.3ab	-57.2±17.6a	0.2±0.0d
WD2	5.8±0.74b	74.4±43.9a	0.2±0.1c	6.3±1.0b	42.1±61.3a	0.1±0.0d

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence

Table 4.2 Pearson's correlation coefficient for selected heavy metal concentration (mg/kg) correlated against EC (mS/cm) across the sampling sites

Sites	Arsenic	Chromium	Copper	Iron	Manganese	Lead	Zinc
				(mg/kg)			
GSM	0.969	0.940	-0.918	0.951	0.933	0.512	0.980
GSM-N	1.000**	-1.000**	1.000**	1.000**	1.000**	1.000**	1.000**
NGD	-0.435	0.482	0.702	-0.482	0.978	0.955	0.537
RB	0.561	1.000**	0.389	0.920	.998*	0.690	0.996*
RGD	-0.909	-0.941	-0.508	-.996*	-0.92	-0.807	-0.214
SM1	-0.538	-0.184	-0.662	-.998*	-.999*	-0.401	0.371
SM2	0.638	0.543	-0.102	0.498	-0.799	0.022	-0.958
WD1	-0.007	-0.212	-0.032	-0.456	0.109	0.242	-0.090
WD2	-0.597	0.505	0.908	-0.592	0.185	1.000**	0.999*

* Correlation is significant at the 0.05 level (1-tailed). ** Correlation is significant at the 0.01 level (1-tailed). SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland.

4.4.2 Bulk density

There was a significant difference across the sampling locations in soil bulk density. GSM had the highest values (1.0776±0.07 g/cm^3), see Figure 4.2. This reflects the impact of cattle grazing on soil compaction. Grazing could also increase the input of plant biomass into the soil, which is likely to affect the accumulation of soil organic matter. The potential use of chemical fertilisers in the grazing land can also have impacts on the soil processes.

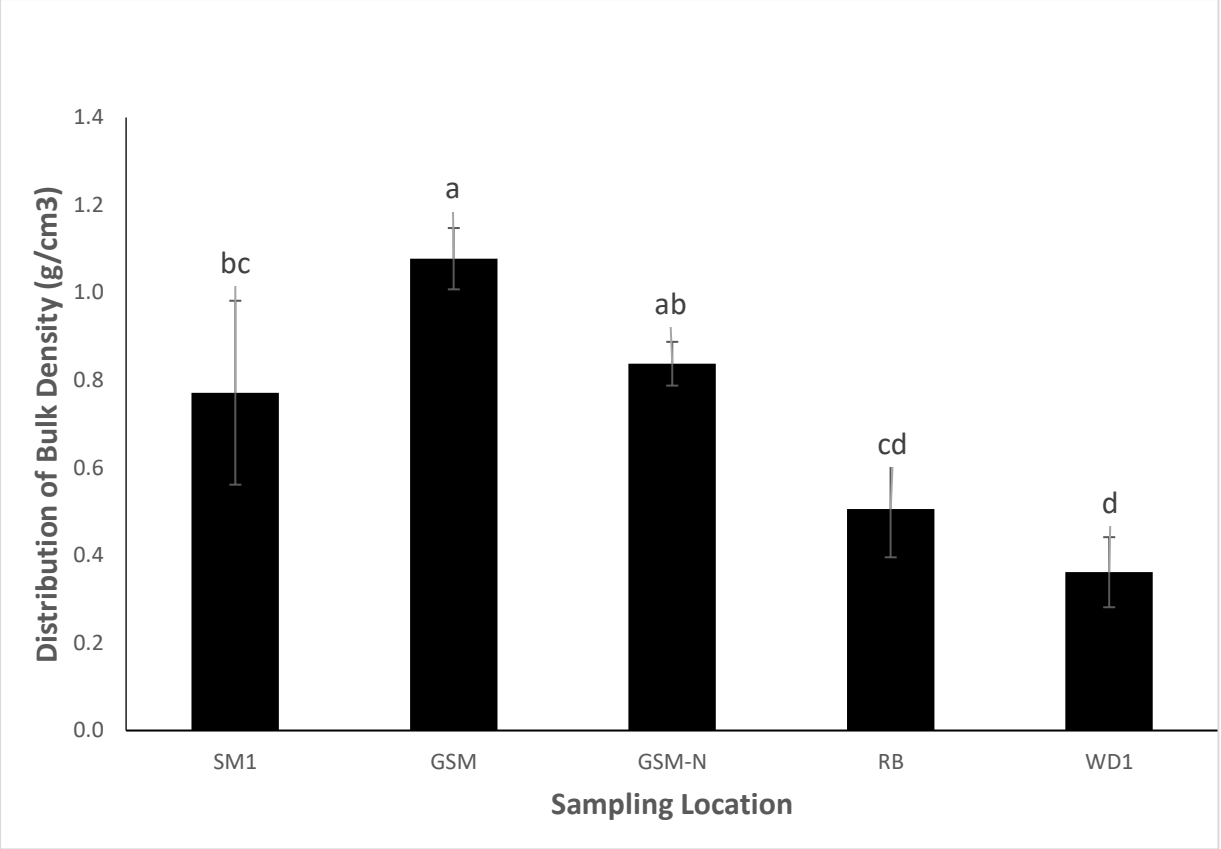


Figure 4. 2 Bulk density (g/cm^3) of selected sampling locations (SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, ab, bc, cd, d= Duncan test at 0.05 level of confidence)

4.4.3 Soil organic carbon (SOC) content

The content of soil organic carbon is shown in Figure 4.3 and its relationship with heavy metal concentration is presented in Table 4.3 using Pearson's correlation coefficient. Soil organic carbon content varied across the sampling location. Soil organic content ranged from 4.0 to 9.4%. SM, GSM and GSM-N have the highest values (Figure 4.2). This may be attributed to waterlogging or poorly drained conditions, which create an anaerobic environment resulting in the accumulation of organic matter in the soils. Results indicated that arsenic, chromium, copper, iron, manganese, and zinc were positively correlated with percentage SOC in GSM and GSM-N compared to other sampling location. NGD, RB, RGD, SM1, SM2, WD1 and WD2 shows negative correlation with percentage SOC compared to other sampling locations (see Table 4.3). This may be an indication that sampling location GSM and GSM-N SOC content is more highly influenced by the concentration of heavy metals compared to other sampling locations. Figure 4.4 shows a relationship between grazing

saltmarsh and the heavy metal contamination within the study area. The relationship indicates that soil organic carbon increases with increasing level of heavy metal contamination (Figure 4.4). Within this land use/land cover, the soil characteristics except for the concentrations of heavy metals and plant biomass were relatively uniform (see Tables 4.1 and 5.4 and Figures 4.2, 4.3, 5.4, 5.5, 5.6, 5.7, 5.9, and 5.11). This gives an opportunity to examine the effects of heavy metal contamination on the soil carbon storage under field conditions.

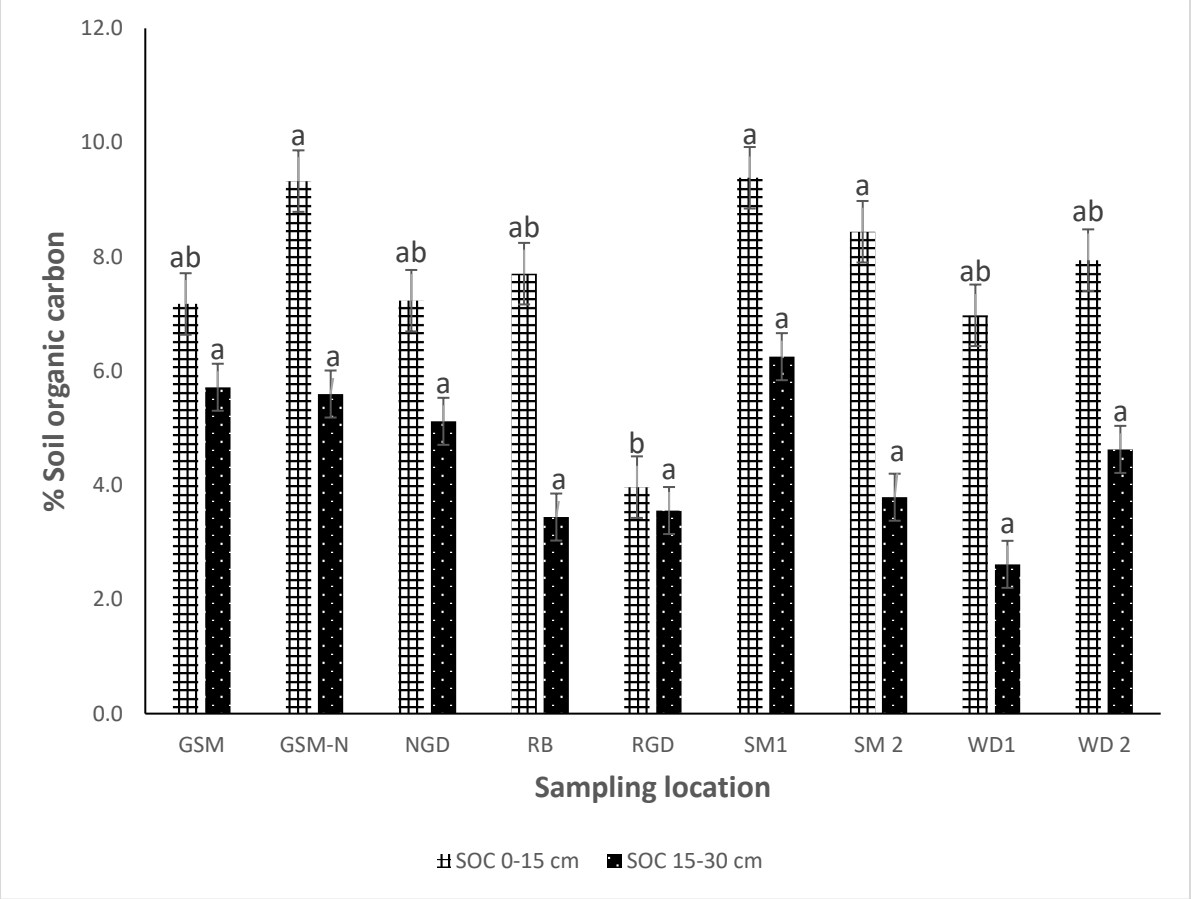


Figure 4. 3 Percentage soil organic carbon distribution under different land uses/land covers (SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, ab= Duncan test at 0.05 level of confidence)

Table 4.3 Pearson's correlation coefficient for selected heavy metal concentrations (mg/kg) correlated against SOC (%) across the sampling sites

Sites	Arsenic	Chromium	Copper	Iron	Manganese	Lead	Zinc
				(mg/kg)			
GSM	0.994*	1.000**	-0.716	0.999*	1.000**	0.783	0.987
GSM-N	1.000**	-1.000**	1.000**	1.000**	1.000**	1.000**	1.000**
NGD	0.686	0.991*	0.917	0.644	0.545	0.621	0.980
RB	-0.941	-0.808	-0.857	-0.974	-0.841	-0.984	-0.856
RGD	-0.980	-0.961	-0.916	-0.858	-0.975	-1.000**	-0.746
SM1	-0.646	-0.313	-0.756	-0.980	-0.983	-0.52	0.243
SM2	-0.654	-0.738	-0.998*	-0.772	-0.726	-0.982	-0.443
WD1	-0.543	-0.36	-0.522	-0.108	-0.637	-0.735	-0.471
WD2	-0.859	0.91	-0.329	0.744	0.996*	0.084	0.140

* Correlation is significant at the 0.05 level (1-tailed). ** Correlation is significant at the 0.01 level (1-tailed).

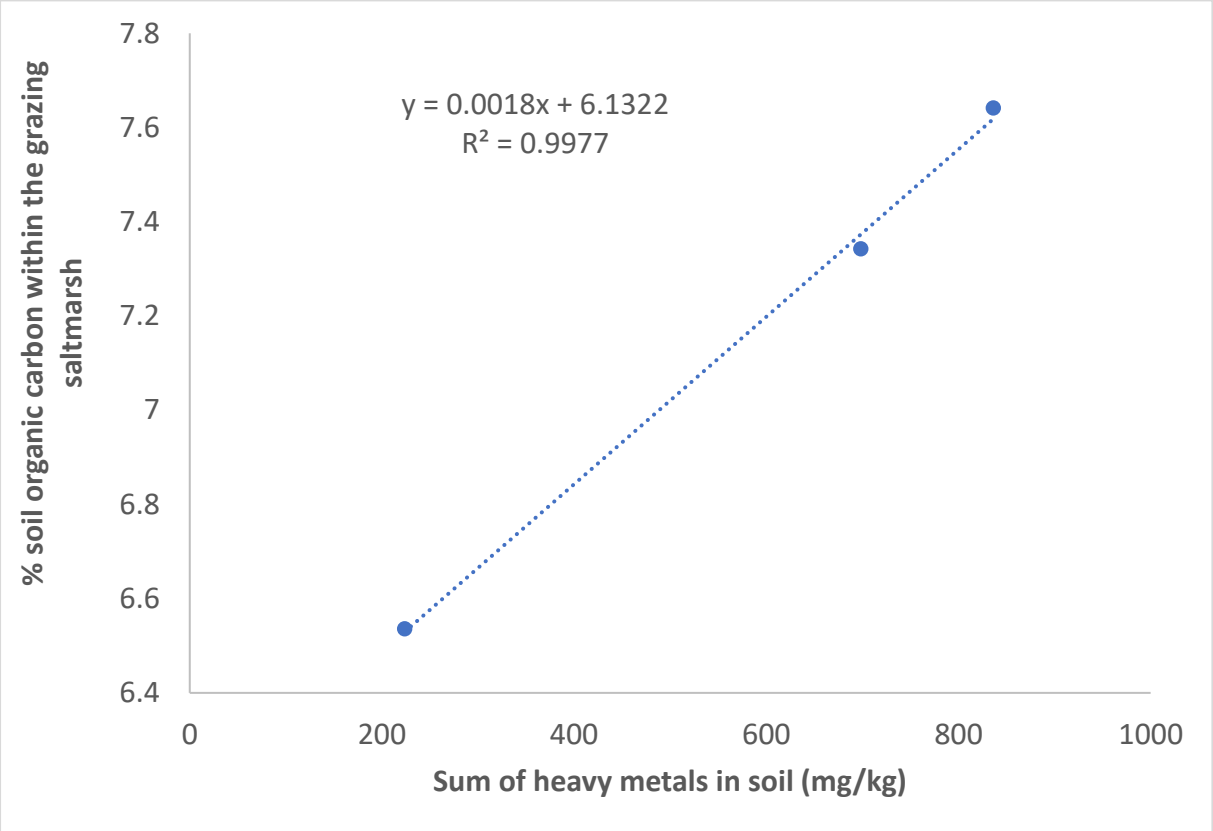


Figure 4. 4 **Relationship between the sum of heavy metals in the soils and the soil organic content within the grazing saltmarsh**

4.4.4 Heavy metal concentrations in soil

Arsenic (As)

The concentration of arsenic indicated that there was a significant variation of arsenic at different depths across the sampling locations, following different methods of analyses used. There was a general increase in the concentration as the depths of sampling increased (that is 15–30 cm > 0–15 cm depth). XRF shows significantly higher concentration of arsenic at GSM, SM1, RB and RGD at 0.05 level of confidence (see Table 4.4) (compared to the different fractions of arsenic from the different metal fractionation methods.

The percentage recovery from the different fractions indicates that F4 > F2 > F1 > F3 except at WD2 sampling location were F4 > F3 > F2 > F1 (see Table 5). The high percentage of recovery shown from F3 indicates that arsenic is easily exchangeable and soluble at the WD2 location. However, F2 within GSM, GSM-N, RB, SM1 and SM2 (see Table 4.5).

Table 4.4 Mean and SE of arsenic concentration (mg/kg) across the study site at different depth using heavy metal fractionation and XRF methods

Methods	Depth (cm)	GSM	GSM-N	NGD	RB	RGD	SM1	SM2	WD1	WD2
					mg/kg					
H_2O	0–15	0.33±0.11c	0.52±0.17c	0.12±0.02c	0.68±0.15b	0.15±0.05b	2.00±1.20c	0.38±0.10c	0.52±0.34a	9.46±4.2b
	15–30	0.17±0.02c	0.22±0.02b	0.10±0.03b	0.43±0.20b	0.12±0.02c	0.28±0.08c	0.32±0.12b	11.30±3.40b	2.82±1.7b
HNO_3	0–15	21.29±1.00bc	13.77±0.86b	1.93±0.24c	14.73±6.14b	8.32±2.72b	24.12±8.06bc	13.61±1.92b	4.14±4.14a	0.02±0.0b
	15–30	40.94±4.77b	8.32±8.32b	1.80±0.34b	27.42±10.76b	7.31±1.57c	45.08±1.35b	29.57±8.52ab	0.20±0.15b	0.03±0.0b
NH_4Cl	0–15	6.02±1.00c	3.02±0.57c	0.03±0.02c	7.50±4.48b	0.02±0.02b	14.32±7.90bc	2.00±1.41bc	1.85±0.21a	32.73±5.6b
	15-30	0.15±0.03c	0.12±0.02b	0.05±0.00b	0.13±0.02b	0.05±0.00c	0.15±0.03c	0.47±0.39b	63.73±13.06b	52.72±9.2b
HF-HNO3	0–15	33.63±14.69ab	34.88±1.33a	37.62±4.97a	69.12±13.34a	43.38±7.29a	34.75±5.85ab	39.38±5.08a	74.47±30.84a	32.85±8.8a
	15–30	71.92±16.38a	60.60±21.70a	38.42±6.60a	41.92±21.77ab	37.70±4.58b	50.17±4.77b	63.73±22.24a	60.26±13.31a	54.87±19.7a
XRF	0–15	44.38±3.20a	28.12±4.81a	28.24±4.39b	60.44±5.34a	47.30±7.96a	52.14±9.12a	36.97±6.77a	80.10±43.68a	15.04±4.4a
	15–30	84.11±6.77a	48.79±0.42a	27.94±4.04a	71.35±17.15a	49.01±5.71a	89.68±8.46a	72.80±23.69a	27.18±8.32a	22.11±7.8a

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence.

Table 4.5 Percentage recovery of arsenic across the study site at different depths

Sites	F1 0–15 cm	F2 0–15 cm	F3 0–15cm	F4 0–15cm	F1 15–30cm	F2 15–30cm	F3 15–30cm	F4 15–30cm
				%				
GSM	1.0	63.3	17.9	100	0.2	56.9	0.2	100
GSM-N	1.5	39.5	8.7	100	0.4	13.7	0.2	100
NGD	0.3	5.1	0.1	100	0.3	4.7	0.1	100
RB	1.0	21.3	10.9	100	1.0	65.4	0.3	100
RGD	0.3	19.2	0.0	100	0.3	19.4	0.1	100
SM1	5.8	69.4	41.2	100	0.6	89.9	0.3	100
SM2	1.0	34.6	5.1	100	0.5	46.4	0.7	100
WD1	0.7	5.6	2.5	100	18.8	0.3	5.8	100
WD2	28.8	0.1	99.6	100	5.1	0.1	96.1	100

F1= soluble fraction, F2= oxidisable phase, F3= soluble + exchangeable, and F4= residual phase, SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland.

Cadmium (Cd)

Results from Table 4.6 indicate that the concentrations of cadmium from different fractions were mostly in the oxidisable and residual phase within GSM, GSM-N, RB, SM1 and SM2 at 0–15 cm depths (7.48 mg/kg, 6.00 mg/kg, 6.35 mg/kg, 7.79 mg/kg and 4.52 mg/kg, respectively). The concentration of cadmium in the oxidisable phase was lower in 0–15 cm depth at GSM, SM1 and SM2, while the concentration of cadmium was higher in 0–15 cm depths at GSM-N and RB when compared to the 15–30 depth, respectively (see Table 4.7). However, GSM shows 66.4% recovery in the oxidisable phase while no percentage of Cd was recovered at GSM-N, RGD and SM2 after different methods of analyses (Table 4.7).

Table 4.6 Mean and SE of cadmium concentration (mg/kg) across the study site at different depths using using heavy metal fractionation and XRF methods

Methods	Depth (cm)	GSM	GSM-N	NGD	RB	RGD	SM1	SM2	WD1	WD2
						mg/kg				
H2O	0–15	0.05±0.00a	0.05±0.00c	0.05±0.00a	0.05±0.00b	0.03±0.02a	0.05±0.00a	0.03±0.02b	0.05±0.00b	nd
	15–30	0.05±0.00b	0.05±0.00a	0.05±0.00a	0.05±0.00b	0.05±0.00a	0.03±0.02b	0.03±0.02b	nd	nd
HNO3	0–15	7.48±0.93a	6.00±0.16a	nd	6.35±3.18b	nd	7.79±1.63a	4.52±2.26a	nd	0.10±0.00a
	15–30	8.91±0.83a	3.96±3.96a	nd	5.64±2.83a	nd	10.53±0.27a	4.90±2.52a	0.07±0.02b	0.13±0.02a
NH4Cl	0–15	0.17±0.04a	0.08±0.03ab	0.10±0.00a	0.20±0.06b	0.08±0.02a	0.28±0.13a	0.13±0.06b	0.08±0.02b	nd
	15–30	0.57±0.07b	0.17±0.02a	0.12±0.02a	0.28±0.07b	0.10±0.03a	1.12±0.12b	0.33±0.26b	nd	nd
HF-HNO3	0–15	11.26±10.14a	nd	2.70±2.70a	nd	nd	5.72±5.72a	nd	nd	0.67±0.67a
	15–30	nd	nd	9.14±9.14a	0.20±0.20b	nd	1.39±1.09b	nd	1.89±0.56b	1.00±1.00a
XRF	0–15	0.71±0.34a	0.58±0.32b	1.73±0.45a	0.79±0.16a	0.05±0.05a	nd	0.65±0.34b	1.56±0.12a	0.16±0.13a
	15–30	0.47±0.00b	2.14±0.54a	1.39±0.26a	1.87±0.59ab	0.25±0.25a	1.19±0.60b	1.09±0.48b	0.40±0.22a	0.24±0.20a

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence, nd= not detected.

Table 4.7 Percentage recovery of cadmium across the study site at different depths

Sites	F1 0–15 cm	F2 0–15 cm	F3 0–15cm	F4 0–15cm	F1 15–30cm	F2 15–30cm	F3 15–30cm	F4 15–30cm
				(%)				
GSM	0.4	66.4	1.5	100	0.0	0.0	0.0	0.0
GSM-N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NGD	1.9	0.0	3.7	100	0.5	0.0	1.3	100.0
RB	0.0	0.0	0.0	0.0	25.0	2820.0	140.0	100.0
RGD	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
SM1	0.9	136.2	4.9	100	2.2	757.6	80.6	100.0
SM2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WD1	0.0	0.0	0.0	0.0	0.0	3.7	0.0	100.0
WD2	0.0	14.9	0.0	100	0.0	13	0.0	100.0

F1= soluble fraction, F2= oxidisable phase, F3= soluble + exchangeable and F4= residual phase, SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland.

Chromium (Cr)

Table 4.8 and Table 4.9 show the concentrations of chromium and percentage recovery of chromium using metal fractionation and XRF methods of analysis across the sampling locations, respectively. Results obtained by the XRF method was statistically significantly higher at 0.05 level of confidence at GSM, GSM-N, NGD, RB, RGD, SM1 and SM1 compared to the different fractions of chromium obtained from metal fractionation procedures (see Table 8). The distribution of chromium seems to follow the trend of arsenic, where the concentrations increase with increasing sampling depths. For example, GSM, GSM-N, SM1 and SM2 at 0–15 cm depth (73.42 mg/kg, 81.37 mg/kg, 116.50 mg/kg and 116.35 mg/kg, respectively) and GSM, GSM-N, SM1 and SM2 at 15–30 cm depth (162.98 mg/kg, 157.78 mg/kg, 124.48 mg/kg and 122.18 mg/kg, respectively) in the residual fractions. However, the RB location shows higher concentration of chromium at 0–15 cm than 15–30 cm depth (137.62 mg/kg and 88.22 mg/kg, respectively).

Table 4.8 Mean and SE of chromium concentration (mg/kg) across the study site at different depths using heavy metal fractionation and XRF methods

Methods	Depth (cm)	GSM	GSM-N	NGD	RB	RGD	SM1	SM2	WD1	WD2
					mg/kg					
H2O	0–15	0.11±0.00c	0.30±0.10d	0.07±0.02c	0.95±0.33b	0.08±0.02c	0.10±0.00c	0.25±0.15b	0.12±0.07b	nd
	15–30	0.17±0.04c	0.15±0.05b	0.12±0.07c	0.63±0.30b	0.08±0.02c	0.10±0.00c	0.42±0.32b	nd	nd
HNO3	0–15	18.32±1.96bc	20.89±1.96c	nd	23.76±9.20b	nd	18.44±3.21c	20.32±4.35b	nd	nd
	15–30	31.20±2.80c	9.27±9.27b	nd	32.56±16.28b	nd	32.08±2.00c	31.28±8.59b	0.02±0.02c	nd
NH4Cl	0–15	0.03±0.02c	nd	nd	nd	nd	0.02±0.02c	0.02±0.02b	nd	24.72±5.22b
	15–30	0.02±0.02c	nd	nd	nd	nd	0.02±0.02c	0.00±0.00b	70.08±6.15c	37.23±8.60c
HF-HNO3	0–15	73.42±37.62b	81.37±1.98b	54.50±4.67b	137.62±38.10a	32.85±5.60b	116.50±20.6b	116.35±37.66a	105.18±49.02a	66.33±18.6b
	15–30	162.98±30.01b	157.78±74.58a	52.00±12.72b	88.22±50.64a	41.88±7.12b	124.48±31.5b	122.18±40.07a	111.94±16.08b	92.30±15.4b
XRF	0–15	158.18±11.11a	120.55±2.79a	98.03±6.56a	167.19±30.08a	98.49±6.17a	182.19±12.7a	154.75±22.77a	101.81±12.63a	18.22±7.7a
	15–30	235.08±6.99a	167.35±14.10a	102.39±7.42a	190.25±47.38a	95.93±4.41a	223.02±7.2a	184.20±32.24a	36.42±12.76a	25.92±10.1a

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence, nd= not detected.

Most of the concentrations of chromium across sampling depths were below detection limits in F1, F2 and F3 but were available in F4. The percentage recovery of chromium from the different fractions was generally low except in F4 and was affected by the limit of detection (see Table 9). This may be due to the fact that the majority of chromium was in the residual form.

Table 4.9 Percentage recovery of chromium across the study site at different depths

Sites	F1 0–15 cm	F2 0–15 cm	F3 0–15 cm	F4 0–15 cm	F1 15–30 cm	F2 15–30 cm	F3 15–30 cm	F4 15–30 cm
				(%)				
GSM	0.1	25.0	0.0	100.0	0.1	19.1	0.0	100.0
GSM-N	0.4	25.7	0.0	100.0	0.1	5.9	0.0	100.0
NGD	0.1	0.0	0.0	100.0	0.2	0.0	0.0	100.0
RB	0.7	17.3	0.0	100.0	0.7	0.0	0.0	100.0
RGD	0.2	0.0	0.0	100.0	0.2	0.0	0.0	100.0
SM1	0.1	15.8	0.0	100.0	0.1	25.8	0.0	100.0
WD1	0.1	0.0	0.0	100.0	0.0	0.0	62.6	100.0
WD2	0.0	0.0	37.3	100.0	0.0	0.0	40.3	100.0

F1= soluble fraction, F2= oxidisable phase, F3= soluble + exchangeable and F4= residual phase, SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland.

Copper (Cu)

Concentrations of copper using XRF and heavy metal fractionation (F1, F2, F3 and F4) procedures follow the sequence XRF > F4 > F2 > F3 > F1 or F1 > F3 in some cases across the study site (see Table 4.10). There is a generally higher trend in the concentration of copper with depth (0–15 cm < 15–30 cm) across lowland sampling locations (GSM, GSM-N, RB, SM1 and SM2).

Results from the percentage recovery from the different fractions indicated that F2 recovered 75.3%, 71.5%, 47.2%, 67.5% and 66.0% at SM2, SM1, RB, GSM-N and GSM, respectively, within 0-15 cm depth and 76.1%, 79.0%, 106.9%, 20.3% and 66.6% at 15–30 cm depth, respectively (see Table 4.11). However, the high percentage recovery in F2 fraction is subject to environmental changes.

Table 4.10 Mean and SE of copper concentration (mg/kg) across the study site at different depths using heavy metal fractionation and XRF methods

Methods	Depth (cm)	GSM	GSM-N	NGD	RB	RGD	SM1	SM2	WD1	WD2
						mg/kg				
H_2O	0–15	0.33±0.16d	0.77±0.28c	0.30±0.10d	3.97±0.54c	0.27±0.03c	0.25±0.03c	0.83±0.32c	0.28±0.09b	18.93±5.0b
	15–30	1.30±0.50c	1.08±0.42b	0.25±0.10c	3.78±1.20b	0.33±0.04c	0.60±0.26c	1.67±1.03b	42.24±11.92b	13.91±13.9b
HNO_3	0–15	57.60±3.49c	38.84±2.14b	17.09±3.43c	85.39±3.72b	21.14±2.6bc	67.34±16.73b	47.98±10.8b	13.76±6.89b	0.03±0.02b
	15–30	182.69±22.3b	26.85±26.08b	17.27±4.32c	117.50±26.7ab	19.63±0.50c	128.49±7.58b	100.14±31.4ab	0.53±0.14b	0.03±0.02b
NH_4Cl	0–15	1.15±0.10d	0.78±0.28c	0.60±0.28d	0.40±0.06c	0.05±0.00c	0.60±0.20c	0.50±0.10c	0.32±0.13b	24.47±7.7b
	15–30	1.38±0.20c	0.87±0.08ab	0.07±0.02c	0.85±0.25b	0.03±0.02c	1.37±0.61c	1.50±0.70b	105.10±26.5b	71.77±36.5b
$HF\text{-}HNO_3$	0–15	87.30±3.83b	57.53±11.4ab	56.95±9.20b	180.97±35.78a	53.85±16.8b	94.17±22.49b	63.72±13.01ab	86.33±30.4a	65.46±18.06
	15–30	274.28±54.8ab	132.43±67.7a	51.25±11.4b	109.93±65.1ab	54.90±11.0b	162.73±13.67b	131.65±61.2ab	130.47±31.5a	109.5±41.2a
XRF	0–15	96.43±3.34a	71.36±16.6a	75.69±4.86a	166.67±15.07a	103.46±20.9a	158.25±33.57a	90.64±21.4a	106.78±36.2a	21.82±7.25a
	15–30	323.69±44.2a	120.52±15.0a	75.01±7.72a	213.03±40.09a	104.22±15.6a	286.33±24.32a	161.35±55.0a	55.71±16.04a	39.10±15.2a

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM–N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence, nd= not detected.

Table 4.11 Percentage recovery of copper across the study site at different depths

Sites	F1 0–15 cm	F2 0–15 cm	F3 0–15 cm	F4 0–15 cm	F1 15–30 cm	F2 15–30 cm	F3 15–30 cm	F4 15–30 cm
				(%)				
GSM	0.4	66.0	1.3	100.0	0.5	66.6	0.5	100.0
GSM-N	1.3	67.5	1.4	100.0	0.8	20.3	0.7	100.0
NGD	0.5	30.0	1.1	100.0	0.5	33.7	0.1	100.0
RB	2.2	47.2	0.2	100.0	3.4	106.9	0.8	100.0
RGD	0.5	39.3	0.1	100.0	0.6	35.8	0.1	100.0
SM1	0.3	71.5	0.6	100.0	0.4	79.0	0.8	100.0
SM2	1.3	75.3	0.8	100.0	1.3	76.1	1.1	100.0
WD1	0.3	15.9	0.4	100.0	32.4	0.4	80.6	100.0
WD2	28.9	0.00	37.4	100.0	12.7	0.0	65.5	100.0

F1= soluble fraction, F2= oxidisable phase, F3= soluble + exchangeable and F4= residual phase, SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland.

Lead (Pb)

The amounts of lead contamination distributed across the study site are presented in Table 12. Results indicated that different methods of analyses have effects on the total concentration within the study area. Results from XRF method were statistically significantly higher at 0.05 level of confidence across the locations (see Table 4.12). There was a clear trend of high concentration levels across the locations at different depths (0–15 cm < 15–30 cm). Different fractions of lead are similar to arsenic, cadmium, chromium, and copper. They are less available in soluble and exchangeable phases and are mostly bound to oxidisable and residual phases across the sampling locations. However, some of the fractions were under detection limits using ICP-OES. For instance, the concentration of Pb was detected in all fractions except in F3 at GSM-N. This may be due to the fact that the metals were not found in that fraction since they were detected in other fractions. The percentage recovery of Pb from the different fractions was affected by the limit of detection (see Table 4.13). Lowland sampling locations show a much higher percentage recovery from F2 at different depths. Values are as follows: GSM (106.0%), GSM-N (113.1%), RB (76.9%), SM1 (123.3%) and SM2 (90.1%) at 0–15 cm depth and GSM (108.5%), GSM-N (30.5%), RB (146.4%), SM1 (145.9%) and SM2 (95.1%) within 15–30 cm depth.

Table 4.12 Mean and SE of lead concentration (mg/kg) across the study site at different depths using heavy metal fractionation and XRF methods

Methods	Depth (cm)	GSM	GSM-N	NGD	RB	RGD	SM1	SM2	WD1	WD2
						mg/kg				
H2O	0–15	0.03±0.02c	0.17±0.03d	0.10±0.08c	1.27±0.16c	0.05±0.00c	0.30±0.18c	0.18±0.11c	0.07±0.07b	45.5±10.6a
	15–30	0.03±0.02c	0.17±0.03c	0.10±0.08c	1.27±0.16b	0.05±0.00c	0.30±0.18d	0.18±0.11b	130.46±52.3b	28.4±20.1b
HNO3	0–15	123.05±5.9b	85.89±0.30b	58.06±14.0bc	181.9±6.53b	48.72±4.2bc	138.71±38.2b	96.09±17.4b	36.75±21.84b	nd
	15–30	298.93±32.6b	58.18±58.2bc	47.86±15.2bc	177.6±58.8ab	44.13±2.70b	260.57±5.53b	162.40±47.0ab	nd	nd
NH4Cl	0–15	nd	nd	0.10±0.10c	2.97±2.97c	nd	0.70±0.65c	0.17±0.09c	nd	231±193.1a
	15–30	0.15±0.13c	nd	nd	nd	nd	0.12±0.07d	0.18±0.16b	249.1±104.3b	92.3±41.3b
HF-HNO3	0–15	116.13±5.01b	75.92±0.22c	130.5±35.8ab	236.5±34.0b	80.92±23.7b	112.52±25.0b	106.63±25.4ab	171.1±71.3ab	112.9±35.0a
	15–30	275.63±53.0b	190.92±89.6ab	97.12±31.15ab	121.3±69.5ab	74.13±18.4b	178.60±15.6c	170.83±75.2ab	343.2±138.9ab	164.3±60ab
XRF	0–15	181.33±4.62a	124.47±5.94a	190.79±37.6a	311.69±20.8a	190.57±30.5a	254.53±54.5a	185.14±49.62a	256.6±103.8a	77.9±40.6a
	15–30	424.59±44.8a	230.55±17.98a	151.01±32.5a	290.26±88.5a	182.54±21.6a	414.54±33.3a	271.75±83.98a	144.6±47.5a	57.0±21.2a

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence, nd= not detected.

Table 4.13 Percentage recovery of lead across the study site at different depths

Sites	F1 0–15 cm	F2 0–15 cm	F3 0–15 cm	F4 0–15 cm	F1 15–30 cm	F2 15–30 cm	F3 15–30 cm	F4 15–30 cm
				(%)				
GSM	0.0	106.0	0.0	100.0	0.0	108.5	0.1	100.0
GSM-N	0.2	113.1	0.0	100.0	0.1	30.5	0.0	100.0
NGD	0.1	44.5	0.0	100.0	0.1	49.3	0.0	100.0
RB	0.5	76.9	0.0	100.0	1.0	146.4	0.0	100.0
RGD	0.1	60.2	0.0	100.0	0.1	59.5	0.0	100.0
SM1	0.3	123.3	0.0	100.0	0.2	145.9	0.1	100.0
SM2	0.2	90.1	0.1	100.0	0.1	95.1	0.1	100.0
WD1	0.0	21.5	0.0	100.0	38.0	0.0	72.6	100.0
WD2	40.3	0.0	0.0	100.0	17.3	0.0	56.2	100.0

F1= soluble fraction, F2= oxidisable phase, F3= soluble + exchangeable and F4= residual phase, SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland.

Zinc (Zn)

XRF data were statistically significantly higher at 0.05 level of confidence than the heavy metal fractionation data in most of the locations. SM2, SM1, GSM, RB and GSM-N were highest in mean values (1056.7 mg/kg, 577.65 mg/kg, 480.5 mg/kg, 605.83 mg/kg, 306.53 mg/kg and 1151.0 mg/kg, 1139.14 mg/kg, 1048.2 mg/kg, 826.94 mg/kg and 600.03 mg/kg, within 0–15 cm and 15–30 cm depth, respectively), see Table 4.14. Zinc fractions were statistically significantly higher at 0.05 level of confidence in F4 and F2 than F3 and F1. This is an indication that zinc fractions are similar to arsenic, cadmium, chromium, copper, and lead in their distribution across the study area. Thus, the metals are sparingly bound to soluble and exchangeable phases and are mostly bound to oxidisable and residual phases across the sampling locations. The sampling locations show a general increased concentration of zinc with depth. Lowland profiles show a higher percentage of recovery from F2 and F4 than F1 and F3 (see Table 4.15). This is because zinc is more bound to oxidisable and residual phases.

Table 4.14 Mean and SE of zinc concentration (mg/kg) across the study site at different depths using heavy metal fractionation and XRF methods

Methods	Depth (cm)	GSM	GSM-N	NGD	RB	RGD	SM1	SM2	WD1	WD2
						mg/kg				
H2O	0–15	0.28±0.04c	0.87±0.33d	0.38±0.12b	4.28±1.18b	0.22±0.02c	1.20±0.85b	1.43±1.23a	0.55±0.21b	33.55±16.6b
	15–30	0.78±0.26d	0.48±0.07b	0.50±0.30b	2.73±1.27b	0.37±0.09c	1.50±0.81c	3.57±3.32a	61.18±6.72b	6.51±0.29b
HNO3	0–15	262.07±11.5b	223.96±2.68c	29.56±4.72b	291.5±125.9b	14.05±3.99c	268.69±33.8ab	549.25±330.8a	29.43±16.79b	0.33±0.06ab
	15–30	486.88±5.72c	123.68±122.3b	33.18±8.01b	387.0±136.8ab	11.06±1.83c	541.15±28.1b	606.04±337.7a	0.23±0.03b	0.27±0.04b
NH4Cl	0–15	1.70±0.08c	0.85±0.10d	0.78±0.61b	3.28±1.64b	0.25±0.03c	15.52±13.24b	3.12±2.09a	0.35±0.25b	99.53±5.5b
	15–30	7.18±2.26d	1.72±0.57b	0.28±0.03b	1.58±0.47b	0.33±0.07c	17.65±8.15c	7.12±4.05a	252.22±30.66b	115.9±21.2b
HF-HNO3	0–15	264.3±126.9b	306.53±14.1b	166.17±27.6a	508.7±189.7a	117.3±12.0b	870.18±555.8a	618.55±282.5a	199.88±31.73a	130.7±73ab
	15–30	739.4±125.6b	582.17±223.6a	157.50±28.3a	537.2±153.8a	105.2±10.4b	547.27±57.8b	848.42±527.6a	289.70±47.69a	122.8±64.6a
XRF	0–15	480.5±11.5a	372.45±32.93a	170.66±14.5a	605.83±233.7a	174.5±26.2c	577.65±72.7a	1056.7±660.1a	253.53±60.94a	52.9±19.0a
	15–30	1048.2±38.2a	600.03±53.53a	181.84±14.9a	826.94±263.6a	172.8±15.6a	1139.14±65.4a	1151.0±623.0a	120.73±34.83a	49.2±19.2a

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence, nd= not detected.

Table 4.15 Percentage recovery of zinc across the study site at different depths

Sites	F1	F2	F3	F4	F1	F2	F3	F4
	0–15 cm	0–15 cm	0–15 cm	0–15 cm	15–30 cm	15–30 cm	15–30 cm	15–30 cm
				(%)				
GSM	0.1	99.2	0.6	100.0	0.1	65.8	1.0	100.0
GSM-N	0.3	73.1	0.3	100.0	0.1	21.2	0.3	100.0
NGD	0.2	17.8	0.5	100.0	0.3	21.1	0.2	100.0
RB	0.8	57.3	0.6	100.0	0.5	72.0	0.3	100.0
RGD	0.2	12.0	0.2	100.0	0.4	10.5	0.3	100.0
SM1	0.1	30.9	1.8	100.0	0.3	98.9	3.2	100.0
SM2	0.2	88.8	0.5	100.0	0.4	71.4	0.8	100.0
WD2	25.7	0.3	76.2	100.0	5.3	0.2	94.4	100.0

F1= soluble fraction, F2= oxidisable phase, F3= soluble + exchangeable and F4= residual phase, SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland.

4.4.5 Percentage distribution of heavy metal distribution across the different land uses/land covers

Figure 4.5 shows the percentage of heavy distribution across the lowland sampling locations (GSM, GSM-N, SM, and RB) and the upland sampling location (NGD, RGD, and WD). Arsenic concentration indicates that WD1 > RB > RGD > SM1 > NGD > GSM-N > SM2 > GSM >WD2. Cadmium concentration was elevated within the lowland sampling location compared to the upland (see Figure 4.5). The majority of the concentrations of chromium, copper, lead, and zinc indicates higher concentration in the lowland compared to the upland sampling locations (see Figure 4.5).

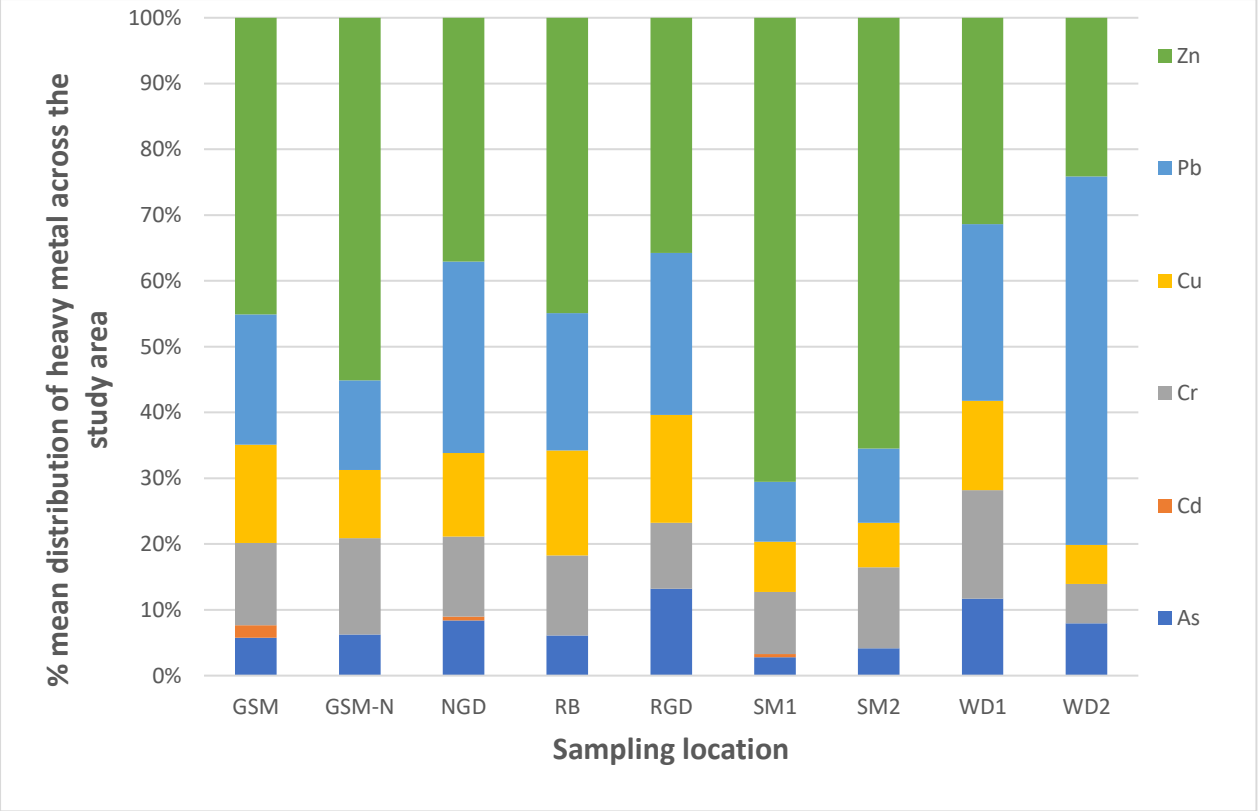


Figure 4. 5 Percentage heavy metal distribution under different land uses/land covers (SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland.

4.4.6 Heavy metal concentrations in plants and their uptake

The heavy metal concentration, and transfer and accumulation of heavy metals from soil to roots and shoots was assessed in terms of Bioaccumulation Coefficient (BAC), Translocation Factor (TF) and Biological Concentration Factor (BCF). Total metal concentrations in roots across the study sites follow the pattern: arsenic and lead were significantly different at $P < 0.05$ (20.16±12.09a mg/kg and 52.78±34.81a mg/kg, respectively) (Table 4.16) at SM1 compared to other sampling locations; higher concentrations of cadmium were found in WD1 and WD2 compared to the other sites; higher concentrations of chromium were found in GSM, RB, WD1 and WD2 compared to the other sites; higher concentrations of copper were found in SM1, RB, WD1, WD2 and RGD compared to the other sampling locations; higher concentrations of Iron were found in all the sampling locations except GSM; higher concentrations of manganese were found in all the sampling locations except NGD; higher concentrations of zinc were found in GSM-N, WD1, WD2 and RGD WD2 compared to the other sites (Table 4.16).

The concentration of heavy metals in the shoot portion shows significant variations across the study site. The concentrations of arsenic were significantly higher in GSM-N (6.63±1.79a mg/kg) compared to location RB, SM2, NGD and RGD (4.56±0.06b mg/kg, 4.44±0.04b mg/kg, 4.54±0.11b mg/kg, 4.67±0.08b mg/kg respectively) (Table 4.17); higher concentrations of cadmium in the shoots were found in all the sampling locations except in WD2, SM2 and RGD; higher concentrations of chromium in the shoots were found in GSM-N location compared to other sites; concentrations of copper found in the plant shoots were not statistically significant across the sampling locations; higher concentrations of iron in the plant shoots were found in all the sampling locations except in GSM and SM; higher concentrations of manganese in the plant shoots were found in all the locations except in GSM and GSM-N; the concentrations of lead were highly significant at GSM-N (27.44±19.95a mg/kg) compared to RB, SM2, NGD and RGD (4.81±0.13b mg/kg, 4.81±0.10b mg/kg, 7.99±2.40b mg/kg and 5.67±0.41b mg/kg respectively); the concentrations of zinc were generally not statistically significant across all the sampling locations (Table 4.17). Generally speaking, there were higher concentrations of the heavy metals found in the root portion compared to the shoot portion except for cadmium, (WD1 and WD2 sampling locations) and chromium (GSM-N, WD1 and WD2 sampling locations) (Tables 4.17 and 4.18).

BCF was < 1 for arsenic, cadmium, copper, iron, and lead but > 1 for chromium, manganese, and zinc (*Elytrigia repens*). This implies that the concentration of chromium, manganese and zinc in roots was greater than that in the soil. TF was < 1 for copper, TF > 1 for chromium, iron, manganese, lead, and zinc (*Elytrigia repens* and *Phragmite australis* – manganese and zinc) and TF= 1 for arsenic and cadmium (*Elytrigia repens* and *Phragmite australis*). This means that, chromium, iron, manganese, lead, and zinc concentrations in shoots are greater than concentrations in roots. BAC was < 1 for arsenic, cadmium, copper, iron, and lead was > 1 for chromium, manganese, and zinc (GSM and WD2) showing that chromium, manganese, and zinc concentrations in shoots are greater than concentrations in soil. Thus, *Elytrigia repens* and *Phragmite australis* have the potential for phytoextraction and phytostabilisation of chromium, iron, manganese, lead, and zinc, while the heavy metals tolerant species with high BCF and low TF can be used for phytostabilisation of contaminated soils.

Table 4.16 Mean and SE of heavy metal concentration (mg/kg) in plant tissues (root portion)

Sites	Arsenic	Cadmium	Chromium	Copper	Iron	Manganese	Lead	Zinc
				mg/kg				
SM1	20.16±12.09a	4.95±0.13a	10.86±3.27ab	36.44±10.27a	7156.68±5084.73a	279.55±124.61a	52.78±34.81a	145.96±38.58ab
GSM	15.79±5.67ab	4.96±0.59a	14.64±6.40a	40.00±13.33ab	4658.92±2108.20ab	306.67±97.88a	35.60±16.38ab	140.35±42.06ab
GSM-N	6.48±1.59ab	6.73±2.30a	5.98±0.44abc	26.51±6.85abc	514.46±264.91b	45.08±4.86b	10.12±3.79ab	155.90±63.13a
RB	4.82±0.13ab	4.73±0.24a	5.22±0.38bc	13.74±2.76cd	132.95±42.48b	47.39±7.20b	6.14±0.43ab	81.93±32.50abc
WD1	1.55±1.55b	1.43±1.43b	1.50±1.50c	3.33±3.33d	41.04±41.04b	9.20±9.20b	2.17±2.17b	12.51±12.51c
SM2	5.02±0.14ab	4.48±0.35a	6.27±0.86abc	18.60±3.83abcd	329.40±90.74b	52.08±7.24b	7.27±0.87ab	142.23±69.34ab
NGD	5.47±0.27ab	4.55±0.29a	6.14±0.73abc	20.69±3.03abcd	623.37±230.90b	159.93±96.10ab	24.30±8.75ab	57.98±3.68abc
WD2	1.71±1.71b	1.29±1.29b	1.68±1.68c	4.44±4.44cd	128.30±128.30b	23.24±23.24b	2.98±2.98b	12.04±12.04c
RGD	6.07±0.25ab	4.45±0.18a	6.21±1.00abc	16.32±2.43bcd	705.85±396.66b	61.86±24.53b	14.66±3.88ab	32.30±5.09bc

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence.

Table 4.17 Mean and SE of heavy metal concentration (mg/kg) in plant tissues (shoot portion)

Sites	Arsenic	Cadmium	Chromium	Copper	Iron	Manganese	Lead	Zinc
				mg/kg				
SM1	5.31±0.55ab	3.93±0.11b	9.67±2.91b	13.53±3.88a	1355.37±983.92ab	192.41±91.62bc	13.01±6.49ab	61.90±34.30a
GSM	5.58±0.38ab	3.90±0.04b	10.89±2.40b	13.25±2.66a	1626.83±650.25ab	282.84±5.15ab	13.02±3.97ab	62.74±22.41a
GSM-N	6.63±1.79a	4.09±0.12b	18.36±9.56a	20.3±10.92a	3580.17±3065.13a	313.91±269.81ab	27.44±19.95a	97.21±62.18a
RB	4.56±0.06b	4.25±0.04b	4.88±0.37b	11.07±1.55a	75.23±11.31b	86.69±3.60bc	4.81±0.13b	72.90±23.18a
WD1	5.65±0.60ab	4.67±0.29ab	5.42±0.43b	20.85±6.31a	518.05±257.12b	103.29±59.32bc	15.77±6.15ab	120.64±62.63a
SM2	4.44±0.04b	4.41±0.07ab	4.96±0.36b	11.73±3.58a	73.77±7.41b	26.18±8.70c	4.81±0.10b	93.11±32.42a
NGD	4.54±0.11b	4.12±0.09b	5.30±0.27b	12.53±0.24a	181.72±77.22b	33.57±8.70c	7.99±2.40b	35.41±5.40a
WD2	5.14±0.38ab	5.71±1.21a	4.96±0.16b	15.55±3.04a	266.75±40.32b	471.49±55.30a	13.33±1.84ab	188.77±117.65a
RGD	4.67±0.08b	4.42±0.04ab	4.72±0.32b	8.69±0.62a	96.74±39.00b	41.39±15.23c	5.67±0.41b	21.66±4.29a

SM= Salt Marsh, GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, WD= Woodland, NGD= Natural Grassland, RGD= Rough Grassland, SE= Standard Error, abc= Duncan test at 0.05 level of confidence.

Table 4.18 Plant uptakes using BAC, TF and BCF indicators

	Sites	Plant species	Arsenic	Cadmium	Chromium	Copper	Iron	Manganese	Lead	Zinc
	GSM	*Elytrigia repens*	0.5	0.6	9.4	0.2	0.7	5.0	0.1	3.0
	GSM-N	*Elytrigia repens*	0.2	0.0	0.2	0.3	0.1	0.1	0.3	0.2
	NGD	*Galium verum*	0.1	0.2	0.1	0.2	0.0	0.1	0.1	0.2
	RB	*Phragmites australis*	0.1	0.0	0.0	0.1	0.0	0.2	0.0	0.2
BAC	RGD	Wildflower	0.1	0.0	0.2	0.2	0.0	0.2	0.1	0.2
	SM1	*Elytrigia repens*	0.2	0.1	0.1	0.2	0.1	0.5	0.1	0.2
	SM2	*Elytrigia repens*	0.1	0.0	0.1	0.2	0.0	0.0	0.1	0.2
	WD1	Broad-leaved woodlands	0.1	0.0	0.1	0.3	0.0	0.2	0.1	0.5
	WD2	Broad-leaved woodlands	0.2	0.0	0.2	0.7	0.0	5.8	0.3	1.8
	GSM	*Elytrigia repens*	0.5	0.8	1.1	0.4	1.8	1.3	0.9	0.6
	GSM-N	*Elytrigia repens*	1.0	0.8	2.5	0.6	4.2	4.6	1.9	0.5
	NGD	*Galium verum*	0.8	0.9	0.9	0.6	0.3	0.4	0.4	0.6
	RB	*Phragmites australis*	1.0	0.9	0.9	0.8	0.7	1.9	0.8	1.1
TF	RGD	Wildflower	0.8	1.0	0.8	0.5	0.3	1.5	0.5	0.7
	SM1	*Elytrigia repens*	0.6	0.8	1.4	0.5	6.7	4.0	1.6	0.7
	SM2	*Elytrigia repens*	0.9	1.0	0.8	0.6	0.3	0.5	0.7	0.8
	WD1	Broad-leaved woodlands	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.4
	WD2	Broad-leaved woodlands	0.3	0.4	0.3	0.2	0.2	1.8	0.4	0.4
	GSM	*Elytrigia repens*	0.7	0.6	4.1	0.5	0.3	2.4	0.3	2.4
	GSM-N	*Elytrigia repens*	0.2	0.0	0.1	0.4	0.0	0.0	0.1	0.4
	NGD	*Galium verum*	0.2	0.2	0.1	0.4	0.0	0.5	0.2	0.4
	RB	*Phragmites australis*	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.2
BCF	RGD	Wildflower	0.2	0.0	0.2	0.4	0.1	0.3	0.2	0.3
	SM1	*Elytrigia repens*	0.8	0.1	0.1	0.5	0.4	1.5	0.7	0.4
	SM2	*Elytrigia repens*	0.1	0.0	0.1	0.4	0.0	0.1	0.1	0.2
	WD1	Broad-leaved woodlands	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
	WD2	Broad-leaved woodlands	0.1	0.0	0.1	0.4	0.0	0.1	0.2	0.1

BAC= Bioaccumulation Coefficient, TF= Translocation Factor and BCF= Biological Concentration Factor.

4.4.7 Multivariate analysis showing interaction effects among selected parameters

From the Pillai's Trace multivariate test Table 4.19, the Pillai's Trace value for intercept is 0.10 with an F value of 2.30. This is significant at 5% level as P value is 0.04 and as such, we reject the null hypothesis that the concentration of heavy metals obtained from methods of heavy metal analyses, sampling location and dependent variable are the same. However, 9% of the variability in the dependent variables is being accounted for by the variability in the Eh and pH based on the Partial Eta Squared (see Table 4.19). This also shows that there are 70% and 71% chances of the pH and Eh values obtained from the study area to be the same and have effects on the concentration of heavy metals based on the observed power values (see Table 4.19). However, the post-test based on Duncan Multiple Range at $P < 0.05$ (see Section 4.4.1) indicated that there was significant difference in pH and Eh at GSM, GSM-N, SM1, WD1 and WD2 compared to other sampling locations.

Table 4.19 Showing Pillai's Trace multivariate test[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Observed Power[c]
Intercept	Pillai's Trace	0.10	2.301b	6	119	0.04	0.10	0.78
pH	Pillai's Trace	0.09	1.950b	6	119	**0.08**	**0.09**	**0.70**
Eh	Pillai's Trace	0.09	1.978b	6	119	**0.07**	**0.09**	**0.71**
Metal analysis type	Pillai's Trace	1.81	8.956	36	744	0.00	0.30	1.00
Sampling location	Pillai's Trace	0.92	2.816	48	744	0.00	0.15	1.00
Metal analysis type * Sampling location	Pillai's Trace	2.24	1.542	288	744	0.00	0.37	1.00

a Design: Intercept + pH + Eh + Metal analysis type + Sampling location + Metal analysis type * Sampling location; b Exact statistic; c Computed using alpha = 0.05, Bold= not significant

Table 4.20 shows the tests of between-subjects effects from the multivariate analysis of variance. The corrected model shows the coefficient of determination or R^2 and the adjusted coefficient of determination. The R^2 values indicated that relationship exists between the dependent variables (concentration of heavy metal) and independent variables (pH, Eh, type of analysis and sampling locations). This shows that 76.8% arsenic, 56.7% cadmium, 90.1% chromium, 88.3% copper, 76.0% lead and 59.2% zinc variances were

associated with the changes in the independent variables. The intercept shows that all the dependent variables were significant at P < 0.05 except cadmium (see Table 20). pH values indicated that no significant difference exist in cadmium and zinc across the study area compared to other dependent variable while Eh values were significantly different at P < 0.05 in all the dependent variables across the study area except cadmium. There was significant difference in the methods of heavy metal analyses used for the soil and plant tissue within the study area. Also, the sampling locations indicated that no significant difference at P < 0.05 exist in cadmium and lead compared to other dependent variables (see Table 4.20). There were no interaction effects among the sampling locations and methods of heavy metal analyses in lead and zinc compared to other dependent variables.

Table 4.20 Showing tests of ANOVA between-subjects effects

Source of variability	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power g
Corrected Model	Arsenic	78553.069a	64	1227.392	6.42	0.00	0.77	1.00
	Cadmium	1328.621b	64	20.760	2.54	0.00	0.57	1.00
	Chromium	497341.523c	64	7770.961	17.67	0.00	0.90	1.00
	Copper	362735.132d	64	5667.736	14.55	0.00	0.88	1.00
	Lead	1302769.619e	64	20355.775	6.14	0.00	0.76	1.00
	Zinc	9422106.425f	64	147220.413	2.82	0.00	0.59	1.00
Intercept	Arsenic	1646.546	1	1646.546	8.61	0.00	0.07	0.83
	Cadmium	0.652	1	0.652	0.08	0.78	0.00	0.06
	Chromium	3475.090	1	3475.090	7.90	0.01	0.06	0.80
	Copper	4506.498	1	4506.498	11.57	0.00	0.09	0.92
	Lead	19501.823	1	19501.823	5.88	0.02	0.05	0.67
	Zinc	203289.679	1	203289.679	3.89	0.05	0.03	0.50
pH	Arsenic	1434.616	1	1434.616	7.500	0.01	0.06	0.78
	Cadmium	0.232	1	0.232	0.03	0.87	0.00	0.05
	Chromium	2895.721	1	2895.721	6.58	0.01	0.05	0.72
	Copper	3761.489	1	3761.489	9.66	0.00	0.07	0.87
	Lead	16753.787	1	16753.787	5.05	0.03	0.04	0.61
	Zinc	181171.537	1	181171.537	3.47	0.07	0.03	0.46
Eh	Arsenic	1366.225	1	1366.225	7.14	0.01	0.05	0.76
	Cadmium	0.726	1	0.726	0.09	0.77	0.00	0.06
	Chromium	3251.386	1	3251.386	7.39	0.01	0.06	0.77
	Copper	3664.703	1	3664.703	9.41	0.00	0.07	0.86
	Lead	17278.228	1	17278.228	5.21	0.02	0.04	0.62
	Zinc	182770.393	1	182770.393	3.500	0.06	0.03	0.46

Metal analysis type	Arsenic	61310.006	6	10218.334	53.40	0.00	0.72	1.00
	Cadmium	572.351	6	95.392	11.66	0.00	0.36	1.00
	Chromium	415082.439	6	69180.406	157.30	0.00	0.88	1.00
	Copper	261269.843	6	43544.974	111.81	0.00	0.84	1.00
	Lead	1034772.869	6	172462.145	52.00	0.00	0.72	1.00
	Zinc	4559028.940	6	759838.157	14.53	0.00	0.41	1.00
Sampling location	Arsenic	4214.830	8	526.854	2.75	0.01	0.15	0.93
	Cadmium	119.923	8	14.990	1.83	0.08	0.11	0.76
	Chromium	12423.439	8	1552.930	3.53	0.00	0.19	0.98
	Copper	28540.709	8	3567.589	9.16	0.00	0.37	1.00
	Lead	51564.090	8	6445.511	1.94	0.06	0.11	0.79
	Zinc	1169756.545	8	146219.568	2.80	0.01	0.15	0.93
Metal analysis Type * Sampling location	Arsenic	11653.386	48	242.779	1.27	0.15	0.33	0.98
	Cadmium	578.055	48	12.043	1.47	0.05	0.36	0.99
	Chromium	52337.260	48	1090.360	2.48	0.00	0.49	1.00
	Copper	61507.653	48	1281.409	3.29	0.00	0.56	1.00
	Lead	191294.129	48	3985.294	1.20	0.21	0.32	0.97
	Zinc	2801974.156	48	58374.462	1.12	0.31	0.30	0.95
Error	Arsenic	23726.285	124	191.341				
	Cadmium	1014.764	124	8.184				
	Chromium	54533.559	124	439.787				
	Copper	48290.765	124	389.442				
	Lead	411287.320	124	3316.833				
	Zinc	6483582.209	124	52286.953				
Total	Arsenic	157199.640	189					
	Cadmium	3230.322	189					
	Chromium	765525.139	189					
	Copper	667638.760	189					
	Lead	2531931.716	189					
	Zinc	20865129.120	189					
Corrected Total	Arsenic	102279.354	188					
	Cadmium	2343.385	188					
	Chromium	551875.081	188					
	Copper	411025.897	188					
	Lead	1714056.940	188					
	Zinc	15905688.630	188					

a R^2 = 0.768 (Adjusted R^2 = 0.648); b R^2 = 0.567 (Adjusted R^2 = .343); c R^2 = 0.901 (Adjusted R^2 = 0.850); d R^2 = 0.883 (Adjusted R^2 = 0.822); e R^2 = 0.760 (Adjusted R^2 = 0.636); f R^2 = 0.592 (Adjusted R^2 = 0.382); g Computed using alpha = 0.05

Table 4.21 shows the relationship between heavy metal distribution with soil pH, Eh, EC and soil organic carbon. The results indicated that the relationship was both positive and negative across the sampling locations. The soil pH was positively correlated with all the heavy metal except arsenic and lead. Eh was negatively correlated with pH, EC, SOC, arsenic, cadmium, chromium, copper and zinc while soil organic content was positively correlated with all the heavy metal except arsenic and Eh (see Table 21). The concentration of arsenic was significantly positively correlated with chromium, copper, lead and zinc at $P < 0.01$ and the coefficient of correlations were (0.793**, 0.854**, 0.798** and 0.490** respectively). Copper, lead and zinc were positively correlated with all the selected heavy metals (see Table 4.21). A positive and negative correlation is an indication that as one variable deviates from the mean, the other variable deviates in the same and opposite directions respectively.

Table 4.21 Showing Pearson's correlation coefficient for heavy metal and metalloid contamination with pH, Eh, EC and SOC

	Arsenic	Cadmium	Chromium	Copper	Lead	Zinc	pH	Eh	EC	SOC
Arsenic	1									
Cadmium	-0.005	1								
Chromium	0.793**	-0.029	1							
Copper		0.087		1						
	0.854**		0.851**							
Lead		0.017	0.754**		1					
	0.798**			0.856**						
Zinc		0.098	0.674**			1				
	0.490**			0.629**	0.539**					
pH	-0.003	0.091	0.096	0.014	-0.015	0.094	1			
Eh	-0.008	-0.104	-0.11	-0.031	0.002	-0.116	-	1		
							0.995**			
EC	0.078		0.143	0.124	0.039		0.048	-0.100	1	
		0.223**				0.178*				
SOC	-0.013	0.048	0.050	0.025	0.001	0.109				
							0.145*	0.169*	0.406**	1

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

4.5 Discussion

4.5.1 Differences in soil pH, redox potential, electrical conductivity, and soil organic carbon across the study area

In this subsection, data presented in Section 4.4 detailing four important soil characteristics and how they vary across the sampling loctations will be discussed. This will include discussion on possible explanations of the variability observed. This improves umderstanding of heavy metal dynamics within the Upper Mersey Estaury and other estusaries.

The present study identified variations in the soil pH, redox potential, and electrical conductivity across the study area and this need explanations. The soil pH within the low-lying saltmarshes ranges from 6.6-8.1 while the woodlands with higher elevations ranged from 5.8 to 6.3 (Table 4.1 and Appendix 1). This may reflect the strong influence of seawater in the formation of saltmarsh soil and the history of caustic soda activities within the study area. Soils with relatively acid pH (< 5) are more likely to leach heavy metals (lead, copper, and zinc) from their profiles than more neutral soils (Rawlins, O'Donnell & Ingham, 2003). According to Kalbitz & Wennrich (1998), cadmium and zinc mobilisation depend on soil pH and mobile element content while Beesley (2010) shows that zinc was positively correlated with pH whereas aresnic was negatively correlated with soil pH. According to Kashem & Singh (2001) soil pH increased with flooding time while the concentration of cadmium, nickel and zinc in soil solution decreased with flooding time. In the present study, the soil pH shows both positive and negative effects on the amount of redox potential, electrical conductivity, soil organic carbon storage, and heavy metal distribution and mobility within the study area (Table 4.21). The redox potential values in the present study vary from -89 to 103 mV (Table 4.1 and Appendix 1). The low-lying sampling locations having more negatives values compared to the higher elevation sampling loctions (Table 4.1 and Appendix 1). This is in corroboration with Pezeshki & DeLaune (2012) report showing negative values of redox potential in wetlands and positive values in upland at different depths. Reddy & Delaune (2008) and Pezeshki & DeLaune (2012) reported that in a typical wetlands soil, redox potential values vary from –300 to 700 mV with a total range of about 1000 mV. The soil electrical conductivity values indicate that low-lying sampling locations were higher than the higher elevation locations across the study area at different sampling depths (Table 4.1), indicating

higher soil electrical soil conductivity within the surface soil compared to subsoils. Friedman (2005) reported that water content and electrical conductivity of a soil solution are indeed the major factors affecting its apparent electrical conductivity, which justifies the assessment of salinity from apparent electrical conductivity measurements. Periodic measurement of soil electrical conductivity is shown to define the controlling geochemical processes and the potential for alteration of these processes by changes in land use/land cover and wetland management (Gerla, 2013). In the present study, the soil electrical conductivity shows positive correlation with heavy metal contamination (Table 4.2) within the low-lying sampling location compared to the higher elevation locations. This may be due to the impact of tidal flooding and high concentration of heavy metal within the lowland sampling location compared to the upland location. Williams et al. (1994) reported that electrical conductivity can affect sediment characteristics due to its indirect effects on particulate, nutrient and contaminant deposition in estuaries and fringing salt marshes. Therefore, the differences observed in the soil pH, redox potential, electrical conductivity across the study area are likely controlled by several factors such as history of industrial activities, concentration and mobility of heavy metal, frequency of tidal inundation, land uses/land cover types and wetland management.

The soil organic carbon was distributed at different depths across the present study. There was a general trend showing that organic carbon content decreased from the surface layer to the subsoil layer (Figure 4.3 and Appendix 1). This may be due to the fact that the surface layer received a higher input of biomass from the plants growing in the soils. Significant positive and negative relationships exist between heavy metal concentration with soil organic carbon across the sampling location (Tables 4.3, and 4.21 and Figure 4.4). The majority of this positive relationship was observed in the low-lying sampling locations compared to the higher elevation locations (Table 4.3). This could due to the influence of high soil bulk density in the low-lying sampling locations, resulting in soil organic carbon storage (Figures 4.2 and 4.3). However, the soil pH, Eh, EC played a more major role than the heavy metal concentration in the amount of soil organic carbon stored in the present study (Table 4.21). The tendency for heavy metals to form a complex with soil organic carbon has been reported by (Stevenson 1994; Kabata-Pendias & Mukherjee, 2007; Reddy & Delaune, 2008). Harland et al. (2000) reported that metal concentrations are strongly correlated with soil

organic carbon and particle size, resulting in distribution patterns that reflect sediment characteristics and dynamics rather than the position of input sources. Thus, the distribution of soil organic carbon in the present study was influenced by several factors including soil sampling depths, heavy metal concentration, soil pH, redox potential, electrical conductivity, bulk density and land uses/land cover types.

4.5.2 Heavy metal concentrations in soil under the field condition

The heavy metal distribution in the present study could be control by several factors such land use, depths, historical contamination, interactions with other metals, soil pH, redox potential, and electrical conductivity. The mean values of arsenic, cadmium, chromium, copper, lead and zinc concentrations were higher in the lowland (grazing saltmarsh, non-grazing saltmarsh, and reed bed) compared to the upland (natural grassland, rough grassland, and broad-leaved woodlands (Figure 4.5 and Appendices 2 and 3). According to Ander, Cave, Johnson & Palumbo-Roe (2012), the normal background contamination level of arsenic for the Upper Mersey estuarine floodplain is 33.4 mg/kg but from the findings reported here arsenic concentration was elevated (Table 4.4). Guo, DeLaune & Patrick (1997) reported that arsenic concentration is influenced by the redox chemistry of iron (III) and manganese (IV) oxides during oxidising conditions while in the present study arsenic concentration was influenced by several factors like the soil pH, redox potential, soil organic carbon and interaction with other metals (Tables 4.19, 4.20 and 4.21). Cadmium concentration in the present study was above the normal background contamination level for English soil (Table 4.6). The normal background concentration of cadmium for the entire English soil was reported to be 6.46 mg/kg while the background contamination level of cadmium for the study area was reported as 0.74 mg/kg (Ander et al., 2012). According to Guo et al. (1997), cadmium transformations were controlled by both iron (III) and manganese (IV) oxides and carbonates. Higher concentrations of cadmium, chromium, copper, lead, nickel, and zinc within the subsoil in sediments were reported by Gwynne (2004). In the present study, higher concentration of arsenic, chromium, copper, lead and zinc was found within 15–30 cm depth compared to 0–15 cm depth across the study area except cadmium (Tables 4.4, 4.6, 4.8, 4.10, 4.12, and 4.14). This is in corroboration with (Li & Shuman, 1996) whose report suggested that cadmium accumulation in the topsoils may be probably because of their affinity with the soil orgnic matter fraction. However, Martin (2000) reported high concentration of heavy

metals in estuarine floodplain with increased depth due to metal deposition during floods. Cundy et al. (2003) reported that higher concentrations of heavy metals is mainly associated with fine sediments while Enya et al. (2011) shows co-migration of iron and fine soil particle in subsoil compared to topsoil. Olafisoye et al. (2013) shows that mobility potential of heavy brings about vertical metals' trends in soils. The generally lower concentration of heavy metals in the surface soil layer, as compared to the subsoil layer, suggests a trend of reduced accumulation of heavy metals in the soils over time (Enya, Lin, & Qin, 2019). This could be explained by the decrease in heavy metal loadings from the upper catchment due to the de-industrialisation in recent decades.

The reasons for the elevated concentration of chromium, copper, lead and zinc in the present study may be due to several possible explanations. The present study shows that chromium behaviour was influenced by the soil pH, redox potential, soil organic carbon and interaction with other metals (Table 4.21). Ridgway et al. (2003) reported high concentration of chromium in sediments in England and Wales which may be due to coastal source and anthropogenic inputs. Jones & Turki (1997) reported elevated concentration of chromium at the upper layers of the sediment in UK estuaries. Santonen, Zitting, Riihimäki, & Howe (2009) reported that chromium behaviour was governed by the redox chemistry of iron (III) and manganese (IV) oxides under oxidising conditions while at reducing conditions chromium was controlled primarily by insoluble large molecular humic material and sulphides. The higher concentration of copper observed in the present study (Table 4.10), may be due the high amount of redox potential obtained across the study area and historical contamination due coal mining, dye production and sewage as detailed in Figure 3.5. This is in corroboration with Guo et al. (1997) which reported that increased in sediment redox potential will result to an increased in the concentration of copper. A history of high concentration of lead within the study area is detailed in Chapter 3. According to Rowlatt & Lovell (1994), lead concentrations were found to be markedly elevated near the north-east coast of England due to industrial inputs and erosion products. Guo et al. (1997) suggested that sediment redox potential will increase the values of lead while the present study also shows a positive correlation beween lead and redox potential (Table 4.21). The concentration of zinc distributed across the study site was generally elevated and shows significant variation with sampling depth (Table 4.14). The normal background concentration of Zinc for English soil is 129.0mg/kg (Appleton et al.,

2013). Rowlatt & Lovell (1994) reported that zinc concentrations were found to be markedly elevated near the north-east coast of England due to industrial inputs and erosion products. Therefore, the current contamination level of chromium, copper, lead, and zinc may be due to continuous pollution of the estuarine floodplain from human factors, resulting to high redox potential and electrical conductivity.

The metal fractionation in the present study could show that heavy metal exists in different forms. Naturally, metals are bound to different matrices in the soil. Some are in soluble, exchangeable, oxidisable and residual forms (Passos, 2010). The different fractions of arsenic, cadmium, chromium, copper, lead, and zinc in the present study indicates that these metals are sparse in soluble and exchangeable fractions and are mostly bound in oxidisable and residual form across the sampling locations (Table 4.5, 4.7, 4.9 4.11, 4.13, and 4.15). This agrees with Castillo, Alonso, Cordero, Pavón, & de Torres (2011) findings. The effects of heavy metal bound to oxidisable and residual forms have also been reported by Hurley et al. (2017) in assessing the metal contamination of bed sediments in the Irwell and Upper Mersey catchments. Ashrafi, Mohamad, Yusoff & Hamid (2015) reported an exchangeable form of cadmium in the soil which was then transformed into the residual fraction in order to lower cadmium mobility in the soil.Tessier et al. (1979) reported that under oxidising conditions in natural waters, organic matter can be degraded, leading to a release of soluble trace metals like cadmium, copper, lead, and zinc, while in the residual phase, metals are not expected to be released in solution over a reasonable time span under the conditions normally encountered in nature. Heavy metals existing in an oxidisable form are likely to have effects on the environment if there are any sudden changes in the environmental factors like pH and temperature changes (Min et al., 2013). This is because in a strongly acidic condition and high temperature most of the trace metals are found in exchangeable forms and can easily be released into the environment. This may imply that the heavy metals in the present study are immobilised in the soil column and as such will not have effects on the environment except there is a sudden changed in soil pH, temperature and salinity.

The present study site is linked with historical contamination due to chemical industries. According to Hurley et al. (2017) the elevated concentration of arsenic and

chromium within Mersey catchment are linked to historical activities coming downstream from Bolton while higher copper found in Mersey catchment is because of historical activities such as blue and green dyes production coming from Rochdale (Figures 3.4 and 3.5). Textiles production, dyeing, bleaching, and pesticides use are linked to elevated concentration of lead and zinc (Ademoroti et al., 1992; West et al., 1999; Deepali & Gangwar, 2010). Harland et al. (2000) reported (arsenic, cadmium, chromium, copper, lead, and zinc) concentration within the Mersey estuarine floodplain from 1974 to 1998. The mean concentration values for arsenic appear to have risen from 0.3 mg/kg in 1975 to 35.1 mg/kg in 1978 and 30.0 mg/kg in 1981 and declined relatively to 0.7 mg/kg in 1998. Cadmium level was higher in 1974 to 1976 compared to 1977 to 1981 and then steadily increased from 1994 to 1998 while the chromium concentration follows a similar trend to arsenic. Copper concentration levels were reduced from 1987 to 1991. Lead values were higher from 1974 to 1991 and were drastically lower in 1992 to 1993 and further increased from 1994 to 1998 while zinc concentration was higher in 1974 to 1975 compared to 1976 to 1994 and further increased from 1995 to 1998. The present study shows that the concentration of arsenic, chromium, copper, lead, and zinc was higher than the values reported by Harland et al. (2000) (Tables, 4.4, 4.6, 4.8, 4.10, 4.12, and 4.14). The concentration of arsenic, chromium, copper, lead, and zinc from the present study were most similar to the values reported by Hurley et al. (2017). However, Harland et al. (2000) only collected samples from 2 cm depths while in the presen study, the sampling collection was carried out to at 30 cm depths. Therefore, the variation in the recent findings may be associated with many factors such as sampling depths, municipal and industrial waste that will easily impact on the estuarine floodplain.

4.5.3 Heavy metal concentration in plant tissue

The plant tissue in the present study shows marked variation in the concentration of heavy metal in the root and shoot portions. The concentration of heavy metals in the root and shoot portions shows significant variation across the study site (Tables 4.17, 4.18 and Appendices 4 and 5). Higher concentration of the heavy metals was stored in the root portion compared to the shoot portion (Tables 4.17 and 4.18). Normal heavy metal concentration levels in shoots of plants for lead, copper and zinc were reported by Yanqun, Yuan, Schvartz, Langlade & Fan (2004) to be 5, 10, and 100 mg/kg, respectively. Concentrations exceeding these levels could induce toxicity in plants and cause toxic effects in animals feeding on them

(Annenkov, 1982). In the present study, the concentration of lead and copper (13.02 and 13.25 mg/kg respectively) at grazing saltmarsh exceeded the normal level (Table 4.17), and as such could have effects on the grazing cattle. According to Statutory Instrument (1995) report, in preparing the animal feeds there is a legal limit set by the Feeding Stuffs Regulations in the UK for the lead content of a complete diet feed (5 mg Pb/kg), with 10 mg Pb/kg allowed in raw material foods. The maximum-permissible level in the feed of cattle or sheep in the USA is 30 mg Pb/kg (National Research Council, 1980). However, death of four cows were reported in the third year of grazing in the present study site due to salt poisoning or poor husbandary from the landowner's view. There was no further assessment to know if this was linked to the level of heavy metal contamination. According to Yoon, Cao, Zhou & Ma (2006) and Li, Luo & Su (2007), BCF, TF and BAC values greater than one are used to assess the potential of plant species for phytoextraction and phytostabilisation. In the present study, BCF was < 1 for arsenic, cadmium, copper, iron, and lead but > 1 for chromium, manganese, and zinc while TF was < 1 for copper but > 1 for chromium, iron, manganese, lead, and zinc (Table 4.18). BAC was < 1 for arsenic, cadmium, copper, iron, and lead was > 1 for chromium, manganese, and zinc (Table 4.18). This is an indication that the study site has the potential for phytoremediation for the elements with BCF, TF, and BAC values greater than one compared to the values less than one. Since the rooting zone of saltmarsh plants could extend beyond the surface soil layer, assessment of soil heavy metal risk to the grazed plants and the grazing animals is not sufficient. For more accurate evaluation of the environmental risk from the contaminated soils in the study area, both surface soils and subsoils need to be considered with reference to the distribution pattern of roots for the plants of concern.

In conclusion, this chapter presents the baseline data that was necessary for the effective selection of sampling locations with elevated concentration of heavy metal. Heavy metal concentration in the soil-plant system within the Upper Mersey estuarine floodplain was high in the lowland compared to the upland. This was greatly influenced by several factors such as the land uses/land covers, soil pH, redox potential, electrical conductivity, interaction with other metals and the matrices or the forms with which the heavy metals exist. This will have implications on the heavy metal accumulation, bioavailability, and immobilisation from the soil to roots and shoots. The present study indicates that the lowland sampling locations (saltmarshes and reed bed land uses/land covers) had an elevated

concentration of arsenic, chromium, copper, lead, and zinc above the background levels for English soil and Wales compared to the upland sampling locations. This may result to harmful effects to the human and animal grazing in this area and, also, cause pollution of the underground water system through leaching. Although, the different fractions of heavy metal indicate that the concentration of the metals was highly bound to oxidisable and residual form compared to the soluble and exchangeable forms. This may cause the heavy metal to be more immobilised and cannot be easily transferred from the soil column to the plant roots and then to the plant shoots and cannot also pollute the underground water thereby not causing any potential effects to the environment. However, any potential change in environmental factors, like soil pH, salinity and temperature, could alter the oxidisable form making the metals to exist in soluble and/or exchangeable form. The soluble and exchangeable form of heavy metal may likely have a negative effect on the underground water and uptake by plant roots thereby causing harm to human and the animal grazing within the site. The soil organic carbon in the present study shows both positive and negative correlation with heavy metals and may be responsible for the complex bond formation. Soil organic carbon (SOC) was generally high within the grazing and non-grazing saltmarsh compared to other sampling locations, having the highest percentage due to the high turnover rate and anaerobic conditions. This has improved our understanding of the heavy metal interactions with soil organic carbon, and plant uptake.

The soil pH, redox potential and electrical conductivity interactions with heavy metal concentration and soil-plant system shows a marked variation across the study area. The soil pH, redox potential and electrical conductivity shows positive correlation with soil organic carbon compared to the heavy metal concentration. The soil pH values within the grazing saltmarsh (GSM), non-grazing saltmarsh (GSM-N) and reed bed (RB) were slightly alkaline, reflecting the influence of tidal inundation in the formation of saltmarsh soil. The redox potential (Eh) (mV) of the study site shows negative concentration across the wetland sampling locations indicating an increase in soil organic matter and anaerobic conditions. Electrical conductivity (EC) of the soil indicate different levels of salinity across the study site, notably the lowland sampling locations (GSM, GSM-N, RB, SM1, and SM2), because of tidal inundation. EC shows both a positive and negative perfect relationship with selected heavy metal concentration indicating that as one the EC deviates from the mean, the heavy metal

deviates in the same and opposite directions respectively. Plant species have shown some potential for phytoremediation for metals like chromiun, iron, manganese, lead and zinc. Hence, otational cattle grazing to areas of low concentration of metals will be beneficial to both the cows' owner and the food chain. The soil pH, redox potential and electrical conductivity played major roles in the understanding of the interactions between heavy metal and soil organic carbon.

5. Variations in Soil Organic Carbon Storage

5.1 Introduction to the chapter

Soil carbon storage and soil respiration were found, in this research, to be important factors affecting global and national carbon budgets as detailed in Chapter 1 and 2. There have been several efforts by international initiatives such as the United Nations programme on reducing emissions (such as carbon dioxide, nitrous oxide, and methane) from deforestation and forest degradation (UN REDD), towards protecting terrestrial green carbon storage through forest conservation. The potential for carbon storage in vegetated coastal ecosystems such as saltmarshes, termed 'blue carbon' has, however, not received such attention (da Silva Copertino, 2011; Fourqurean et al., 2012; Luisetti, Jackson, & Turner, 2013). According to UK National Ecosystem Assessment (NEA) (2011), saltmarshes remain one of the ecosystems that can store large amounts of soil carbon. The potential of this ecosystem provides a strong argument for the protection and restoration of this habitat (Mcleod et al., 2011).

The global soil organic carbon map V1.0 was launched on World Soil Day 2017 (5 December 2017) by the Global Soil Partnership (GSP) and the Intergovernmental Technical Panel on Soils (ITPS). It is an important stepping stone to understand better the current soil organic carbon stock stored and soil's potential for further sequestration (FAO, 2017). Soil differences dictate that the soils must be managed differently and will behave differently when used for agriculture, forestry, sewage disposal, foundations, pavements, and other purposes (Olson, 1981). Soil characteristics are greatly influenced by climate, topography, parent materials, vegetation and disturbance from human activity (Jenny 1941). The morphological, physical and chemical properties will help to characterise and classify the pedons into specific soil order or type. Soil characterisation is necessary to interpret the effects of land use/land cover management on soil organic carbon stocks (Wiesmeier et al., 2015). Soil profile descriptions have been used to classify the soil into different uses (Buol, Southard, Graham & McDaniel, 2011). The dynamics of soil organic carbon and carbon dioxide emission based on the four UK seasons have not been completely studied. Hence, models for local, national, or regional carbon budget are required. This need is reflected in objective 2 of this research: to assess the dynamics of soil carbon storage based on seasonal monitoring by looking at the input of carbon and evaluating the avoided emission and costs for storing

carbon and is the focus of this chapter. Addressing this objective will improve our understanding as to whether an estuarine floodplain with a history of contamination of inorganic pollutants will act as a carbon sink or source of carbon. The research reported in this chapter is phase two and three of the research in Section 3.4.

5.2 Material and methods

This section provides information on the methods used for field sampling, sample preparations, and laboratory analyses. The site description and sampling designs have already been detailed in Chapter 3 (Section 3.3.1). More detailed studies were conducted within grazing saltmarsh, non-grazing saltmarsh, and reed beds located at Widnes Warth, on the north bank of the Upper Mersey Estuary, shown in the research flow charts (Figures 3.6 and 3.7). This site is a 45-hectare land area dominated by *Elytrigia repens* (common couch) and *Phragmite australis* (common reed, locally called reed bed by land owners). The sampling designs in Widnes Warth were based on the existing designs constructed by Smith (2013) for his PhD research which was completed in 2013.The design incorporates exclosures, which prevent cattle grazing the vegetation within the exclosure and hence provides an opportunity to study grazed and un-grazed land that are near to each other (Figure 5.1). In his studies, Smith (2013) used a grazing density used was between 1 and 1.25 cows per hectare, in line with generally accepted conservation grazing guidelines. This level of grazing has been maintained after the completion of Smith's work on this site.

Several authors have used this method to evaluate vegetation in grazing trials in saltmarshes (Jensen, 1985; Bazley and Jefferies, 1986; Esselink, Fresco, & Dijkema, 2002; Bouchard et al., 2003; Smith, 2013). Smith (2013) used a total of 5 hectares for grazing area and six 10 m × 10 m size exclosures (see Figure 5.1). The 1 m × 1 m area within and outside the non-grazing area are the quadrats used in this research to achieve the sampling objectives considering different land use/land cover in an effective management regime. Mini pits (45 cm x 45 cm x 70 cm) have been used to describe soil profiles in a valley bottom with a shallow water table (Enya, Omueti, & Akinbola, 2011). Various laboratory procedures were used for soil sample analysis: the loss on ignition method has been used by many researchers to measure the percentage of total organic carbon in soils and sediments (Matejovic, 1997; Gywnne, 2004; Smith, 2013). The method used in this study is from Smith (2013) and Walkley

& Black (1934) while the particle size distribution was determined by the hydrometer method (Bouyoucos, 1951). In the study reported here soil samples were taken from two soil layers: 0-15 cm and 15-30 cm to have a two-view approach. The locations were those used by Smith (2013) in his PhD studies.

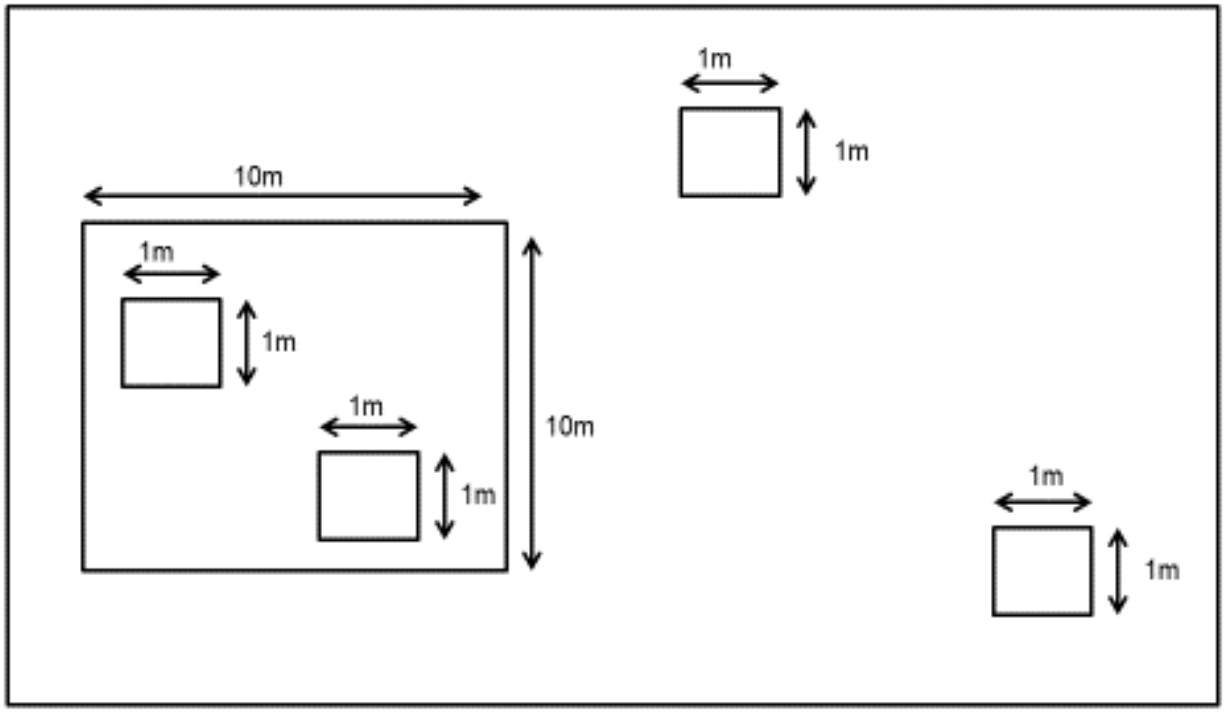


Figure 5. 1 The arrangement of the design of the exclosures (10m X 10m) and quadrats (1m X 1m), sampling was carried out in 3 replicates within the grazing and non–grazing sampling locations

5.2.1 Field monitoring

In 2016, field monitoring was only undertaken in winter, spring and summer. The year-round field monitoring covering the four seasons (winter, spring, summer and autumn) was completed in 2017 with a total of three hundred and sixty samples collected. At each monitoring season/period, ninety composite soil samples were collected within 0–15 cm and 15–30 cm depths across grazing, non-grazing and reed bed land uses/land covers located at the lowlands of the estuarine floodplain (that is, 3 replicates from 5 sampling points within the two depths respectively). The whole plants (shoot and root) within 1 m^2 around the auger hole were collected for determination of biomass (see Figure 5.1). Three mini pits (45 cm x 45 cm x 70 cm) were sunk, one at each land use/land cover, and the morphological properties

were described under moist field conditions using Munsell colour chart. The mini pits samples were collected using a knife based on the genetic horizon, starting from the lower horizon to the top. This is to avoid contamination of soil from different horizons, and a soil auger was used to collect an additional 30 cm depths. Soil samples for measurement of bulk density were collected using core samplers. The empty cores had the volume of 106.043 cm^3 and were driven into the soil until the tubes were level in the ground and bulk samples were collected with the help of a knife without disturbing the soil inside the cores and were transferred into labelled bags and then taken to the laboratory. Plant biomass was collected within the B area (see Figure 5.2) packed into sampling bags and was taken to the University of Salford laboratory.

Figure 5. 2 Sampling techniques for soil and plant biomass (A= soil auger, B= length of quadrat in a 1 m^2 area, Cs= 5 sampling points bulked to have a composite sample that is representative and the point where the plant biomass was collected).

5.2.2 Sample preparation

Soil samples were sorted out in the laboratory into labelled envelopes and were oven dried at 40 °C until a constant weight was obtained. Soil samples were crushed and sieved and were passed through a 2 mm sieve for laboratory analyses. Plants samples collected in

m^2 quadrats were weighed fresh (W1) before being oven dried at a maximum temperature of 70 °C using labelled envelopes. The oven dried weight was also recorded (W2) and the water content of the vegetation was calculated as W1 - W2 to get dry weight of the plant W3 in grams (g). The dry weight was presented in kilograms, the area in square metres was converted into hectares and the results were presented in kg/ha. The dry weight value is then used to convert recorded fresh weights from the field to dry weights for the calculation of the plant biomass according to Coombs, Hall, & Long (2014).

5.2.3 Laboratory analysis

Laboratory analyses for physical and chemical properties was carried out using different laboratory protocols, as listed below.

5.2.3.1 Bulk density

The bulk density of the soil was determined using a combination of Gywnne (2004) and Smith (2013) procedures. The core samples from the field were weighed fresh and recorded and then put into the oven at 105 °C and the dried weight was recorded until a constant weight was obtained. The bulk density was calculated as the dry weight of the core samples in grams/the volume of the core samples used in centimetres cubed. The bulk density values were used to calculate the soil carbon stock or pool within the study area by multiplying the bulk density values by the carbon distribution within the study site. The porosity of the study area was also calculated from the bulk density using 1-bulk density/particle density. Particle density is the weight of an individual soil particle per unit volume (g/cm^3). The particle density of the soil in the temperate region is constant (2.65 g/cm^3).

5.2.3.2 Particle size distribution

The particle size distribution was determined by the hydrometer method (Bouyoucos, 1951). Fifty grams of air-dried soil samples which passed through a 2 mm sieve were poured into a 250 ml conical flask which served as a dispersion cup, 10 ml of 5% calgon solution was added. The calgon used helps to disperse the soil particles into different sizes or fractions. One hundred millilitres of deionised water were poured into the conical flask and mixed using a mechanical stirrer for 5 minutes. The mixture was then transferred into a 1,000 mL cylinder with all soil particles rinsed into the cylinder with deionised water until the volume in the

cylinder was made up to the 1,000 ml mark (see Figure 5.3). The cylinder was sealed using a stopper and then thoroughly mixed. A hydrometer was inserted gently into the mixture in the cylinder and the first hydrometer reading (H_1) was recorded at 40 seconds along with the corresponding temperature reading (T_1). The second hydrometer reading (H_2) was recorded with corresponding temperature reading (T_2), using a thermometer, after standing for two hours. H_1 gives the weight of the silt plus the clay fractions in the suspension while H_2 gives the clay fraction in the mixture only. The hydrometer was calibrated at 20 °C so any temperature above or below this requires a temperature correction factor. The correction factor is ± 0.3 x t °C (where t= temperature in degrees Celsius). For temperatures above 20 °C, the values will be corrected using + 0.3 x t °C, while temperatures below 20 °C will be corrected with -0.3 x t °C. The textural class of the soil was determined using a textural triangle according to FAO, 2011 guidelines.

Figure 5. 3 **Different soil horizon samples used for particle size analysis**

5.2.3.3 Soil pH, Eh and EC

Meters were used to measure soil pH, Eh and EC. pH and Eh were measured using an HI-2020 edge meter and EC was measured with a Mettler Toledo EC meter. The procedure involves the weighing of 5 g of 2 mm sieved soil into 150 mL bottles. Twenty-five millilitres of

deionised water were added and shaken with a mechanical stirrer for 5 minutes before inserting the pH, Eh and EC probes.

5.2.3.4 Soil organic carbon determination

The soil organic carbon content was determined using the loss on ignition and Walkley-Black methods. The loss on ignition method was carried out in the University of Salford laboratory, the procedures involved have been detailed in Chapter 4. The Walkley & Black (1934) wet oxidation method using 20 mL conc. Sulphuric acid (H_2SO_4) and 10 ml potassium dichromate (KCr_2O_7), later titrated with standard ferrous sulphate solution according to Nelson and Sommers (1982) was conducted using the external resource of the University of Ibadan laboratory. This method destroyed the carbonates and silicate bound to organic carbon. The data from the two methods were correlated across the sampling locations (GSM, GSM-N and RB) and were positively correlated ($R^2 = 0.9681$, $R^2 = 0.7687$ and $R^2 = 0.6503$ respectively). This is to know how reliable the loss on ignition method is to the Walkley-Black method

5.3 Statistical analysis

Data analyses were carried out using IBM SPSS 24.0 version statistical tool to test for significant differences among the different land uses/land covers using multivariate analysis, one-way ANOVA, while the interaction between the data was determined using Pearson's correlation coefficient. A Post hoc multiple comparison for observed means from the IBM SPSS 24.0 was carried out using Duncan Multiple Range Test to further separate the mean to test for significant differences using lower case alphabets (a, b, c, d, etc.), were the alphabets indicates different degree of significance (at $P < 0.05$). Integrated Valuation of Ecosystem Services and Tradeoffs (InVEST) blue carbon model (developed by the Natural Capital project) was used to predict avoided carbon dioxide emission and avoided emission valuation (costs) for storing carbon in the land use/land cover types according to Bernhardt (2015). The blue carbon model used the carbon stock and the annual carbon sequestration to calculate the avoided emission and avoided emission valuation (Tallis et al., 2011; Sharp et al., 2014 and Bernhardt, 2015). The original data were extrapolated to tonnes per hectare and the price of carbon was based on current government prices from 2016 to 2021.

5.4 Results

5.4.1 Soil morphological characteristic of Widnes Warth pedons under three land uses/land covers

The soil colour of Widnes Warth pedons under moist conditions were dark greyish brown (2.5Y 4/2) surface horizons to dark olive brown (2.5Y 2.5/3) subsoil at GSM location, GSM–N had dark grey (2.5Y 4/1) surface horizons to greyish brown (2.5Y 5/2) subsoil, while RB pit shows grey (2.5Y 6/1) topsoil, and black (2.5Y 6/1) and greyish brown (2.5Y 6/4) subsoil. The soil structures were medium sub angular blocky, angular blocky and platy, while the soil consistence was friable, slightly sticky, slightly plastic, firm, or very firm (Table 5.1). The horizon boundaries were clear smooth with mottled inclusions within the subsoil and many fine and medium roots at the topsoil.

5.4.2 Physical and chemical properties of Widnes Warth pedons under three land uses/land covers

Results indicated that soil texture was sandy across the three land uses/land covers (GSM, GSM-N and RB) except GSM-N at 55–70 cm depth which was loamy sand (Table 5.2). This may be because of soil transportation and deposition which are characteristic of estuarine floodplains. pH ranged from 6.8 to 7.6 indicating that the pedons were nearly neutral to slightly alkaline (Table 5.2). This may be because of the influence of seawater from tidal inundation and the historical contamination from the production of caustic soda from chemical industries located within the study area. There was no statistically significant difference in pH within the GSM location while GSM-N and RB locations showed statistically significantly higher pH values in topsoil than subsoil. Eh values indicated that there was variation in redox potential. GSM had statistically significantly higher (9.5±3.50a) Eh at topsoil than subsoil (1.7±1.87b). GSM-N and RB data showed statistically significantly higher Eh in subsoil (-13.8±0.70a and -3.7±1.05a, respectively) than topsoil (-29.1±0.06b and -28.3±1.10c, respectively) (Table 5.2). Electrical conductivity values within the Widnes Warth pedons were generally statistically significantly higher within the topsoil than subsoil (Table 5.2). Soil organic carbon content across the pedons indicated that there was an increase in the percentage of organic matter from topsoil horizon A, AB and B than in Bg1 and Bg2 (Table 5.2).

Table 5. 1 Soil morphological characteristic of Widnes Warth pedons under three land uses/land covers

Location	Depth (cm)	Horizon	Colour (moist)	Structure	Consistence (dry and wet)	Boundary	Inclusion	Roots
SJ 52917	GSM							
BNG 85058	0–25	A	2.5Y 4/2	m-sbk	fr, sl st, sl plt	cs	non	M f
	25–30	AB	2.5Y 4/2	abk	fr, sl st, sl plt	cs	non	M f
	30–40	Bt1	2.5Y 2.5/1	abk	fm, st, plt	cs	non	fw f
	40–70	Btg1	2.5Y 2.5/3	abk	vfm, st, plt	cs	mottles	non
	70–100	Btg2	2.5Y 2.5/3	plty	vfm vst, vplt	cs	mottles	non
SJ 52759	GSM-N							
BNG 84975	0–20	A	2.5Y 4/1	m-sbk	fr, sl st, sl plt	cs	non	M f
	20–39	AB	2.5Y 2.5/1	abk	fr, sl st, sl plt	cs	non	M f
	39–55	Bt1	2.5Y 5/2	abk	fm, st, plt	cs	non	fw f
	55–70	Btg1	2.5Y 5/2	abk	vfm, st, plt	cs	mottles	fw f
	70–100	Btg2	2.5Y 5/2	plty	vfm vst, vplt	cs	mottles	non
SJ 52851	RB							
BNG 85046	0–38	A	2.5Y 6/1	m-sbk	fr, sl st, sl plt	cs	non	M m
	38–46	AB	2.5Y 2.5/1	abk	fr, sl st, sl plt	cs	non	M m
	46–54	Bt1	2.5Y 6/4	abk	fm, st, plt	cs	non	fw m
	54–70	Btg1	2.5Y 6/4	abk	vfm, st, plt	cs	mottles	fw m
	70–100	Btg2	2.5Y 6/4	plty	vfm vst, vplt	cs	mottles	fw f

m-sbk= medium sub angular blocky, abk= angular blocky, fr= friable, sl st= slightly sticky, sl plt= slightly plastic, plty= platy, fm= firm, vfm= very firm, vst= very sticky, vplt= very plastic, cs= clear smooth, M= many, f= fine, fw= few, m= medium, GSM= Grazing Salt Marsh, GSM–N= Non–Grazing Salt Marsh, RB= Reed Bed, g= greying evidenced by mottling.

Table 5. 2 Selected soil physical and chemical properties of Widnes Warth pedons under three land uses/land covers

Depth (cm)	Horizon	Clay	Silt	Sand	Texture	pH	Eh (mV)	EC (mS/cm)	SOC (%)
			%						
GSM									
0–25	A	2.6	12.8	84.6	Sand	6.8±0.04a	9.5±3.50a	2.5±0.07a	7.3±0.05c
25–30	AB	2.6	6.8	90.6	Sand	6.9±0.03a	2.9±1.39b	2.5±0.02a	8.2±0.02b
30–40	Bt1	3.6	8.5	87.9	Sand	6.9±0.02a	1.6±0.99b	2.3±0.02b	10.0±0.13a
40–70	Btg1	4.6	16.2	79.2	Sand	6.9±0.03a	0.6±1.51b	1.6±0.00c	4.1±0.03d
70–100	Btg2	6.6	15.6	77.8	Sand	6.9±0.04a	1.7±1.87b	1.4±0.01d	4.0±0.03d
GSM-N									
0–20	A	2.6	10.8	86.6	Sand	7.6±0.01a	-29.1±0.06b	3.6±0.03a	7.8±0.05b
20–39	AB	2.6	8.8	88.6	Sand	6.9±0.05c	-15.4±4.32a	3.4±0.02b	8.9±0.02a
39–55	Bt1	4.6	14.2	81.2	Sand	6.9±0.07c	-10.4±5.21a	2.0±0.01d	3.8±0.05c
55–70	Btg1	12.6	17.8	69.6	Loamy sand	7.2±0.03b	-14.1±1.87a	2.0±0.02d	3.4±0.07d
70–100	Btg2	6.6	15.6	77.8	Sand	7.2±0.01b	-13.8±0.70a	2.3±0.01c	2.3±0.04e
RB									
0–38	A	3.6	12.5	83.9	Sand	7.5±0.03a	-28.3±1.10c	2.1±0.04a	7.7±0.11b
38–46	AB	3.6	12.5	83.9	Sand	7.1±0.03c	-9.8±0.85a	1.7±0.01b	8.9±0.13a
46–54	Bt1	6.6	17.6	75.8	Sand	7.1±0.00c	-9.4±0.57a	1.3±0.00d	2.5±0.05e
54–70	Btg1	6.6	19.6	73.8	Sand	7.3±0.09b	-18.1±4.42b	1.4±0.01c	3.5±0.06c
70–100	Btg2	6.6	19.6	73.8	Sand	6.9±0.03d	-3.7±1.05a	1.3±0.01e	3.0±0.06d

GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, abc= Duncan test at 0.05 level of confidence.

5.4.3 Seasonal variation in soil pH during the year-round monitoring in 2017

Results from the seasonal monitoring at the three selected study sites indicated that soil pH varied over time for both the surface soil layer (0–15 cm) and the subsoil layer (15–30 cm). pH ranged from 7.1 to 8.3, indicating slightly alkaline to moderately alkaline. Different seasonal variation patterns were observed between the surface soil layer and the subsoil layer at the sample study site, and among the different study sites. For the surface soil layer, pH was significantly higher (at $P < 0.05$) in autumn in the following order, RB > GSM > GSM-N compared to other seasons. The subsoil layer showed no significant difference in pH between summer and autumn across the locations compared to other seasons (Figure 5.4).

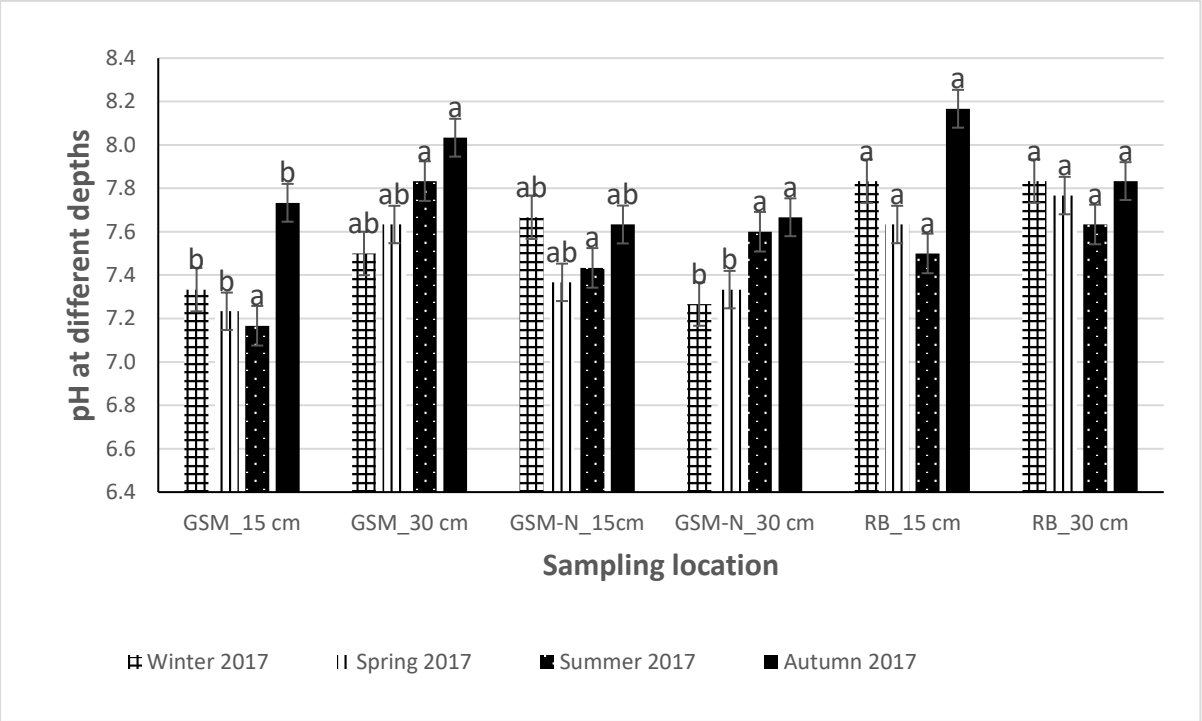


Figure 5. 4 Seasonal pH values within GSM, GSM-N, and RB sampling (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as as a, b, ab= Duncan test at 0.05 level of confidence)

5.4.4 Seasonal variation in soil redox potential during the year-round monitoring in 2017

Redox potential (Eh) values indicated that significant variation exists across the sampling locations at different depths after each season monitoring. Eh values were all negative and were statistically significantly lower in autumn than other seasons at 0–15 cm

depth within GSM-N and RB (see Figure 5.5). There was no significant difference in Eh within GSM and GSM-N at 15–30 cm depth.

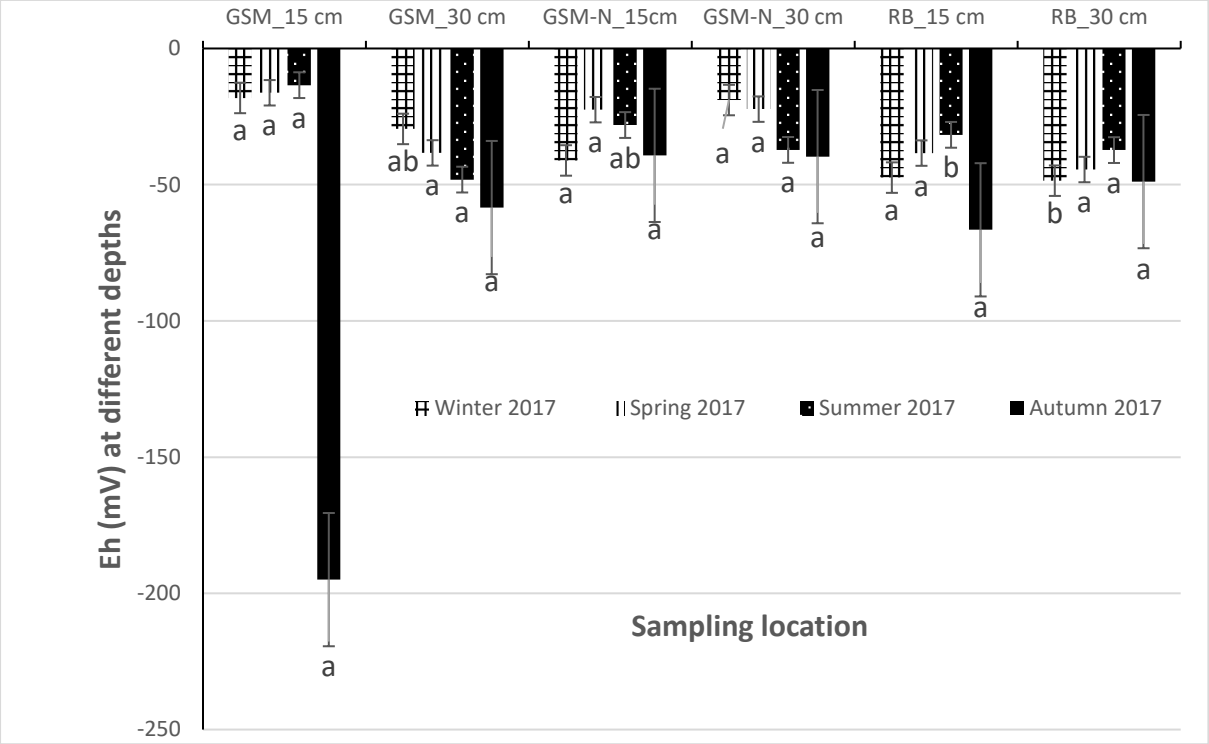


Figure 5. 5 Seasonal Eh (mV) values within GSM, GSM-N, and RB sampling locations (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, ab= Duncan test at 0.05 level of confidence)

5.4.5 Seasonal variation in soil electrical conductivity during the year-round monitoring in 2017

Electrical conductivity (EC) results indicated that there were significantly (at $P < 0.05$) higher values of EC during spring within GSM and GSM-N at 0–15 cm depth compared to other seasons. However, GSM 0–15 cm and 15–30 cm depths did not show any significant difference in EC. The RB location showed significantly higher EC values in winter and summer across the different depths compared to other seasons (Figure 5.6).

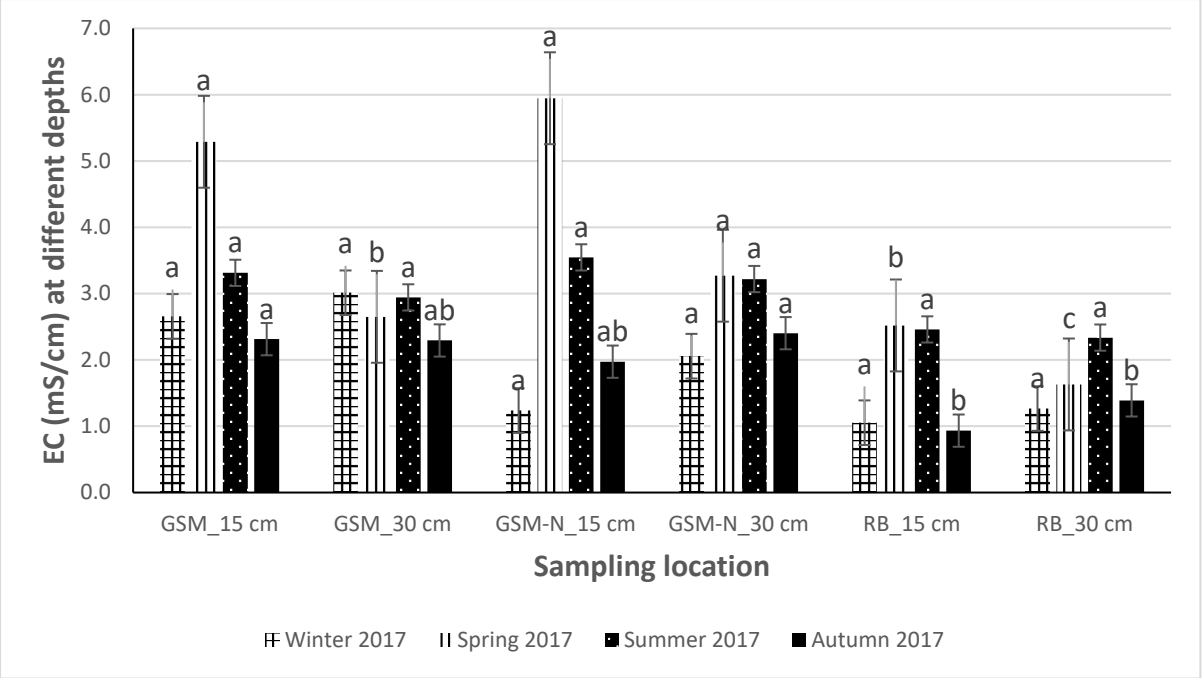


Figure 5. 6 Seasonal EC values within GSM, GSM-N, and RB sampling locations (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, ab, c= Duncan test at 0.05 level of confidence)

5.4.6 Seasonal variation in soil bulk density during the year-round monitoring in 2017

Variation exists in bulk densities following different sampling periods across the different land uses/land covers (GSM, GSM-N, and RB). GSM and RB showed higher values of bulk density compared to the GSM-N location (Figure 5.7). This may be due to trampling by grazing cattle. There were significantly higher values in bulk density during the summer than the other seasons compared to other seasons.

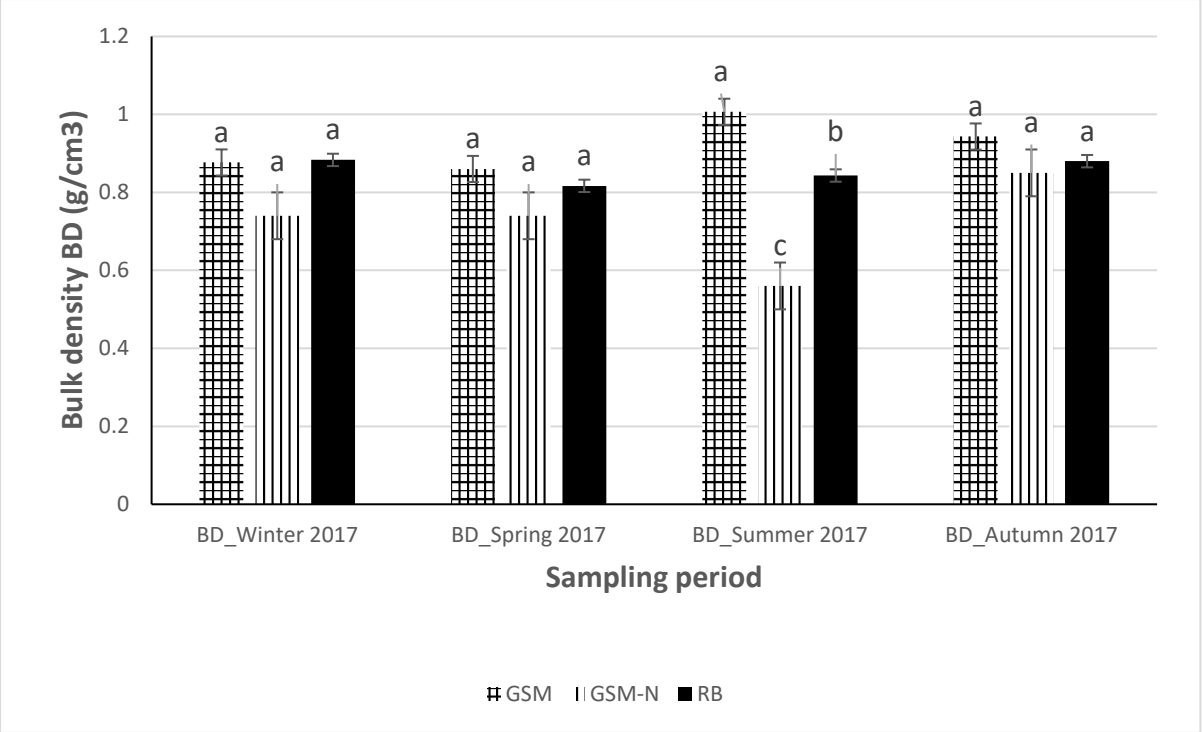


Figure 5. 7 Seasonal bulk density within the sampling locations (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, c= Duncan test at 0.05 level of confidence)

5.4.7 Seasonal variation in soil porosity during the year-round monitoring in 2017

Percentage porosity of the study area varies across sampling locations in different seasons. Generally speaking, the trend in percentage porosity was in the opposite direction to that of the bulk density. This means that as the bulk density values increased, the porosity decreased (Figures 5.7 and 5.8). The GSM-N location showed significantly higher values in pore space compared to GSM and RB during the summer. There was no significant difference in porosity during winter, spring and autumn compared to summer season.

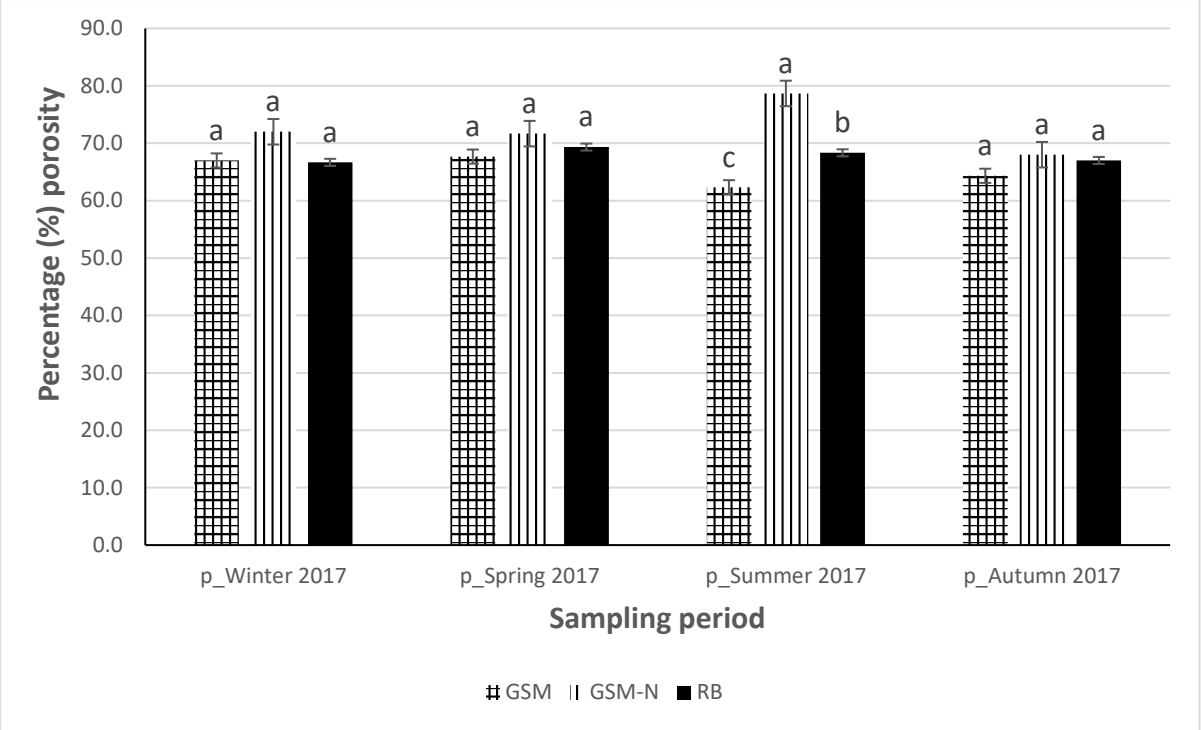


Figure 5. 8 Seasonal percentage porosity within the sampling locations (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, c= Duncan test at 0.05 level of confidence)

5.4.8 Seasonal variation in plant biomass during the year-round monitoring in 2017

Results from the plant biomass across the study area indicated that there were statistically significantly higher values in the GSM location than RB during the winter season (Figure 5.9). The GSM-N location was generally high in plant biomass during spring, summer and autumn but there was no statistically significant difference in these seasons. This may be attributed to grazing activities taking place within GSM and RB. There was a general decrease in plant biomass within the saltmarshes and reed bed during winter due to their ability to go dormant in response to cold weather conditions, causing the plant to dry up. Figure 5.10 shows the relationship between the plant biomass and the sum of heavy in soil across the different monitoring season in 2017. The results indicated that the coefficient of correlation was less than 0.5 across the different sampling seasons, indicating that the heavy metal concentration had minimal impact on the plant biomass during the different monitoring seasons.

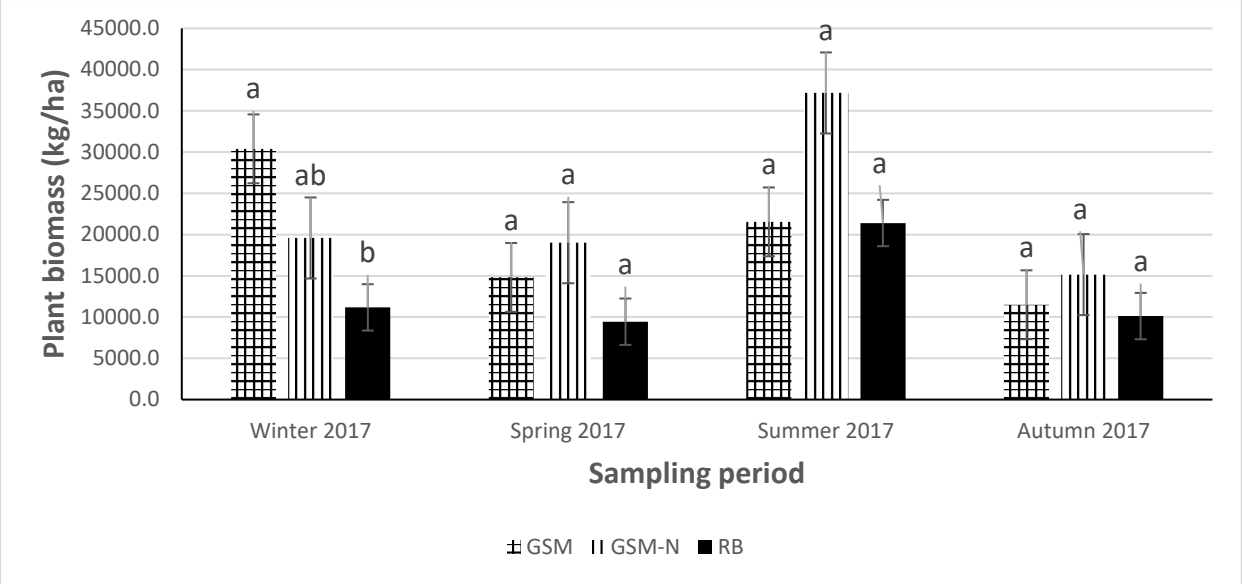


Figure 5. 9 Seasonal plant biomass within the sampling locations (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, ab= Duncan test at 0.05 level of confidence)

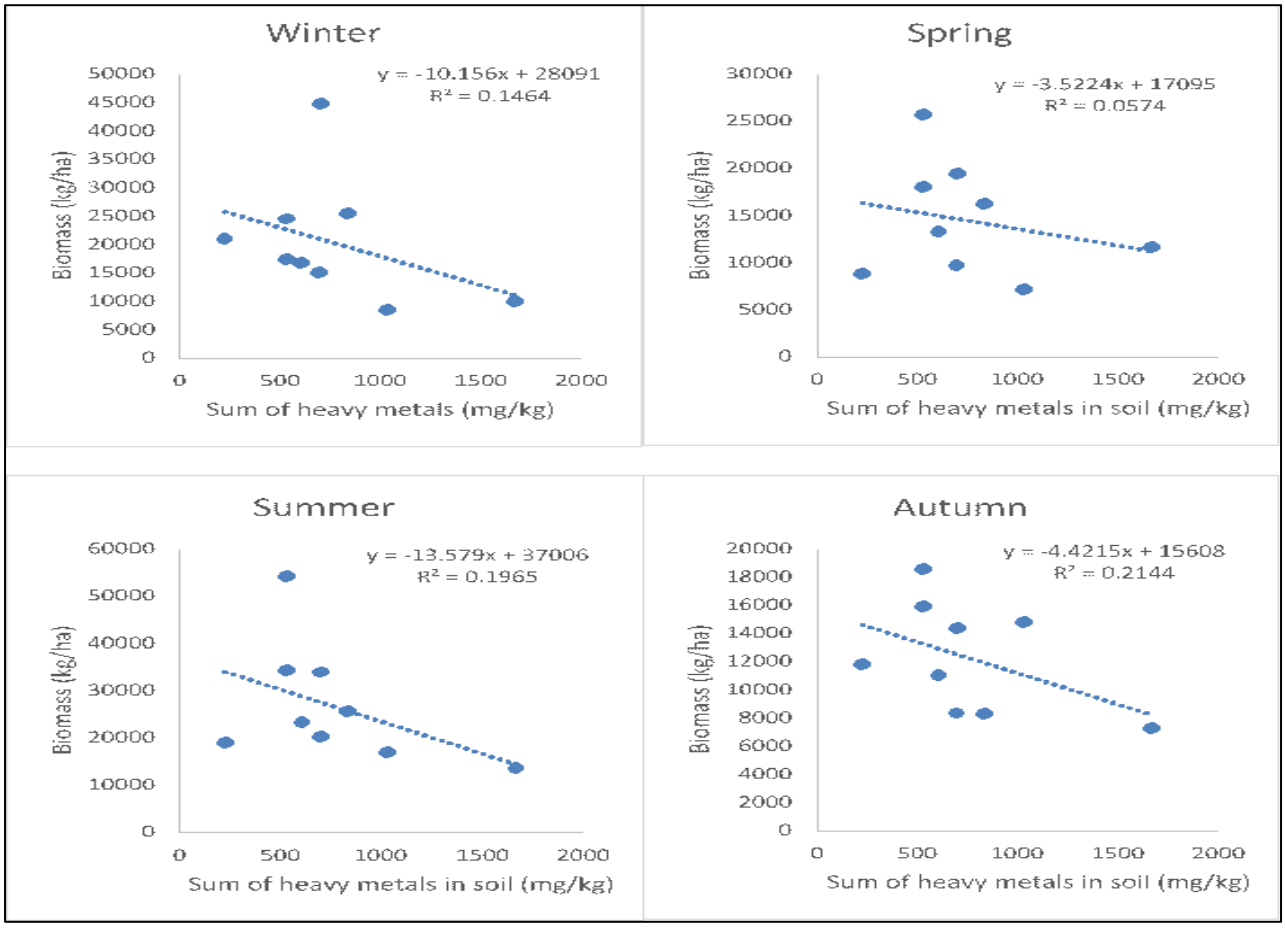


Figure 5. 10 **Relationship between the sum of heavy metals in soils and the plant biomass at different seasons for the three monitoring sites**

5.4.9 Variation in soil organic carbon storage at different study sites

This section will look at the soil carbon dynamics based on different sampling periods to assess the carbon sequestration during the long-term monitoring across the different land use/land cover types. The distribution of soil organic carbon content for the entire estuary is detailed in Chapter 4 (Section 4.3.3), showing that saltmarsh locations were higher in carbon stock than other sampling locations. Seasonal carbon stock results from the Widnes Warth study location indicated that GSM was generally higher across the sampling periods and, as such, GSM locations were higher during winter and summer seasons than spring and autumn (Figure 5.11). The annual carbon sequestration results show that variation exists in the amount of carbon storage in different years during the summer period (Figure 5.12). The amount of carbon stored was higher in GSM during 2015, 2016 and 2017 summers compared to GSM-N and RB. Environmental factors such as frequency of tidal inundation accompanied by material deposition (Figure 5.14) were greater in 2016 prior to the summer sampling period and could have had a positive impact on the carbon stored.

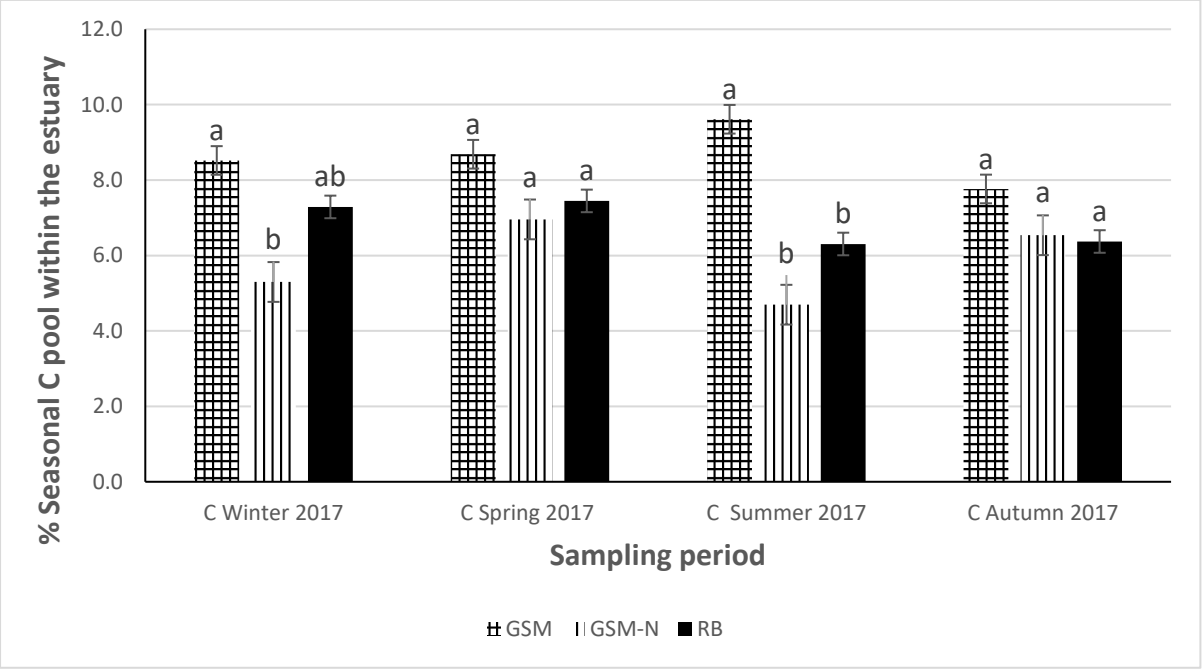


Figure 5. 11 Seasonal carbon pool within the sampling locations (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, ab= Duncan test at 0.05 level of confidence)

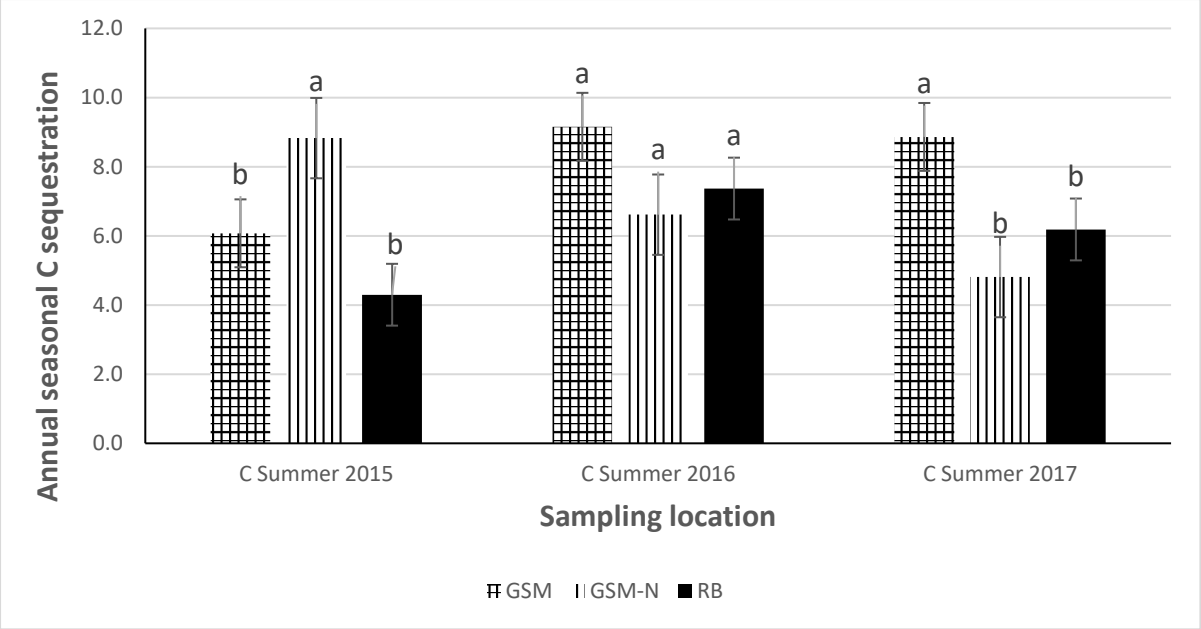


Figure 5. 12 Yearly carbon pool within the sampling locations (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b= Duncan test at 0.05 level of confidence)

Figure 5. 13 **Deposition of materials by tidal impact, date 9th April 2016**

5.4.10 Relationship between SOC, pH, Eh, EC, bulk density, porosity and plant biomass at the monitoring sites

Pearson's correlation coefficient indicated that a relationship exists among SOC, pH, Eh and across the sampling locations and with the annual seasonal monitoring parameters (Table 5.3). The amount of soil organic carbon stored within the sampling locations was negatively correlated by the pH while the Eh and EC were positively correlated across GSM, GSM-N and RB (Table 5.3). Redox potential Eh was more highly correlated with EC (r= 0.972*) and was significant at $P < 0.05$ within the GSM-N sampling location compared to other locations. EC shows a positive correlation with soil organic carbon, but the relationship was not perfect since the coefficient of correlation was less than 0.5. Eh and pH indicated a strong positive perfect relationship with each other (r= -0.983* and r= -0.999**) within the GSM and RB sampling locations compared to GSM-N. The annual seasonal monitoring shows that the soil pH was negatively correlated with Eh, EC, porosity, soil organic carbon and plant biomass (Table 5.3). The annual plant biomass across the sampling locations were positively correlated with soil organic carbon, porosity, Eh and EC. The soil bulk density was negatively correlated porosity (r= -0.998** at $P < 0.01$) whereas the relationship was negative with soil organic carbon and EC (Table 5.3).

Table 5. 3 Pearson's correlation coefficient for SOC, pH, Eh and EC across sampling locations and with SOC, pH, Eh, EC, bulk density, porosity and plant biomass in the annual seasonal monitoring

GSM	SOC (%)	pH	Eh (mV)	EC mS/cm
GSM_SOC	1			
GSM_pH	-0.838	1		
GSM_Eh	0.789	-0.983*	1	
GSM_EC	0.348	-0.646	0.544	1

GSM-N	SOC (%)	pH	Eh (mV)	EC mS/cm
GSM-N_SOC	1			
GSM-N_pH	-0.083	1		
GSM-N_Eh	0.219	-0.949	1	
GSM-N_EC	0.437	-0.877	0.972*	1

RB	SOC (%)	pH	Eh (mV)	EC mS/cm
RB_SOC	1			
RB_pH	-0.269	1		
RB_Eh	0.224	-0.999**	1	
RB_EC	0.105	-0.864	0.859	1

	pH	Eh (mV)	EC mS/cm	Bulk density g/cm3	Porosity (%)	SOC (%)	Plant biomass kg/ha
pH	1						
Eh	-0.347*	1					
EC	-0.618**	0.146	1				
Bulk density	0.159	-0.225	-0.279	1			
Porosity	-0.159	0.237	0.265	-0.998**	1		
SOC	-0.614**	0.261	0.304	-0.057	0.068	1	
Plant biomass	-0.371*	0.184	0.176	-0.266	0.262	0.066	1

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); GSM = Grazing Saltmarsh, GSM-N = Non-grazing Saltmarsh, RB = Reed bed, SOC= Soil organic carbon.

5.4.11 Tonnes of soil carbon stock per hectare at 15 cm sampling depth

The seasonal soil carbon stock and plant biomass carbon were extrapolated to tonnes per hectare (Figures 5.14, 5.15 and Appendices 19, 20, 21 and 22) to determine the avoided emission and costs according to each land use/land cover ability to store carbon within the study area. From the British House of Commons library (House of Commons, 2018), the price of carbon was capped by the Government at a maximum of £18/tCO_2 from 2016 to 2021 to limit the competitive disadvantage faced by businesses and reduce energy bills for consumers. Avoided emission and cost data were calculated based on the addition of the mean annual seasonal carbon stock data for winter 2016 and 2017, spring 2016 and 2017 and summer 2016 and 2017 (Figures 5.14, 5.15 and Appendices 19, 20, and 21). Avoided emission results show that winter 2017 and summer 2017 were higher than spring 2017 while the avoided emission valuation or costs follows the order GSM > RB > GSM-N across the different seasons (Table 5.4). Under the grazing land use, a carbon credit of 288 to 326 tCO_2/ha was achieved, equivalent to a capital gain of £5184 to £5863 per hectare in 2017 based on a price of £18 per tonne of carbon dioxide. This indicated that more carbon dioxide emission was avoided during winter compared to other seasons (Table 5.4)

Table 5. 4 Avoided emission and costs for storing carbon within Widnes Warth site

Avoided Emission and Costs	GSM	GSM-N	RB
AE_Winter 2017 (tCO_2)	325.76	226.82	238.52
AE V_Winter 2017 (£/ha)*	5863.61	4082.73	4293.33
AE_Spring 2017 (tCO_2)	288.09	224.81	229.73
AE V_Spring 2017) (£/ha)*	5185.68	4046.64	4135.13
AE_Summer 2017 (tCO_2)	303.07	206.80	226.50
AE V_Summer 2017 (£/ha)*	5455.19	3722.41	4077.06

AE= Avoided emission, AE_V = Avoided emission valuation or cost, GSM = Grazing Salt Marsh, GSM-N = Non-Grazing Salt Marsh, RB= Reed Bed. * based in £18/tCO_2

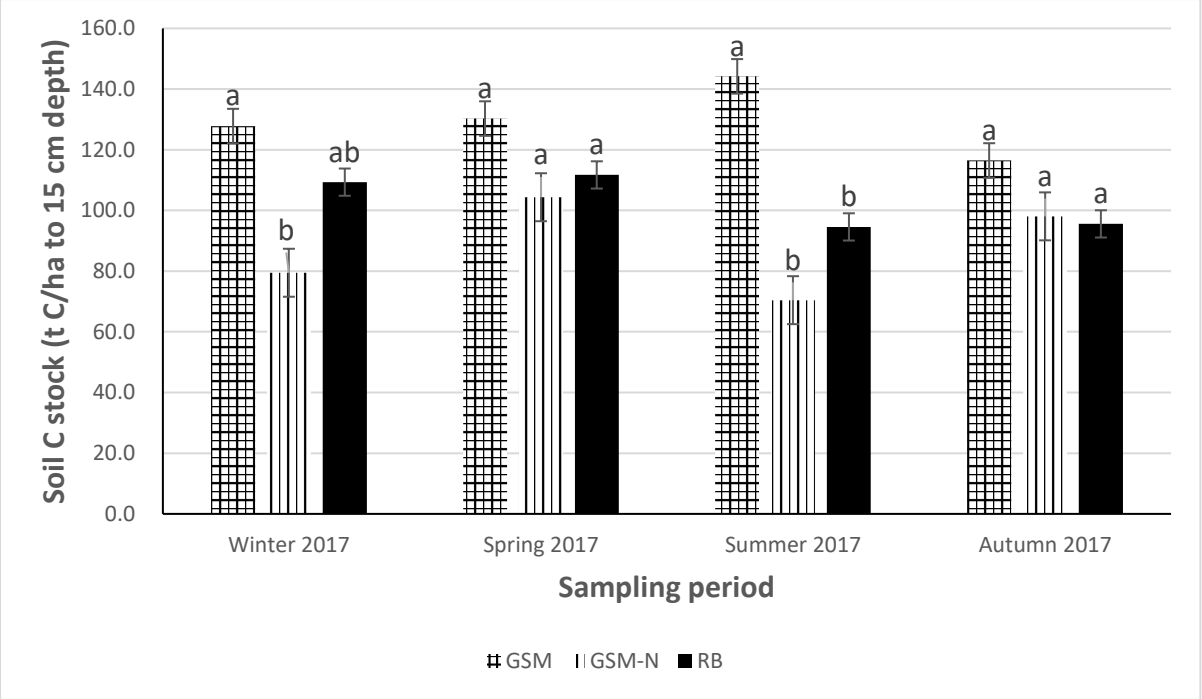


Figure 5. 14 Seasonal carbon stock (t C/ ha to 15 cm depth) (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, ab= Duncan test at 0.05 level of confidence)

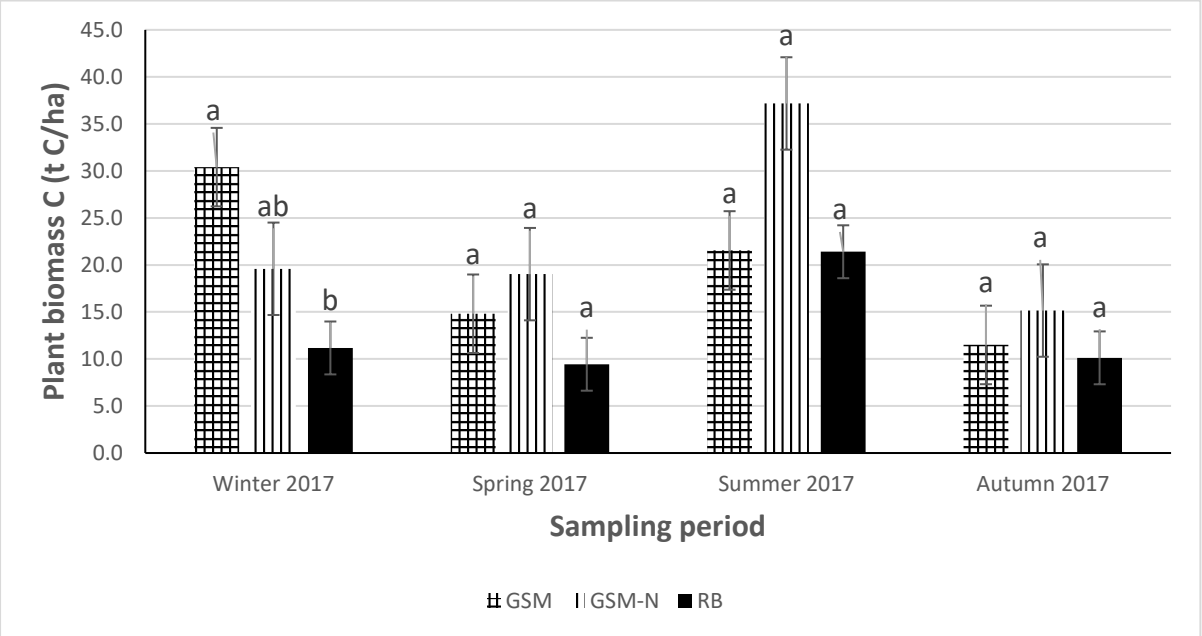


Figure 5. 15 Seasonal plant biomass carbon stock (t C/ha) (GSM= Grazing Salt Marsh, GSM-N= Non-Grazing Salt Marsh, RB= Reed Bed, error bars are presented as standard error of the mean (n= 3), statistical significance are presented as a, b, ab = Duncan test at 0.05 level of confidence)

5.4.12 Multivariate analysis showing interaction effects among selected parameters

Table 5.5 shows the Pillai's Trace multivariate test. The results indicated that, intercept, sampling seasons, and sampling locations were significant at 5% level as P value is 0.00. This means that there is different in the values of the dependent variables obtained from the different seasons and the sampling locations. Therefore, we reject the null hypothesis and accept that there is different in the values of the dependent variables obtained from the different seasons and the sampling locations. However, 28.7% of the variability in the dependent variables is responsible for the variability in the interaction between the different seasons and the sampling locations based on the Partial Eta Squared (see Table 5.5).

Table 5. 5 Pillai's Trace multivariate tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Observed Power[c]
Intercept	Pillai's Trace	1.000	442010.871b	7	18	0.00	1.00	1.00
Season	Pillai's Trace	1.597	3.251	21	60	0.00	0.53	1.00
Location	Pillai's Trace	1.387	6.137	14	38	0.00	0.69	1.00
Season * Location	Pillai's Trace	1.724	1.325	42	138	**0.12**	0.29	0.98

a Design: Intercept + Season + Location + Season * Location; b Exact statistic; c Computed using alpha = 0.05; Bold and underlined = not significant

The tests of between-subjects' effects from the multivariate analysis of variance table indicate that there was significant difference (at $P < 0.05$) in the intercept among the dependent variables. This means that values of the dependent variables are different across the study area. The effects of seasonal variation on the dependent variables were significant (at $P < 0.05$) on the soil pH, EC and plant biomass compared to other dependent variables (see Table 5.6). However, there were 13.6%, 13.1%, and 23.6% variabilities observed in bulk density, porosity and soil organic carbon respectively due to the variability in the different sampling seasons based on the Partial Eta Squared (Table 5.6). This may be the reason why

bulk density and porosity were only significant (at P < 0.05) in summer while the SOC was significant (at P < 0.05) during winter (Section 5.4.6 and 5.4.7) and summer (Section 5.4.9) compared to other seasons. The different sampling locations did not show any significant difference in the redox potential Eh and soil organic carbon content compared to other dependent variables. The interaction effects between the different seasons and the sampling locations from the tests of between-subjects effects table indicate that the was no significant difference in the dependent variables. However, a post test analysis using Duncan multiple range test at P < 0.05 shows which season or location was significant (Section 5.4.3, 5.4.4, 5.4.5, 5.4.6, 5.4.7, 5.4.8 and 5.4.9).

Table 5. 6 Tests of between-subjects effects among the dependent and independent variables

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power h
Corrected Model	pH	2.603a	11	0.237	5.34	0.00	0.71	1.00
	Eh	79608.217b	11	7237.111	1.21	0.33	0.36	0.49
	EC	81.078c	11	7.371	8.71	0.00	0.80	1.00
	Bulk Density	.427d	11	0.039	3.26	0.01	0.60	0.95
	Porosity	.057e	11	0.005	3.00	0.01	0.58	0.92
	SOC	32.651f	11	2.968	1.44	**0.22**	0.40	0.58
	Plant Biomass	2368214427.813g	11	215292220.700	3.79	0.00	0.63	0.97
Intercept	pH	2056.320	1	2056.320	46386.07	0.00	0.99	1.00
	Eh	77797.296	1	77797.296	13.02	0.00	0.35	0.93
	EC	276.280	1	276.280	326.60	0.00	0.93	1.00
	Bulk Density	250	1	25.000	2102.31	0.00	0.99	1.00
	Porosity	16.933	1	16.933	9784.85	0.00	0.99	1.00
	SOC	2638.363	1	2638.363	1280.72	0.00	0.98	1.00
	Plant Biomass	12246305840.000	1	12246305840.000	215.40	0.00	0.90	1.00
Season	pH	1.285	3	0.428	9.67	0.00	0.55	0.99
	Eh	35360.758	3	11786.919	1.97	**0.15**	0.20	0.44
	EC	51.546	3	17.182	20.31	0.00	0.72	1.00
	Bulk Density	0.045	3	0.015	1.26	**0.31**	0.14	0.29
	Porosity	0.006	3	0.002	1.20	**0.33**	0.13	0.28
	SOC	15.288	3	5.096	2.47	**0.09**	0.24	0.54
	Plant Biomass	1138744704.000	3	379581567.900	6.68	0.00	0.46	0.95
Location	pH	1.069	2	0.535	12.06	0.00	0.50	0.99
	Eh	4706.021	2	2353.011	0.39	**0.68**	0.03	0.11
	EC	19.329	2	9.664	11.42	0.00	0.49	0.99
	Bulk Density	0.247	2	0.124	10.39	0.00	0.46	0.98
	Porosity	0.033	2	0.016	9.40	0.00	0.44	0.96
	SOC	12.064	2	6.032	2.93	**0.07**	0.20	0.52
	Plant Biomass	586995392.000	2	293497696.000	5.16	0.01	0.30	0.78
Season * Location	pH	0.249	6	0.041	0.93	**0.49**	0.19	0.30
	Eh	39541.438	6	6590.24	1.10	**0.39**	0.22	0.35
	EC	10.203	6	1.701	2.01	**0.10**	0.33	0.61
	Bulk Density	0.134	6	0.022	1.88	**0.13**	0.32	0.58

	Porosity	0.018	6	0.003	1.77	**0.15**	0.31	0.55
	SOC	5.300	6	0.883	0.43	**0.85**	0.10	0.15
	Plant Biomass	642474332.100	6	107079055.3	1.88	**0.13**	0.32	0.58
Error	pH	1.064	24	0.044				
	Eh	143366.387	24	5973.599				
	EC	20.302	24	0.846				
	Bulk Density	0.285	24	0.012				
	Porosity	0.042	24	0.002				
	SOC	49.441	24	2.06				
	Plant Biomass	1364497307.000	24	56854054.45				
Total	pH	2059.987	36					
	Eh	300771.901	36					
	EC	377.661	36					
	Bulk Density	25.712	36					
	Porosity	17.032	36					
	SOC	2720.456	36					
	Plant Biomass	1597901757.0004	36					
Corrected	pH	3.667	35					
Tota	Eh	222974.605	35					
	EC	101.381	35					
	Bulk Density	0.712	35					
	Porosity	0.099	35					
	SOC	82.093	35					
	Plant Biomass	3732711735.000	35					

a R^2 = .710 (Adjusted R^2 = 0.577); b R^2 = .357 (Adjusted R^2 = 0.062); c R^2 = .800 (Adjusted R^2 = 0.708); d R^2 = .599 (Adjusted R^2 = 0.415); e R^2 = .579 (Adjusted R^2 = 0.386); f R^2= .398 (Adjusted R^2 = 0.122); g R^2 = .634 (Adjusted R^2 = 0.467); h Computed using alpha = 0.05;

Bold and underlined = not significant

The results from the residual sums of squares and cross products matrix (SSCP) matrix indicates that covariance from the dependent variables were both positive and negative across the different monitoring seasons and the sampling locations. This implies that as one dependent variable deviate from the mean, the other variable deviates from both the same and opposite directions. For instance, the soil pH will have a positive effect on the bulk density and plant biomass compared to other dependent variables (Table 5.7). The correlation coefficient (r) follows a similar trend with the covariance. Porosity and SOC shows a perfect negative relationship (at r= -0.997 and -0.521 respectively) when correlated with bulk density and pH (Table 5.7).

Table 5. 7 Residual sums of squares and cross products matrix (SSCP) matrix based on type III sum of squares

		pH	Eh	EC	Bulk Density	Porosity	SOC	Plant Biomass
Sum-of-Squares and Cross-Products	pH	1.064	-92.821	-1.087	0.208	-0.078	-3.775	57.449
	Eh	-92.821	143366.4	-107.86	-38.206	15.322	544.634	-246971
	EC	-1.087	-107.86	20.302	-1.04	0.379	-9.364	-13787
	Bulk Density	0.208	-38.206	-1.04	0.285	-0.109	-0.988	3453.964
	Porosity	-0.078	15.322	0.379	-0.109	0.042	0.4	-1284.68
	SOC	-3.775	544.634	-9.364	-0.988	0.4	49.441	-12090
	Plant biomass	57.449	-246971	-13787	3453.964	-1284.68	-12090	1.36E+09
Covariance	pH	0.044	-3.868	-0.045	0.009	-0.003	-0.157	2.394
	Eh	-3.868	5973.599	-4.494	-1.592	0.638	22.693	-10290.4
	EC	-0.045	-4.494	0.846	-0.043	0.016	-0.39	-574.46
	Bulk Density	0.009	-1.592	-0.043	0.012	-0.005	-0.041	143.915
	Porosity	-0.003	0.638	0.016	-0.005	0.002	0.017	-53.528
	SOC	-0.157	22.693	-0.39	-0.041	0.017	2.06	-503.749
	Plant iomass	2.394	-10290.4	-574.46	143.915	-53.528	-503.749	56854054
Correlation	pH	1.000						
	Eh	-0.238	1.000					
	EC	-0.234	-0.063	1.000				
	Bulk Density	0.378	-0.189	-0.432	1.000			
	Porosity	-0.373	0.199	0.412	-0.997	1.000		
	SOC	-0.521	0.205	-0.296	-0.263	0.279	1.000	
	Plant biomass	0.002	-0.018	-0.083	0.175	-0.171	-0.047	1.000

5.5 Discussion

5.5.1 Soil characterisation

The dynamics of soil organic carbon in a historically contaminated estuarine floodplain in the present study were affected by many factors such as the morphological characteristics, land uses/land covers, soil physical and chemical properties, and sampling periods. The soil morphological properties show that the sampling locations were poorly drained, sandy, and different elevation above the sea level (Table 5.1 and Appendices 6, 7, 8, and 9). The sandy properties of the study area may be associated with to the overlying sandstone parent materials and the continuous movement of materials out of the soil horizons whenever the soils are saturated due after tidal inundation. This is in corroboration with Griffiths et al. (2003) whos report shows that the study area is underlain by sandstone bedrock or parent material and may be responsible for the sandy soil texture and the differences in elevation

above the sea observed in the study area. Eluviation (movement of materials out of a portion of soil profile) and illuviation (movement of materials into a portion of soil profile) have been reported to have effects on the colour and mottles formation and soil texture under anaerobic conditions due to redox action (Payton 1993; Lindbo, Stolt, & Vepraskas, 2010; Kühn, Aguilar, & Miedema, 2010; Enya et al., 2011). The soil within the grazing saltmarsh, non-grazing saltmarsh and the reed bed can be classified as Gleysol from the morphological, physical and chemical properties (Figure 3.3, Tables 5.1, 5.2 and Appendices 6, 7, 8, 9, and 10). Gleysol is known for irregular decreases in organic carbon content with depth (Soil World Reference System, 2014). This is because during horizons formation more materials are depositional rather pedogenic as shown in Figure 5.13 where organic materials are carried and can be deposited within the estuarine floodplain after the tidal period). Therefore, understanding the soil morphological properties has helped to characterise and classify the the soils in the present study into specific soil type.

5.5.2 Differences in pH, redox potential, electrical conductivity, and bulk density after the year-round monitoring

There were variations in the soil pH, redox potential, electrical conductivity, and bulk density after the year-round monitoring. The variations in the soil pH, redox potential, and electrical conductivity observed in Figures 5.4, 5.5, 5.6, and Appendices 11, 12 and 13. The soil properties are controlled by factors such as influence of the soils by seawater, different seasons, land uses, and past industrial activities associated with the study site (Figures 5.4, 5.5, 5.6 and 5.13) . The production of caustic soda in the area which was reported in Chapter 4 may also have greater influence on the soil pH values observed from the study site. According to Pezeshki & DeLaune (2012) wetland soils are associated with lowering of soil redox potential (Eh) which leads to the production of soil phytotoxins that are by-products of soil reduction, thus imposing potentially severe stress on plant roots. The present study also observed negative redox potential during the year-round monitoring (Figure 5.5). This may be due to the low infiltration rate resulting from high bulk density caused by cattle grazing and the frequency of tidal inundation (Figures 5.7 and 5.13). The soil electrical conductivity was similar to the trend reported in Chapter 4, indicating the impact of tidal inundation on the lowland sampling locations (Figure 5.6). The soil bulk density within the study area was higher in the grazing sampling location compared to the non-grazing location and this was

influenced by the different sampling season (Figure 5.7). This may be attributed to the stock density of the trampling cattle during grazing periods and this may have a negative effect on the amount of carbon stored within the study area because the higher the bulk density resulted to low porosity across the study area (Figure 5.7 and Appendix 16) and as such soil aeration and microbial activities may be impeded. A similar trend in soil bulk density was detailed in Chapter 4 and the high amount of bulk within the grazing saltmarsh could not affect the soil carbon storage. This contradicted Chaudhari et al. (2013) report which was stated in Chapter 4 that soil bulk density is independent of the soil organic carbon content indicating a low carbon storage when there is high bulk density. Thus, the majority of the variations observed in the soil physical and chemical properties in the present study may include changes in hydrological cycle, past industrial activities and sampling seasons.

There was an interaction between the sampling periods and the different land uses/land covers and soil carbon storage. In the present study, there was no significant difference between the sampling periods and the different land uses (Tables 5.5 and 5.6). This is an indication that not all the land uses/land covers and different seasons were the same in the amount of soil carbon stored within the study area. It was generally expected that the high plant biomass during spring, summer, and winter 2017 (Figure 5.8 and Appendix 18) and low bulk densities (Figure 5.6 and Appendix 16) and high pore space (Figure 5.7) within the non- grazing saltmarsh could have positive effects on the turnover of organic carbon. However, the reverse was observed in this study area, indicating that cattle grazing plays a major role in the carbon turnover (Figures 5.9 and 5.10). This is in corroboration with Teixeira et al. (2011) whose findings shows that soil organic carbon is enhanced by animals returning undigested fibre to the soil during grazing. According to Medina-Roldán, Paz-Ferreiro & Bardgett (2012) grazing exclusion (non-grazing location) has no impact on soil carbon storage in an upland grassland in northern England after 7 years of grazing field experiment. According to Chirinda et al. (2014) elevation is an important factor to consider to quantify or predict carbon storage in coastal wetlands while Hayes et al. (2017) reported that soil organic carbon increases with an elevation within coastal wetland sediment. According to Chaplot et al. (2001) and Hook & Burke (2000), differences in elevation account for over 75% of the variation in soil organic carbon distribution along the profile. Gregory et al. (2014) reported high subsoil organic carbon stocks in the inundated grey soil as a reflection of incomplete

decomposition of organic matter under anoxic conditions and illuviation of dissolved and particulate organic carbon from upper horizons. According to Xiong et al. (2014), the amount of soil organic carbon sequestered depends on land use/land cover and is controlled by climate factors interacting with land use/land cover. Therefore, the contribution of the cow dungs during grazing in relation to the number of cattle grazing in the field in a given period may be responsible for the high amount of soil organic carbon in grazing saltmarsh compared to other land uses/land covers.

5.5.3 Avoided emission and avoided emission valuation for carbon storage

There are economic benefits associated with soil carbon storage in the present study. Carbon stored in soil and plant biomass are often used in predicting carbon sequestration (Bernhardt, 2015). This is because carbon storage is a function of pools of carbon, and the four pools within saltmarshes are the aboveground biomass, belowground biomass, litters and carbon in the soil in a given depth and sampling area (Bernhardt, 2015). The present study used the InVEST blue carbon model to predict avoided carbon dioxide emission and avoided emission valuation (costs) for storing carbon in the study area according to Tallis et al. (2011), Sharp et al. (2014) and Bernhardt (2015). The avoided emission because of storing high soil organic carbon shown in Table 5.4 follow the trend of storing carbon within the study area shown in Figure 5.9, showing that more carbon emission was avoided during winter 2017 season and summer 2017 within the grazing saltmarsh. This was in corroboration with the findings from Smith (2013) which reported that higher avoided cost of carbon emission was found in the grazing location compared to the non-grazing sampling location. This may be attributed to the higher carbon stored in the grazing sampling location which prevented more carbon to be emitted compared to the non-grazing sampling location (Figures 5.9, 5.11, 5.12, 5.14 5.15 and and Appendices 15, 19, 20, 21 and 22). According to Morris & Camino (2011) carbon storage benefits are about £220/ha/year for peatbogs based on estimated annual sequestration rates of about 4.1 tCO_2e/ha and department of energy and climate change (DECC) current prices of £52/tCO_2e for non-traded from 2008 -2030 period. In the present study, a carbon credit of 288 to 326 tCO_2/ha was achieved, equivalent to a capital gain of £5184 to £5863 per hectare in 2017 based on a UK Government price for 2016-2022 of £18 per tonne of carbon dioxide. There have been several suggestions relating to factors affecting carbon storage. According to Davidson et al. (2002), photosynthesis and soil respiration

affects carbon balance. Ostle et al. (2009) recommended that to maintain the UK carbon balance it is necessary to protect peatland and soil organic carbon stock, and manage cropland, grassland and forest soils, which increases carbon sequestration. Soil respiration could not be measured in the present study due to limited funding and several efforts for collaborative research failed. The present findings have provided an understanding on the amount of carbon sequestration and this will serve as basline data for the future prediction for avoided emission and avoided emission valuation based on the four seasons (winter, spring, summer and autumn) in the UK.

In conclusion, this research has provided baseline carbon data for future prediction of avoided carbon emission and avoided emission valuation. In this chapter the dynamics of soil organic carbon from seasonal monitoring under different land uses/land covers in an historically contaminated Upper Mersey estuarine floodplain is described. Soil carbon storage within the study area was in the order grazing saltmarsh > reed bed > non-grazing saltmarsh during winter and summer sampling periods. This does not follow a similar trend to the heavy metal concentration at 0–15 cm depth reported earlier in Chapter 4 in the order reed bed > grazing saltmarsh > non-grazing saltmarsh. This may be an indication of other factors such as land uses/land covers, soil type, soil pH, Eh, EC, plant biomass, evapotranspiration rate, sampling depths, sampling periods, temperature, and tide inundation influencing the soil carbon storage within the study area. Redox potential and electrical conductivity values show perfect positive relationships with the content of carbon stored within the study area, while pH values show little or no effect on the amount of carbon stored. Soil bulk density and porosity were found not to have much influence on the amount of carbon stored, since GSM with higher bulk density values stored more carbon than expected. The amount of soil carbon stored increases with continuous cattle grazing which may be an indication of rapid plant biomass turnover. Also, the avoided emission and avoided emission valuation were greater during winter in the grazing saltmarsh than other seasons, indicating that the grazing saltmarsh can result in more carbon credits compared to other land uses/land covers during the winter season. Therefore, soil organic carbon dynamics based on seasonal monitoring is not only important in predicting future carbon storage but also necessary to make useful decisions on both regional and national carbon budgets.

6. Differential Effects of Heavy Metal Contamination on Organic Matter Decomposition in Inundated Soils

6.1 Introduction to the chapter

It has been long recognised that the presence of heavy metals in soils could adversely affect microbial activities (Ohya, Fujiwara, Komai, & Yamaguchi, 1988; Hiroki, 1992; Yeates, Orchard, Speir, Hunt, & Hermans, 1994; Post & Beeby, 1996; Oorts, Bronckaers & Smolders, 2006; Lejon et al., 2008; Ranjard, Nowak, Echairi, Faloya, & Chaussod, 2008; Lenart-Boron & Wolny-Koładka, 2015). As such, microbially mediated soil processes are likely to be affected by soil-borne heavy metals. For example, it has been reported that microbially driven nitrogen transformation in soils was markedly inhibited by heavy metals (Slater & Capone, 1984 and Yan, Quan, & Ding, 2013). Soil organic matter is decomposed predominantly through microbial degradation. In the past, there have been several investigations into the effects of heavy metal contamination on decomposition of soil organic matter (Ruhling & Tyler, 1973; Zwoliński, 1994; Hattori, 1996; Quenea, Lamy, Winterton, Bermond, & Dumat, 2009; Marchand, Allenbach, & Lallier-Vergès, 2011; Chen, Liu, Liu Jia, & He, 2014). Zwoliński (1994) demonstrated that the decomposition rate of organic matter in Scots pine (*Pinus silvestris*) stands decreased with increasing level of heavy metal contamination, resulting in increased emission of carbon dioxide and accumulation of soil organic matter. This has implications for estimating global carbon budget given the fact that the area of heavy metal-contaminated soils is rapidly increasing with the expansion of industrialised areas around the world.

Despite these previous efforts made to understand the impacts of heavy metals on soil organic matter decomposition, much of the work has so far been focussed on forest soils (Ruhling & Tyler, 1973; Zwoliński, 1994; Köhler, Wein, Reiss, Storch, & Alberti, 1995; Bernard et al., 2009; Birge et al., 2015) with contaminated soils under other land uses/land covers being not sufficiently covered. Estuarine floodplains/coastal wetlands such as the Mersey estuarine floodplain are likely sinks for heavy metals due to their close association with human civilization and industrial activities (Gwynne, 2004 & Smith, 2013). The soils in such landscape are subject to tidal inundation and, thus, are markedly different from the upland soils in terms of organic matter decomposition. No work has been identified that reported on

the effects of heavy metal contamination on organic matter decomposition in soils under water inundation. This does not allow evaluation of heavy metal impacts on soil organic carbon dynamics and storage in estuarine lowlands. Furthermore, it is expected that different heavy metals have different impacts on the decomposition processes of soil organic matter. There is currently a lack of information on the effects of individual heavy metals on the soil organic matter degradation.

Soil organic matter consists of all the organic fractions in soil such as litter, light fraction, microbial biomass, dissolved organic matter and humus (stabilised organic matter) (Stevenson, 1994). They are classified into low-molecular-weight (oxalic, malic, and citric acids) and high-molecular-weight components, such as humic and fulvic acids (known as humic substances). Hsu & Lo (1999), reported that transformations of soil organic matter such as decomposition and mineralisation bring about changes in functional group chemistry, such as the relative increase in aromatic to aliphatic groups during decomposition. Soil carbohydrates are important because of their ability to bind with inorganic soil particles into stable aggregates and form complexes with metal ions. Also, soil carbohydrates serve as building blocks for humus synthesis (Stevenson, 1994). According to Kumpiene et al. (2008), the reduction of chromium from its toxic and mobile hexavalent form chromium (VI) to stable chromium (III) in natural environments is accelerated by soil organic matter. However, what will happen to the functional groups or carbon species, such alkyl, O-alkyl, and carbonyl carbon, during the decomposition of soil organic matter when the soil is contaminated with heavy metals is not fully understood. The combination of spectroscopic techniques with thermolytic and chemolytic methods will add substantially to the understanding of the characterisation of soil organic matter (Kögel-Knabner, 2000; Bull, Nott, van Bergen, Poulton & Evershed, 2000; Poirier et al., 2005; Dungait et al., 2010). According to Stevenson (1994), the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of a humic substance has been hypothesised to provide an inventory of the different components of which the material is composed. It is expected that different heavy metals will have different impacts on the decomposition of soil organic matter and formation of humic substances.

The overall objective in this chapter is aimed at evaluating the effects of heavy metal contamination on soil organic carbon storage and characteristics of soil humic substances

during the decomposition of soil organic matter under water inundation condition using a laboratory simulation experiment. This will improve our understanding of the amount of organic carbon stored and the different species of soil organic carbon (such as carbohydrates, lignin, cellulose, fats and lipids, and protein) during the decomposition process of organic matter under heavy metal contamination. This work relates to the phase three of the research (see Figures 3.6 and 3.7).

6.2 Experimental design

The experimental plastic cups were made of up thoroughly mixed representative composite 100 grams moist soil samples and 20 grams of fresh plant biomass (*Elytrigia repens*) collected from the Upper Mersey estuarine floodplain. These were collected using a soil auger at 0–30 cm depth and knife. The soil type within the sampling location are term gleysol according to the World Reference Base system (2014) (Chapter 5 in this thesis) while the dominant land use/land cover is detailed in Chapter 3. The experimental design used was a completely randomised design with six treatments: five heavy metals ((arsenic, chromium, copper, lead, zinc, and Mx, a combination of arsenic, chromium, coper, lead, and zinc; and a control in which there was no artificial addition of a heavy metal. The heavy metals treatments were of the following compounds: sodium arsenate dibasic heptahydrate ($Na_2HASO_4.7H_2O$), potassium chromate (K_2CrO_4), copper II chloride dihydrate ($CuCl_2.2H_2O$), lead (II) nitrate ($Pb(NO_3)_2$) and zinc nitrate (N_2O_6Zn) while the control treatment represents soil that was not artificially contaminated with heavy metal. Each treatment had three treatment levels with different concentrations of heavy metal representing low (L) concentration, medium (M) concentration and high (H) concentration except for the control (Table 6.1). The treatment levels were in triplicate (1, 2 and 3) for effective statistical analysis (Figure 6.1).

The choice of the contamination levels was in line with the European Community permissible metal concentration in soil directive 86/278/EEC, and also based on the initial heavy metal concentration reported in Chapter 4 in this thesis. The procedure for the randomisation was carried out before the soil was spiked with heavy metal treatments and it involved the following steps:

(a) ranking 57 numbers to equate to the total number of the treatment levels.

(b) randomly picking from the numbers after mixing them effectively, picking seven numbers to represent each treatment.

(c) picking of three numbers to represent different treatment levels. (d) picking triplicates from each treatment level.

The concentrations of arsenic, chromium, coper, lead, and zinc were then calculated considering the atomic weight of the compound, the percentage by mass of the elements within the compound and the desired concentration of each treatment. The selection of the desired concentration was based on the soil background study detailed in Chapter 4.

The soil was then spiked with the concentration of the different treatment levels (low, medium and high doses), and incubated for 200 days at a temperature of 20 °C and a moisture content of about 81% was maintained using deionised water. Readings were taken after 60 days, 120 days and 200 days for soil organic carbon content and selected chemical properties. Estimation of soil microbial biomass was measured after 30 days, 120 days and 200 days of incubation. This was due to the hiring of the carbon emission equipment, while the 60 days reading was only used to calculate the retention capacity of the metals because it is assumed that a normal compost period is 60 days. The reasons for selecting the different heavy metal treatments, duration of the experiment, and the temperature for the incubation were detailed in Chapter 3. Ten grams of soil was taken for the 60 days and 120 days readings, while 80 g of soil was taken for the 200 days readings, this was oven dried, sieved with a 2 mm sieve and was then ready for analyses. The control treatment showed that no plant biomass was left after 120 days and 200 days incubation which was an indication for complete decomposition of organic matter (Appendix 27).

Table 6. 1 Concentration of heavy metal added to the soil before incubation

Treatment	Low dose (mg/kg)	Medium dose (mg/kg)	High dose (mg/kg)
As-contaminated soils	50	100	200
Cr-contaminated soils	125	250	500
Cu-contaminated soils	125	250	500
Pb-contaminated soils	125	250	500
Zn-contaminated soils	125	250	500
Multi-contaminated soils	550	1100	2200
Control	0	0	0

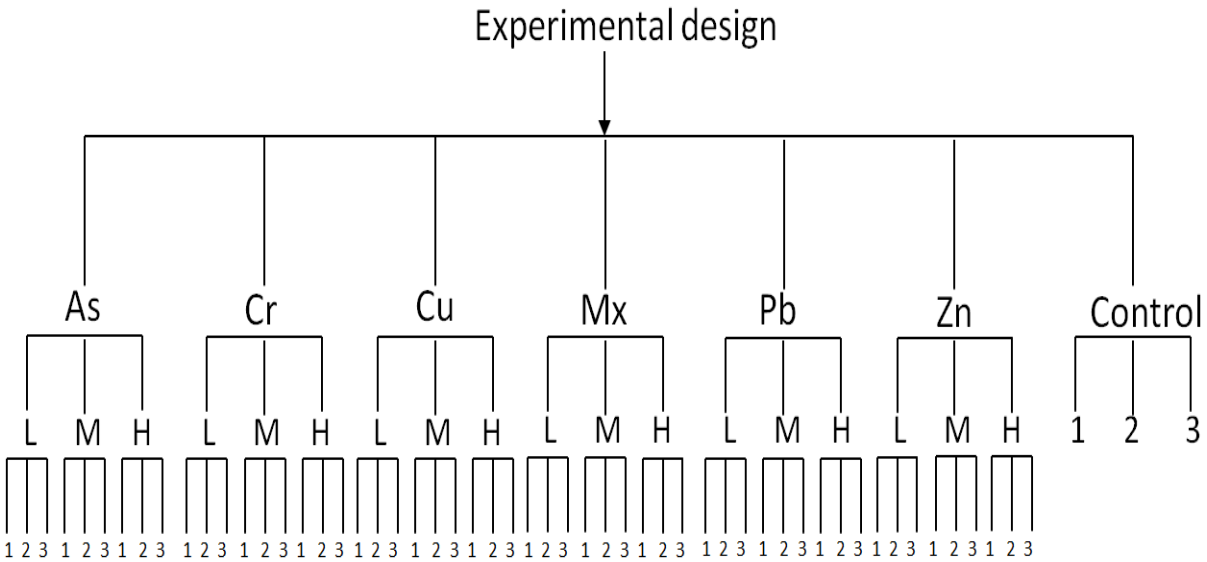


Figure 6. 1 Experimental design showing treatments and corresponding treatment levels (L= low dose, M= medium dose and H= high dose)

6.3 Laboratory analysis

A total of fifty-seven samples were analysed from 3 replicates within the three treatment levels for six treatment and three replicates for the control treatment (Figure 6.1). The following laboratory analyses were carried out: pH, redox potential and electrical conductivity; soil organic carbon content; soil microbial biomass; retention capacity of the heavy metal used; and soil organic matter characterisation.

6.3.1 pH, Eh and EC determination

Meters were used to measure soil pH, Eh, and EC. pH and Eh were measured using a HI-2020 edge meter and EC was measured with a Mettler Toledo EC meter. The procedures

for the determination of pH, Eh and EC were the same as the ones detailed in Chapters 4 and 5.

6.3.2 Determination of soil organic carbon content

The organic carbon content was determined using the loss on ignition procedures detailed in Chapter 4 and 5.

6.3.3 Heavy metal absorbed after 60 days incubation

The amount of heavy metal retained or absorbed in the soil after 60 days of incubation was calculated. This was done using initial concentration before incubation (which includes soil background concentration and desired concentration for each treatment) minus the final concentration after the 60 days incubation period, divided by the initial concentration, and multiplied by 100 (that is heavy metal retained = $C_i - C_f/C_i$ *100). The procedures for heavy metal determination are fully detailed in Chapter 4.

6.3.4 Characterisation of soil humic substances

This was measured using a combined method of Stevenson (1994) and Conte, Piccolo, Van Lagen, Buurman, & De Jager (1997) procedures. It involves two steps: extraction of humic substances and NMR analysis.

6.3.4.1 Extraction of humic substances

Two hundred grams of air-dried treatments were dissolved in 1 M sodium hydroxide (NaOH) and shaken overnight. The content was centrifuged at 6,000 revolutions per minute (rpm) for 30 minutes and supernatant was taken. Half molar (0.5 M) sodium hydroxide (NaOH) was added to the supernatant and further centrifuged for total removal of soil particles and then decanted. The clear solution was divided into two parts. One molar (1 M) hydrochloric acid (HCl) was added to the first portion, precipitated and was further centrifuged and filtered with Whatman filter paper (grade 93, pore size 125 mm). The second portion was only filtered with Whatman filter paper (grade 93, pore size 125 mm). Hydrolysis of the two acids increased the aromaticity of the humic materials, in part by removing proteinaceous components and carbohydrates. It also lowered the COOH content of two out of three humic preparations, probably because of acid decarboxylation (Schnitzer and Preston, 1983). The samples were dialysed for a maximum of 6 hours against water using

snakeskin dialysis tubing to further remove impurities (Figure 6.2) and the samples were fridge dried to ensure purified concentrate for analyses. Dialysis according to Stevenson (1994), is a procedure which eliminates salts and low-molecular-weight components of humic substances. However, some research has shown that up to one-half of the carbohydrate material is low in molecular weight and as such cannot be removed by dialysis (Stevenson, 1994).

Figure 6. 2 Dialyzed humic and fulvic samples before freeze drying process (A= dialysis process, B and C= humic acid (HA) fractions before and after dialysis, respectively, D and E= fluvic acid (FA) fractions before and after dialysis, respectively)

6.3.4.2 NMR analysis

Half molar of sodium deuterium oxide (0.5 M NaOD) was added to the pure sample and sonicated for 30 minutes. It was then centrifuged at 6,000 rpm for 15 minutes and the

clear supernatant was used for NMR analysis of proton and nuclei using Bruker 400 AV at 100 Hz NMR machine with different accusation times and with about 17,000 as the number of scans.

6.4 Statistical analysis

The hypothesis was tested with IBM SPSS 24.0 version statistical tool using multivariate analysis, one-way ANOVA and Pearson's correlation coefficient to test for significant differences among the different treatments. A Post hoc multiple comparison for observed means from the IBM SPSS 24.0 was carried out using Duncan Multiple Range Test to further separate the mean to test for significant differences using lower case alphabets (a, b, c, d etc), were the alphabets indicates different degree of significance (at $P < 0.05$). For example, letter a is more significant compared to other letters.

6.5 Results

6.5.1 Soil characteristics used for incubation

The soil type is gleysol with characteristic high organic carbon content, sandy soil texture, slightly alkaline nature, high salinity due to sea level rise, and negative to a few positive values of redox potential. Heavy metal concentration was significantly higher across the lowland area compared to the upland part of the estuarine floodplain. Heavy metal fractionation within the study area indicated that the majority of the metals are stored in oxidising and residual form and, as such, a perfect positive relationship exists between soil organic carbon content and heavy metal distribution. The concentration of chromium, copper, and lead on the plant shoot used for the incubation experiment was higher than normal for grazing land use/land cover (Chapter 4, Section 4.3.5).

6.5.2 Selected chemical properties after the incubation process

The pH was moderately acid to strongly alkaline, ranging from 5.6 to 9.7 (Table 6.2). Soil contaminated with arsenic was moderately alkaline across the different concentration levels over the incubation process, while chromium treatment was moderately alkaline to strongly alkaline at low concentration after 120 and 200 days of incubation. Copper and Mx treatments were slightly alkaline at lower concentration and slightly acid at higher concentration, while lead was slightly alkaline within the different concentrations over the

incubation periods. Soil treated with zinc showed significantly lower pH values (moderately acid) with increased concentration of zinc, while the control did not show any significant change in pH from the original soil pH values after the incubation periods. The Eh values ranged from -119.3 mV to 77.3 mV (Table 6.3), indicating an anaerobic condition within the experimental set up. Eh values were higher at 200 days compared to 60 and 120 days of incubation across the different treatments. High concentration of heavy metal treatment increased the Eh values compared to those lower concentrations except for soil treated with As, while there was no significant difference in the control treatment after the incubation period (Table 6.3). Table 6.4 shows the mean and standard error of soil electrical conductivity (EC) after heavy metal-soil incubation at different periods. Soil EC values ranged from 2.0 mS/cm to 23.5 mS/cm. The values increased with the concentration of the treatment when compared to the control treatment except in arsenic and lead where the EC in the control was higher. Generally, soil EC values were higher after 60 days of incubation than 120 and 200 days except in high concentration of copper treatment. This is an indication that the decomposition process affects the values of EC.

Table 6. 2 Mean and SE of pH under different concentrations of heavy metal

Treatment	Days	Low	Medium	High	Control
Arsenic	60	8.6±0.11	8.8±0.02	8.9±0.03	7.7±0.01
	120	8.8±0.21	8.9±0.44	8.6±0.12	7.0±0.02
	200	8.2±0.00	8.5±0.00	8.6±0.00	7.7±0.00
Chromium	60	8.9±0.01	8.6±0.01	8.6±0.08	7.7±0.01
	120	9.7±0.04	9.2±0.14	8.9±0.09	7.0±0.02
	200	9.3±0.00	8.9±0.00	8.8±0.00	7.7±0.00
Copper	60	7.2±0.03	7.0±0.03	6.6±0.03	7.7±0.01
	120	7.1±0.05	6.8±0.28	6.6±0.18	7.0±0.02
	200	7.5±0.00	6.7±0.00	6.5±0.00	7.7±0.00
Mx	60	7.7±0.01	7.6±0.02	6.6±0.01	7.7±0.01
	120	7.3±0.03	7.9±0.17	7.0±0.04	7.0±0.02
	200	7.7±0.00	7.8±0.00	6.9±0.00	7.7±0.00
Lead	60	7.9±0.02	7.8±0.02	8.0±0.00	7.7±0.01
	120	7.9±0.27	7.5±0.19	7.6±0.02	7.0±0.02
	200	7.9±0.00	7.8±0.00	7.8±0.00	7.7±0.00
Zinc	60	8.4±0.01	6.6±0.01	5.9±0.01	7.7±0.01
	120	7.7±0.05	6.4±0.01	5.6±0.02	7.0±0.02
	200	7.9±0.00	6.8±0.00	6.1±0.00	7.7±0.00

Mx = Combination of arsenic, chromium, copper, lead, and zinc

Table 6. 3 Mean and SE of Eh (mV) under different concentrations of heavy metal

Treatment	Days	Low	Medium	High	Control
	60	-81.7±6.59b	-113.8±1.42c	-119.3±1.55c	-60.9±0.50a
Arsenic	120	-98.6±2.73c	-118.1±1.74d	-85.0±4.31b	7.0±0.02a
	200	-69.8±0.00a	-85.7±0.00a	-93.3±0.00a	-41.7±0.00a
	60	-120.9±0.7c	-102.8±0.84b	-101.1±3.46b	-60.9±0.50a
Chromium	120	-126.4±2.5c	-99.2±7.13b	-93.8±1.93b	7.0±0.02a
	200	-134.2±0.0a	-108.9±0.00a	-104.2±0.00a	-41.7±0.00a
	60	-31.7±0.85c	-15.2±1.76b	6.3±0.46a	-60.9±0.5d
Copper	120	-1.5±1.50a	14.8±13.79a	6.3±0.66a	7.0±0.02a
	200	-28.0±0.00a	13.8±0.00a	25.2±0.00a	-41.7±0.00a
	60	-61.3±0.77b	-59.4±0.61b	-5.3±0.28a	-60.9±0.50b
Mx	120	-14.6±0.73b	-40.7±8.60c	2.6±2.34a	7.0±0.02a
	200	-42.1±0.00a	-46.4±0.00a	5.0±0.00a	-41.7±0.00a
	60	-72.8±0.99c	-69.3±0.94ab	-75.2±0.03d	-60.9±0.50a
Lead	120	-41.7±13.1b	-19.9±9.61ab	-32.6±5.78b	7.0±0.02a
	200	-50.8±0.00a	-47.8±0.00a	-49.8±0.00a	-41.7±0.00a
	60	-96.0±0.41d	-3.1±0.32b	35.9±0.61a	-60.9±0.50c
Zinc	120	-34.8±1.79d	36.7±1.84b	77.3±1.56a	7.0±0.02c
	200	-52.4±0.00a	8.0±0.00a	49.7±0.00a	-41.7±0.00a

Mx = Combination of arsenic, chromium, copper, lead, and zinc; statistical significance is presented as a, b, ab, c= Duncan test at 0.05 level of confidence)

Table 6. 4 Mean and SE of EC (mS/cm) under different concentrations of heavy metal

Treatment	Days	Low	Medium	High	Control
	60	2.1±0.02d	2.9±0.01c	3.0±0.01b	4.0±0.04a
Arsenic	120	2.0±0.03d	2.4±0.01c	2.5±0.04b	7.0±0.02a
	200	2.3±0.01c	2.2±0.01c	2.7±0.02a	2.6±0.01b
	60	6.3±0.05c	14.7±0.06b	23.5±0.21a	4.00.04d
Chromium	120	4.9±0.01d	9.7±0.27b	15.8±0.45a	7.0±0.02c
	200	4.9±0.01c	10.7±0.04b	18.7±0.04a	2.6±0.01d
	60	5.4±0.11c	5.9±0.02b	7.8±0.03a	4.0±0.04d
Copper	120	4.0±0.20d	5.5±0.02c	8.3±0.05a	7.0±0.02b
	200	4.7±0.02c	6.3±0.01b	9.2±0.01a	2.6±0.01d
	60	7.8±0.16c	10.0±0.28b	14.0±0.27a	4.0±0.04d
Mx	120	3.8±0.08d	5.0±0.13c	8.5±0.08a	7.0±0.02b
	200	4.2±0.02c	6.1±0.01b	9.9±0.03a	2.6±0.01d
	60	3.4±0.01b	3.0±0.01d	3.2±0.01c	4.0±0.01a
Lead	120	2.3±0.01c	2.5±0.04b	2.2±0.02d	7.0±0.02a
	200	2.4±0.01c	2.6±0.01a	2.5±0.00b	2.6±0.01a
	60	3.1±0.00d	7.9±0.04b	14.8±0.05a	4.0±0.04c
Zinc	120	2.5±0.06d	5.2±0.08c	9.3±0.18a	7.0±0.02b
	200	3.0±0.00c	5.6±0.01b	11.0±0.01a	2.6±0.01d

Mx = Combination of arsenic, chromium, copper, lead, and zinc; statistical significance is presented as a, b, ab, c= Duncan test at 0.05 level of confidence)

6.5.3 Soil organic carbon content during and after the incubation process

The content of soil organic carbon (SOC) from soil spiked with heavy metals is shown in Figure 6.3 to Figure 6.8. The amount of SOC varies with the types of heavy metal used, the concentration of the metals and the periods of incubation. The amount of SOC in soil spiked with arsenic and copper shows significantly higher values after 60 days incubation compared to 120- and 200-days incubation across different concentrations or treatment levels (Figure 6.3 and Figure 6.5) when compared to the control treatment. The soil organic content in soil treated with chromium was higher after 200 days incubation across the treatment levels (L, M and H) and the control treatment (Figure 6.4). There was no clear trend in the amount of soil organic carbon when soils were spiked with Mx (combination of arsenic, chromium, copper, lead, and zinc treatment) after the incubation periods (Figure 6.6). However, the amount of soil organic content was significantly higher at 60 days within the M level, while the amount of soil organic content was higher after 200 days as the concentration of the treatment increased.

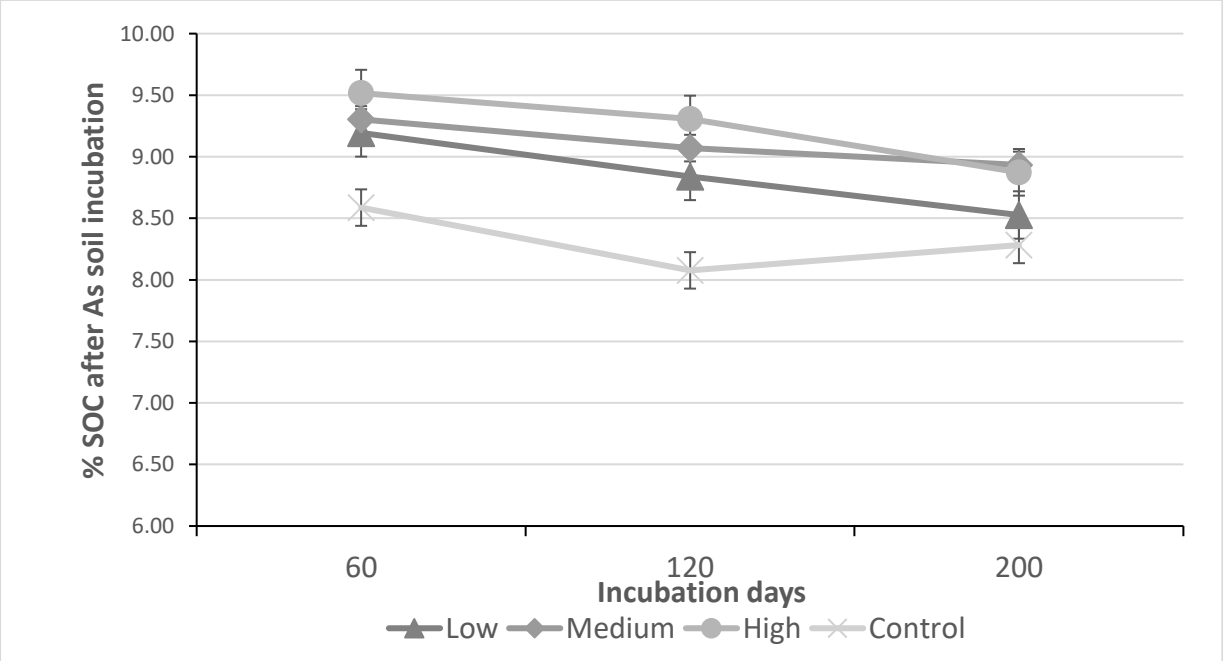


Figure 6. 3 Soil organic carbon content (%) in the arsenic-contaminated soil at different sampling times for the control and the treatments (error bars are presented as standard error of the mean (n= 3), statistical significance is presented according to Duncan test at 0.05 level of confidence)

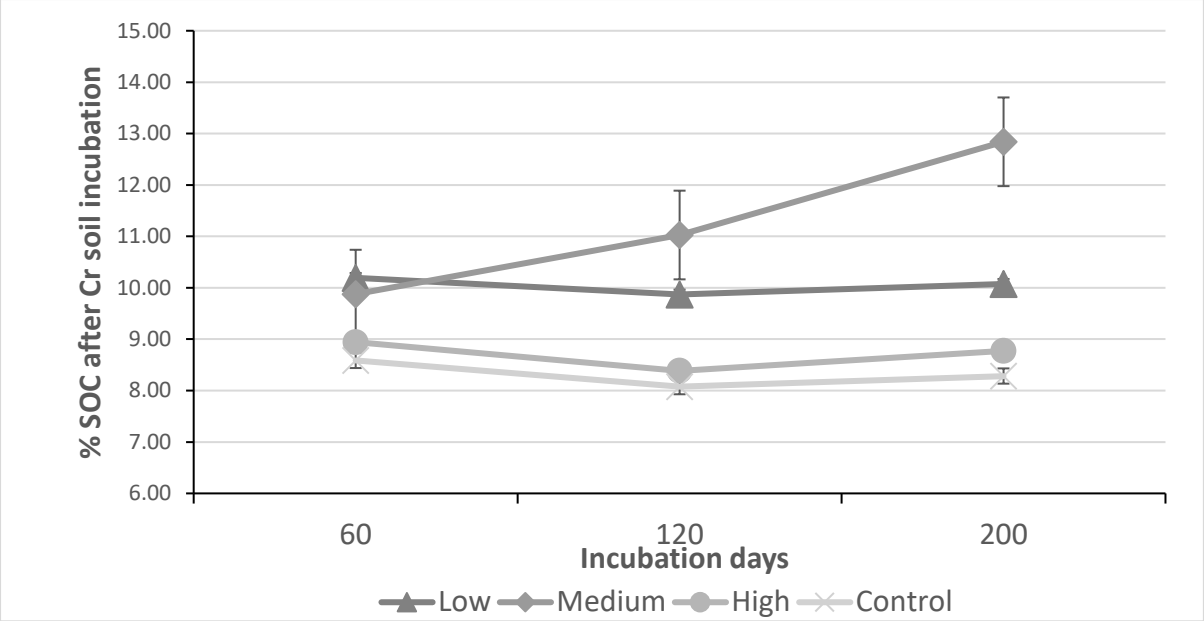


Figure 6. 4 Soil organic carbon content (%) in the chromium-contaminated soil at different sampling times for the control and the treatments (error bars are presented as standard error of the mean (n= 3), statistical significance is presented according to Duncan test at 0.05 level of confidence)

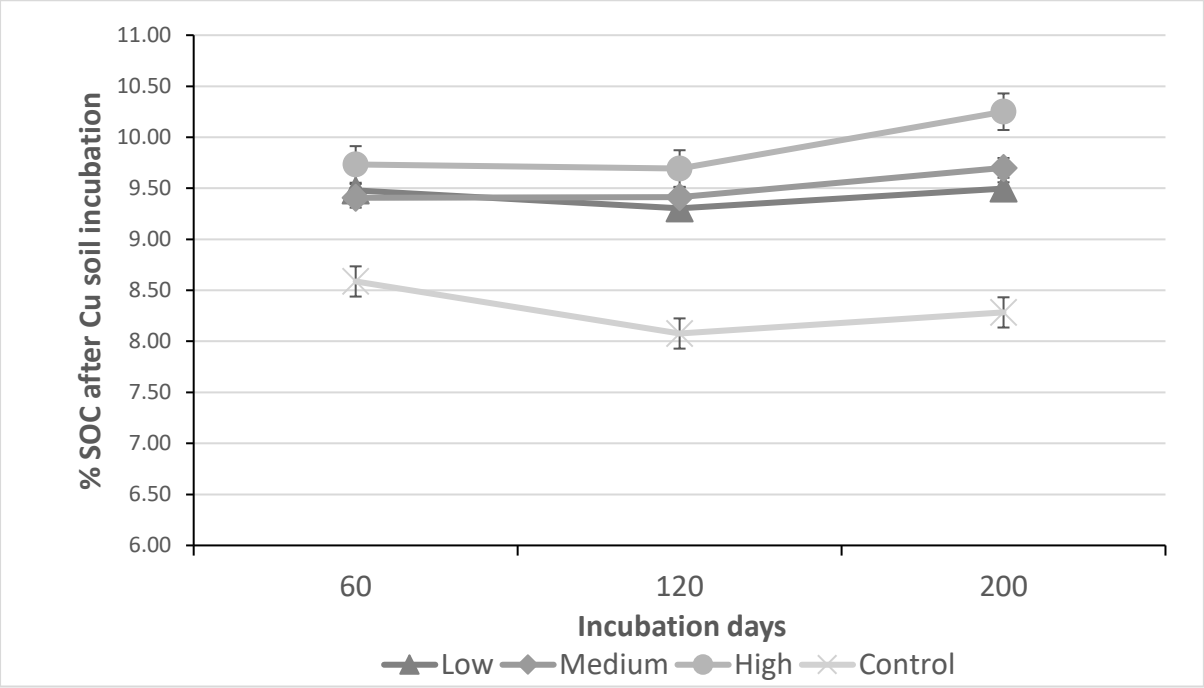


Figure 6. 5 Soil organic carbon content (%) in the copper-contaminated soil at different sampling times for the control and the treatments (error bars are presented as standard error of the mean (n= 3), statistical significance is presented according to Duncan test at 0.05 level of confidence)

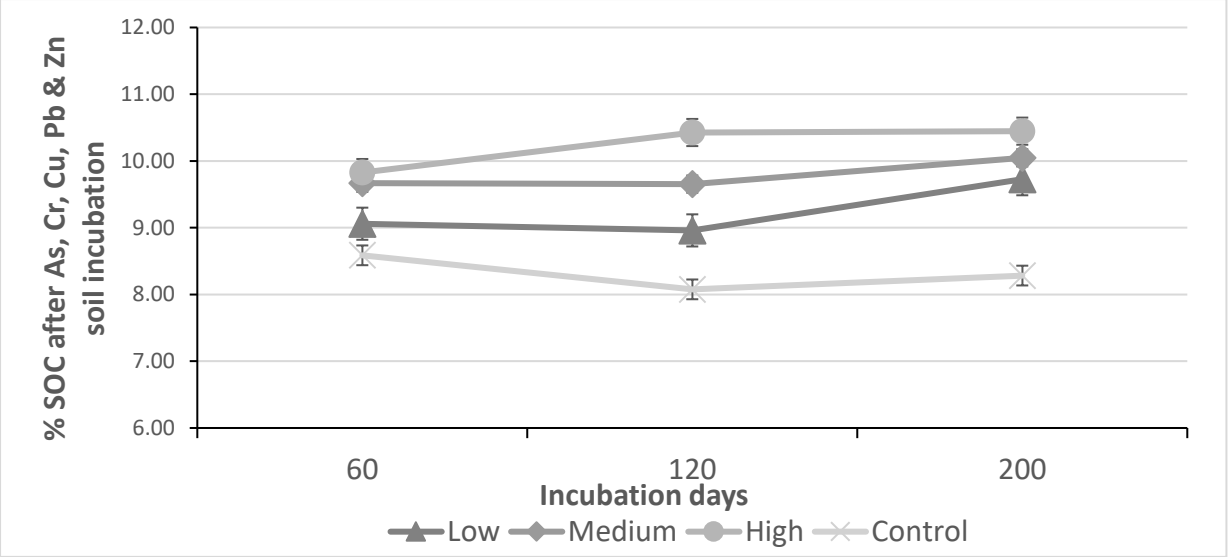


Figure 6. 6 Soil organic carbon content (%) in the Mx-contaminated soil at different sampling times for the control and the treatments (error bars are presented as standard error of the mean (n= 3), statistical significance is presented as according to Duncan test at 0.05 level of confidence)

Soil spiked with lead shows no statistical difference in soil organic content among the treatment levels over the incubation period except at low concentration were 60- and 120-days incubation was higher than 200 days (see Figure 6.7). Soil treated with zinc was statistically significantly higher in SOC as the concentration increased across the different incubation periods and from the control treatment (Figure 6.8).

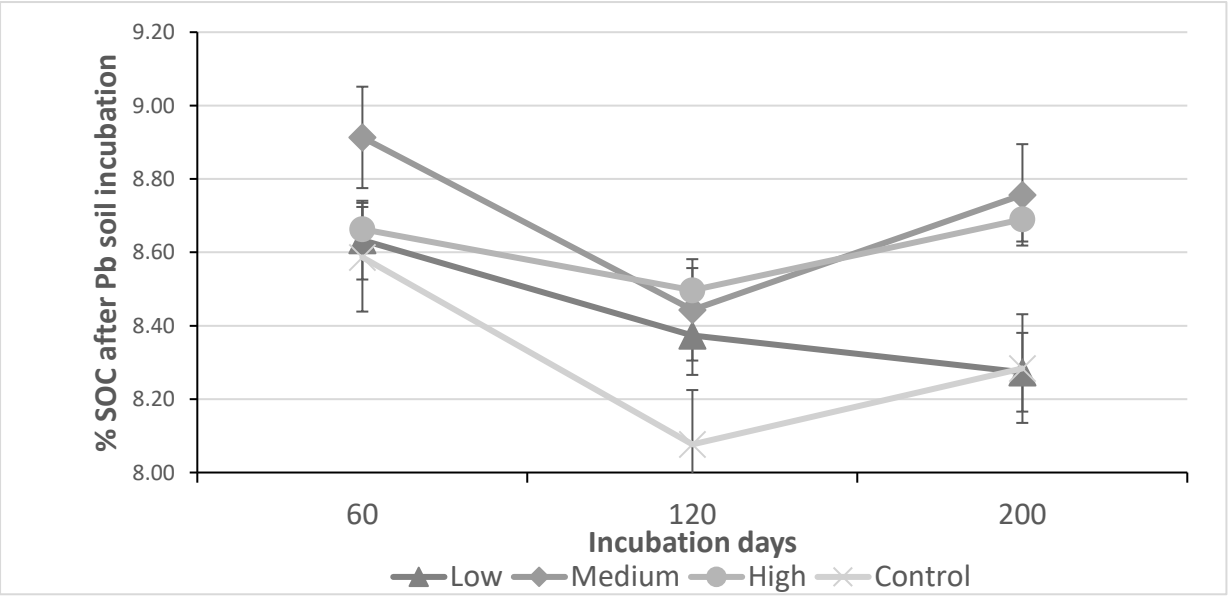


Figure 6. 7 Soil organic carbon content (%) in the lead-contaminated soil at different sampling times for the control and the treatments (error bars are presented as standard error of the mean (n= 3), statistical significance is presented according as Duncan test at 0.05 level of confidence)

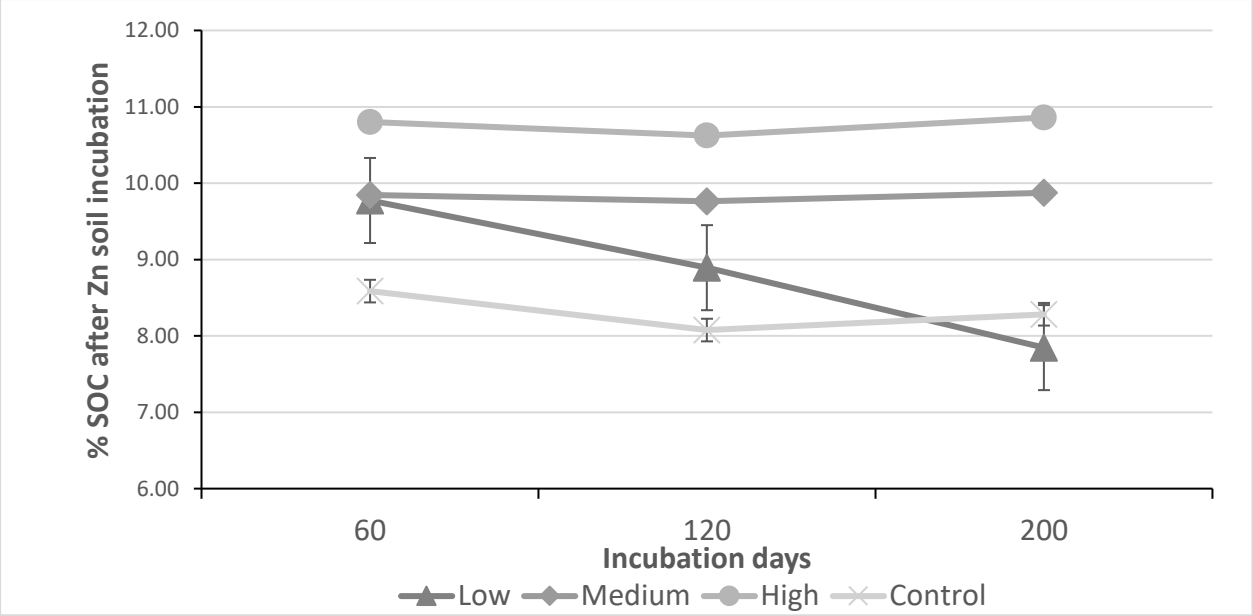


Figure 6. 8 Soil organic carbon content (%) in the zinc-contaminated soil at different sampling times for the control and the treatments (error bars are presented as standard error of the mean (n= 3), statistical significance is presented according to Duncan test at 0.05 level of confidence)

6.5.4 Heavy metal adsorbed after incubation

The pH and Eh values from the incubation periods detailed in Section 6.5.2 indicated that, the heavy metal used occurs in the following forms or speciation: As^{3+}, Cr^{6+}, Cu^{2+}, Pb, and Zn^{2+} due to variations in pH and redox potential (Eh). The percentage amount of heavy metal adsorbed in the soil after 60 days incubation varied across the treatment levels (L, M and H) as shown in Figure 6.9. The amount of metals absorbed increased with increased concentration in the order lead > arsenic > chromium > copper > zinc. Soil polluted with high concentration of arsenic and Mx treatments had significant higher percentages of heavy metal adsorbed in the soil compared to the soil polluted with low concentration. Soil spiked with chromium, lead, and zinc did not show any significant difference with the level of concentration.

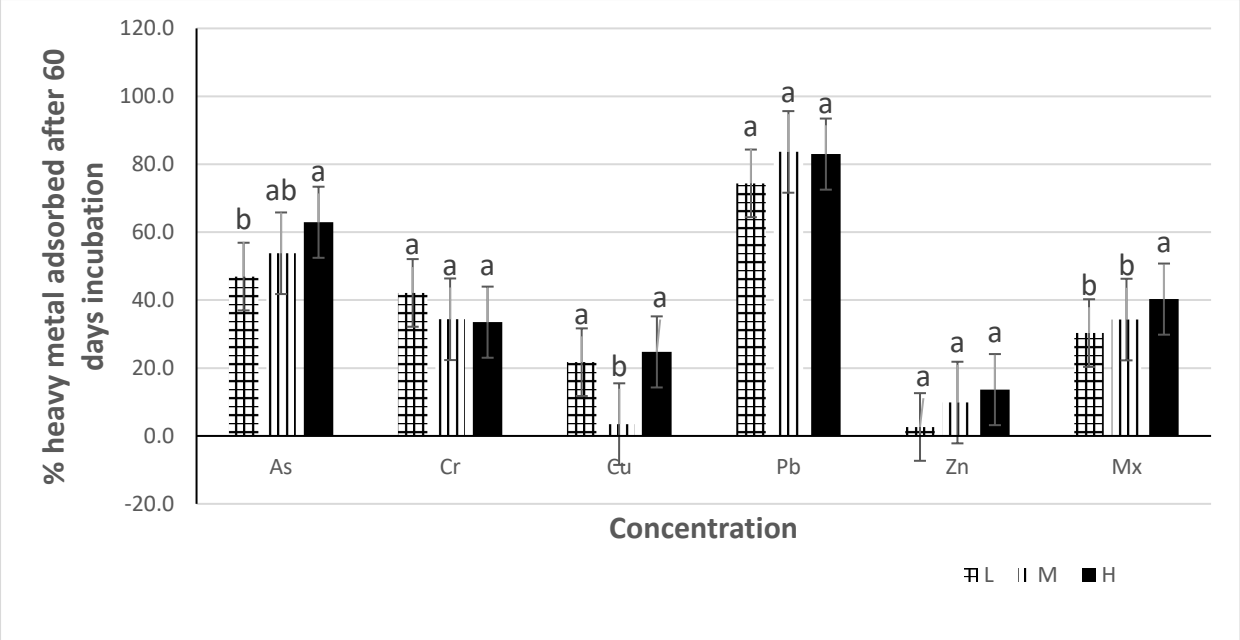


Figure 6. 9 Percentage amount of metals adsorbed after 60 days incubation (error bars are presented as standard error of the mean (n= 3), statistical significance is presented as a, b, ab= Duncan test at 0.05 level of confidence), L= Low dose, M= Medium dose, H= High dose

6.5.5 Soil humic substance characterisation

There was a higher amount of carbon in the carbonyl carbon functional group than O-alkyl carbon and alkyl carbon except for the Mx treatment in which the highest carbon was within O-alkyl with no carbon in alkyl-C across the humic acids fraction, while copper, Mx and zinc treated soil had a higher amount of carbon in the O-alkyl functional group than in the carbonyl functional group in the fulvic acids fraction. The control treatment in the humic acids fraction was greater in carbonyl carbon in the following order: 18 times > Mx treated soil, 13 times > zinc treated soil, 9 times > copper treated soil, 7 times > lead treated soil, 5 times > arsenic treated soil and 1 time > chromium treated soil, while the control treatment carbonyl carbon in the fulvic fraction follows the pattern: Control = lead treated soil > 14 times Mx treated soil > 12 times copper treated soil > 8 times chromium treated soil > 6 times zinc treated soil > twice As treated soil (Figure 33). Generally, the soil without any heavy metal treatment has high organic carbon distribution using the liquid-state ^{13}C-NMR spectroscopy. The ratio of A-alkyl carbon to O-alkyl carbon indicated that a greater amount of organic matter was decomposed in the control treatment than the soil treated with heavy metal (Figure6.11).

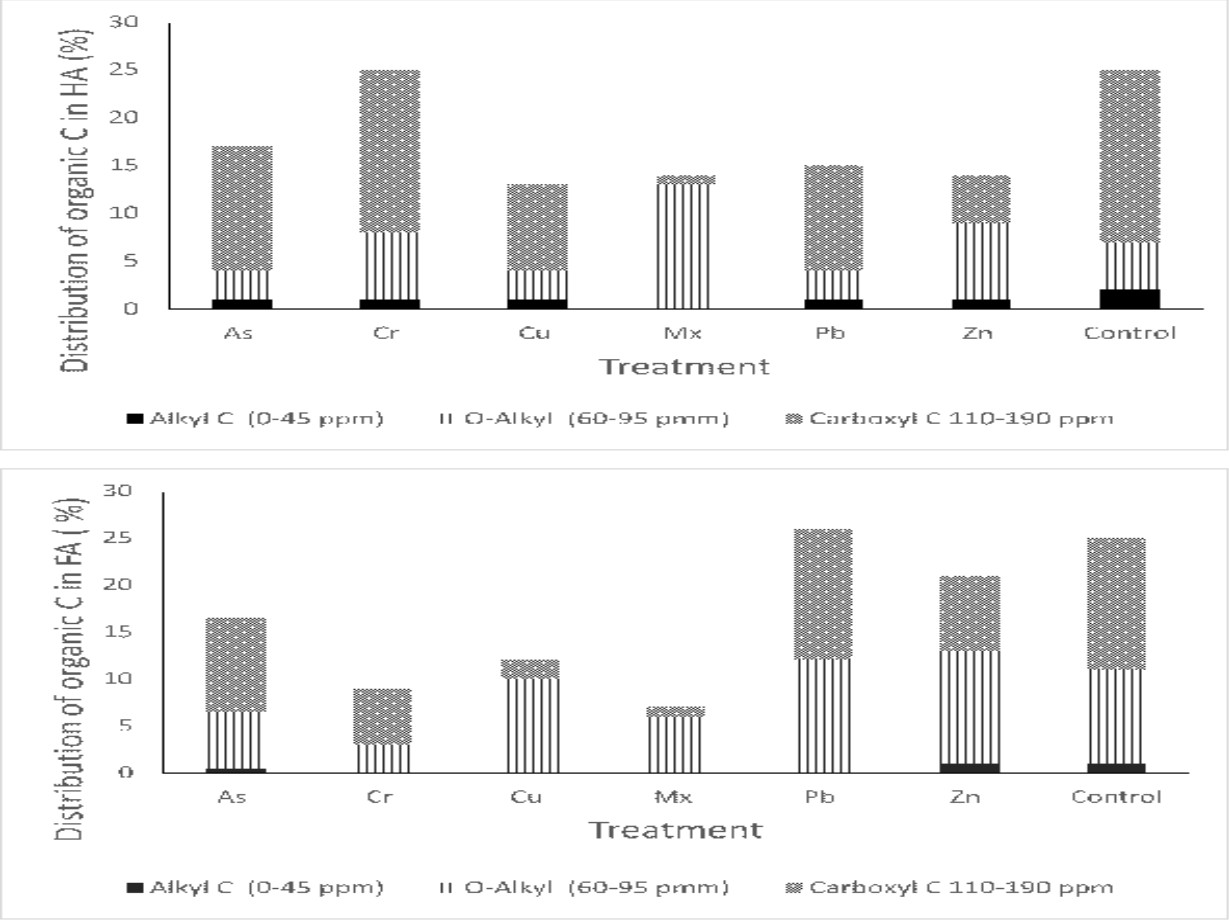


Figure 6. 10 Distribution of organic carbon in the humic acids (HA) and fulvic acids (FA) from soil spiked with heavy metals + control (Mx= arsenic + chromium + copper + lead + zinc)

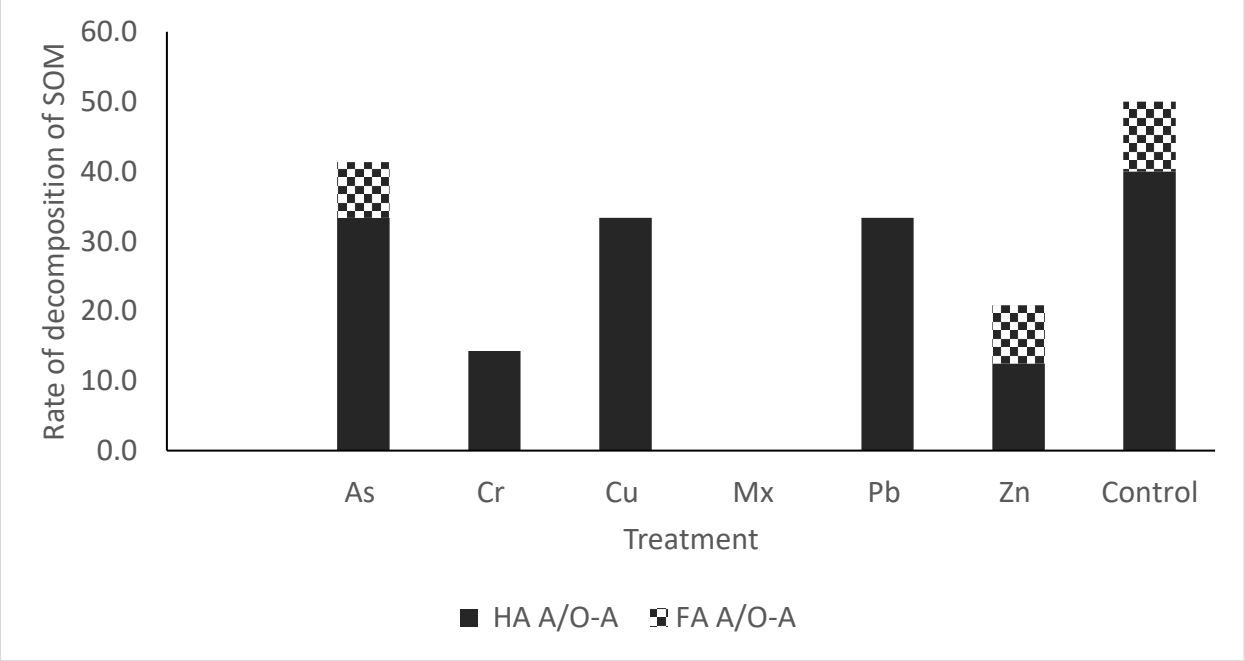


Figure 6. 11 Decomposition rate of soil organic matter in humic acids (HA) and fulvic acids (FA) from soil spiked with heavy metals and control

6.5.6 Multivariate analysis showing interaction effects among selected parameters

From the Pillai's Trace multivariate test (Table 6.5), the results indicated that, the intercept, incubation period, treatment level, and interaction effects (incubation period * treatment level) were significant at the 5% level as P value is 0.00. This is an indication that there is significant difference in the values of the dependent variables obtained from the different incubation periods and treatment levels.

Table 6. 5 Pillai's Trace multivariate tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Observed Power c
Intercept	Pillai's Trace	1.000	9610499.505b	23	2	0.00	1.00	1.00
Incubation period	Pillai's Trace	2.000	62419.793	46	6	0.00	1.00	1.00
Treatment level	Pillai's Trace	3.000	10268.555	69	12	0.00	1.00	1.00
Incubation period * Treatment level	Pillai's Trace	5.906	19.092	138	42	0.00	0.98	1.00

a Design: Intercept + incubation period + Treatment level + incubation period * Treatment level; b Exact statistic; c Computed using alpha = 0.05

The tests of between-subjects' effects from the multivariate analysis of variance is presented in (Tables 6.6, 6.7, 6.8, and 6.9). There was a significant difference (at $P < 0.05$) in the intercept and treatment level among all the dependent variables (Tables 6.6 and 6.8). This is an indication that values obtained under different treatment level and the intercept in all the dependent variables were different. The incubation period shows a significant difference in all the dependent variables except pH under arsenic contamination (As_pH), soil organic carbon under chromium contamination (Cr_SOC) and soil organic carbon under zinc contamination (Zn_SOC) (Table 6.7). This means that not all the values in the dependent variables are different during the incubation periods as detailed in Section 6.5.3. Table 6.9 shows the interaction effects between the different incubation periods and the treatment levels. The results indicated that majority of the dependent variables were significant (at $P < 0.05$) except As_SOC, Cr_SOC, Mixed_SOC, Pb_SOC, Zn_SOC, Cr_MC, Mixed_MC, and Pb_MC (Table 6.9). However, Duncan multiple range for soil organic carbon at $P < 0.05$ shows which treatment and incubation period was significant (Section 5.4.3).

Table 6. 6 Tests of between-subjects effects among the dependent and independent variables

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power ae
Intercept	As_pH	2518.8769	1	2518.869	37278.22	0.00	1.00	1.00
	Cr_pH	2670.650	1	2670.650	279486.60	0.00	1.00	1.00
	Cu_pH	1782.528	1	1782.528	63166.67	0.00	1.00	1.00
	Mixed_pH	1975.802	1	1975.802	239733.40	0.00	1.00	1.00
	Pb_pH	2138.908	1	2138.908	77527.89	0.00	1.00	1.00
	Zn_pH	1752.399	1	1752.399	1990106.00	0.00	1.00	1.00
	As_Eh	230829.000	1	230829.000	11976.87	0.00	1.00	1.00
	Cr_Eh	295497.336	1	295497.300	15983.77	0.00	1.00	1.00
	Cu_Eh	2789.072	1	2789.072	56.56	0.00	0.70	1.00
	Mixed_Eh	31986.130	1	31986.130	1576.20	0.00	0.99	1.00
	Pb_Eh	77106.182	1	77106.180	1028.68	0.00	0.98	1.00
	Zn_Eh	1376.163	1	1376.163	556.62	0.00	0.96	1.00
	As_EC	316.425	1	316.425	275152.00	0.00	1.00	1.00
	Cr_EC	3760.756	1	3760.756	45406.04	0.00	1.00	1.00
	Cu_EC	1246.208	1	1246.208	88019.38	0.00	1.00	1.00
	Mixed_EC	1713.960	1	1713.960	32569.31	0.00	1.00	1.00
	Pb_EC	354.130	1	354.130	325221.10	0.00	1.00	1.00
	Zn_EC	1436.157	1	1436.157	120883.00	0.00	1.00	1.00
	As_SOC	2839.646	1	2839.646	70951.74	0.00	1.00	1.00
	Cr_SOC	3302.035	1	3302.035	907.66	0.00	0.97	1.00
	Cu_SOC	3105.276	1	3105.276	87774.75	0.00	1.00	1.00
	Mixed_SOC	3180.208	1	3180.208	28234.36	0.00	1.00	1.00
	Pb_SOC	2614.277	1	2614.277	77339.11	0.00	1.00	1.00
	Zn_SOC	3206.579	1	3206.579	8236.25	0.00	1.00	1.00
	As_MC	142847.462	1	142847.500	14071.19	0.00	1.00	1.00
	Cr_MC	167884.136	1	167884.100	4307.00	0.00	1.00	1.00
	Cu_MC	146576.675	1	146576.700	4279.79	0.00	0.99	1.00
	Mixed_MC	187224.963	1	187225.000	11621.90	0.00	1.00	1.00
	Pb_MC	193098.725	1	193098.700	11689.12	0.00	1.00	1.00
	Zn_MC	158875.317	1	158875.300	23569.90	0.00	1.00	1.00

_ = Under heavy metal contamination; MC = Moisture content; Mixed = combination of arsenic, chromium, copper, lead, and zinc.

Table 6. 7 Tests of between-subjects effects among the dependent and independent variables

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Square d	Observed Power ae
incubation period	As_pH	0.430	2	0.215	3.18	0.06	0.21	0.55
	Cr_pH	0.457	2	0.228	23.90	0.00	0.67	1.00
	Cu_pH	0.350	2	0.175	6.19	0.01	0.34	0.85
	Mixed_pH	0.290	2	0.145	17.60	0.00	0.60	1.00
	Pb_pH	0.891	2	0.445	16.14	0.00	0.57	1.00
	Zn_pH	1.483	2	0.741	841.84	0.00	0.99	1.00
	As_Eh	3451.582	2	1725.791	89.55	0.00	0.88	1.00
	Cr_Eh	2809.506	2	1404.753	75.99	0.00	0.86	1.00
	Cu_Eh	6167.180	2	3083.590	62.53	0.00	0.84	1.00
	Mixed_Eh	7506.740	2	3753.370	184.96	0.00	0.94	1.00
	Pb_Eh	13709.230	2	6854.615	91.45	0.00	0.88	1.00
	Zn_Eh	16723.249	2	8361.625	3382.07	0.00	0.99	1.00
	As_EC	6.048	2	3.024	2629.74	0.00	0.99	1.00
	Cr_EC	64.593	2	32.296	389.94	0.00	0.97	1.00
	Cu_EC	1.761	2	0.880	62.18	0.00	0.84	1.00
	Mixed_EC	76.642	2	38.321	728.19	0.00	0.98	1.00
	Pb_EC	6.900	2	3.450	3168.29	0.00	0.99	1.00
	Zn_EC	23.770	2	11.885	1000.36	0.00	0.99	1.00
	As_SOC	1.637	2	0.818	20.45	0.00	0.63	1.00
	Cr_SOC	3.1260	2	1.563	0.43	0.66	0.04	0.11
	Cu_SOC	0.588	2	0.294	8.30	0.00	0.41	0.94
	Mixed_SOC	0.928	2	0.464	4.12	0.03	0.26	0.67
	Pb_SOC	0.826	2	0.413	12.21	0.00	0.50	0.99
	Zn_SOC	1.941	2	0.970	2.49	0.10	0.17	0.45
	As_MC	4717.761	2	2358.881	232.36	0.00	0.95	1.00
	Cr_MC	4416.005	2	2208.002	56.65	0.00	0.83	1.00
	Cu_MC	4215.538	2	2107.769	61.54	0.00	0.84	1.00
	Mixed_MC	6036.272	2	3018.136	187.35	0.00	0.94	1.00
	Pb_MC	7059.852	2	3529.926	213.68	0.00	0.95	1.00
	Zn_MC	6532.724	2	3266.362	484.58	0.00	0.98	1.00

_ = Under heavy metal contamination; MC = Moisture content; Mixed = combination of arsenic, chromium, copper, lead, and zinc.

Table 6. 8 Tests of between-subjects effects among the dependent and independent variables

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Square d	Observed Power ae
Treatment level	As_pH	10.173	3	3.391	50.19	0.00	0.86	1.00
	Cr_pH	17.636	3	5.879	615.21	0.00	0.99	1.00
	Cu_pH	4.390	3	1.463	51.85	0.00	0.87	1.00
	Mixed_pH	4.261	3	1.420	172.33	0.00	0.96	1.00
	Pb_pH	0.944	3	0.315	11.40	0.00	0.59	1.00
	Zn_pH	23.727	3	7.909	8981.98	0.00	0.99	1.00
	As_Eh	30303.587	3	10101.200	524.11	0.00	0.99	1.00
	Cr_Eh	45377.590	3	15125.860	818.17	0.00	0.99	1.00
	Cu_Eh	11698.295	3	3899.432	79.08	0.00	0.91	1.00
	Mixed_Eh	12531.317	3	4177.106	205.84	0.00	0.96	1.00
	Pb_Eh	2923.84	3	974.613	13.00	0.00	0.62	1.00
	Zn_Eh	69598.396	3	23199.470	9383.60	0.00	0.99	1.00
	As_EC	30.731	3	10.244	8907.51	0.00	0.99	1.00
	Cr_EC	1271.425	3	423.808	5116.91	0.00	0.99	1.00
	Cu_EC	88.201	3	29.400	2076.54	0.00	0.99	1.00
	Mixed_EC	212.145	3	70.715	1343.75	0.00	0.99	1.00
	Pb_EC	22.907	3	7.6436	7012.49	0.00	0.99	1.00
	Zn_EC	394.877	3	131.626	11079.09	0.00	0.99	1.00
	As_SOC	4.465	3	1.488	37.19	0.00	0.82	1.00
	Cr_SOC	48.240	3	16.080	4.42	0.01	0.36	0.81
	Cu_SOC	12.331	3	4.110	116.18	0.00	0.94	1.00
	Mixed_SOC	18.342	3	6.114	54.28	0.00	0.87	1.00
	Pb_SOC	0.836	3	0.279	8.24	0.00	0.51	0.98
	Zn_SOC	31.686	3	10.562	27.13	0.00	0.77	1.00
	As_MC	4133.497	3	1377.832	135.72	0.00	0.94	1.00
	Cr_MC	2310.069	3	770.023	19.76	0.00	0.71	1.00
	Cu_MC	3788.532	3	1262.844	36.87	0.00	0.82	1.00
	Mixed_MC	1380.129	3	460.043	28.56	0.00	0.78	1.00
	Pb_MC	829.207	3	276.402	16.73	0.00	0.68	1.00
	Zn_MC	2789.092	3	929.697	137.93	0.00	0.95	1.00

_ = Under heavy metal contamination; MC = Moisture content; Mixed = combination of arsenic, chromium, copper, lead, and zinc.

Table 6. 9 Tests of between-subjects effects among the dependent and independent variables

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Square d	Observed Power ae
incubation	As_pH	1.625	6	0.271	4.01	0.01	0.50	0.92
period*	Cr_pH	1.968	6	0.328	34.33	0.00	0.90	1.00
Treatment	Cu_pH	0.934	6	0.156	5.52	0.00	0.58	0.98
level	Mixed_pH	1.254	6	0.209	25.37	0.00	0.86	1.00
	Pb_pH	0.462	6	0.077	2.79	0.03	0.41	0.78
	Zn_pH	0.653	6	0.109	123.57	0.00	0.97	1.00
	As_Eh	8929.435	6	1488.239	77.22	0.00	0.95	1.00
	Cr_Eh	5118.335	6	853.056	46.14	0.00	0.92	1.00
	Cu_Eh	5255.074	6	875.846	17.76	0.00	0.82	1.00
	Mixed_Eh	3868.690	6	644.782	31.77	0.00	0.89	1.00
	Pb_Eh	1612.557	6	268.760	3.59	0.01	0.47	0.89
	Zn_Eh	1765.158	6	294.193	118.99	0.00	0.97	1.00
	As_EC	26.053	6	4.342	3775.85	0.00	0.99	1.00
	Cr_EC	103.091	6	17.182	207.45	0.00	0.98	1.00
	Cu_EC	36.258	6	6.043	426.82	0.00	0.99	1.00
	Mixed_EC	75.468	6	12.578	239.01	0.00	0.98	1.00
	Pb_EC	28.256	6	4.709	4324.93	0.00	0.99	1.00
	Zn_EC	68.986	6	11.498	967.78	0.00	0.99	1.00
	As_SOC	0.384	6	0.064	1.60	0.19	0.29	0.50
	Cr_SOC	11.410	6	1.902	0.52	0.79	0.12	0.17
	Cu_SOC	0.656	6	0.109	3.09	0.02	0.44	0.83
	Mixed_SOC	1.552	6	0.259	2.30	0.07	0.37	0.68
	Pb_SOC	0.278	6	0.046	1.37	0.27	0.26	0.43
	Zn_SOC	4.134	6	0.689	1.77	0.15	0.31	0.55
	As_MC	460.025	6	76.671	7.55	0.00	0.65	0.99
	Cr_MC	484.123	6	80.687	2.07	0.10	0.34	0.63
	Cu_MC	727.332	6	121.222	3.54	0.01	0.47	0.88
	Mixed_MC	170.621	6	28.437	1.77	0.15	0.31	0.55
	Pb_MC	82.821	6	13.803	0.84	0.56	0.17	0.27
	Zn_MC	228.889	6	38.148	5.66	0.00	0.59	0.99

_ = Under heavy metal contamination; MC = Moisture content; Mixed= combination of arsenic, chromium, copper, lead, and zinc.

Table 6.10 shows the Pearson's correlation coefficient for pH, Eh, EC, SOC and MC under different arsenic, chromium, copper, lead, zinc, and a combination of arsenic, chromium, coper, lead, and zinc. This indicated that a relationship exists among pH, Eh, EC, SOC and MC under different heavy metal contamination. The pH under arsenic (AS_pH) contamination was positively correlated with chromiumr and lead (0.796** and 0.472** respectively) at $P < 0.01$) compared to other elements. The coefficient of correlation of pH under chromium contamination (Cr_pH) shows a negative correlation with copper and zinc while pH under copper (CU_pH) shows a negative correlation with lead compared to others. This means that as Cr_pH and Cu_pH increases, copper, zinc, and lead will be decreased respectively. Soil redox potential Eh varies in a relationship when soil was artificially spiked with heavy metals. Eh under arsenic contamination correlated positively with Eh under chromium and lead contamination (0.788** and 0.546** respectively) at $P < 0.01$ compared to Eh under chromium contamination. EC values under arsenic contamination (As_EC) correlated negatively with Cr_EC while EC values under chromium contamination (Cr_EC) correlated negatively with Pb_EC (see Table 6.10). Soil organic carbon content under different heavy metal contamination shows a perfect positive correlation among the different heavy metal contamination except when chromium was contaminated. The soil moisture content (MC) shows a perfect positive correlation among the different heavy metal contamination (Table 6.10). A positive correlation implies that any increased in the soil moisture content resulting from the contamination of one metal will lead to an increased in the soil moisture content due to the contamination of the other metal.

Table 6. 10 Pearson's correlation coefficient for pH, Eh, EC, SOC and MC under different heavy metal contamination

Correlations						
	As_pH	Cr_pH	Cu_pH	Mixed_pH	Pb_pH	Zn_pH
As_pH	1					
Cr_pH	0.796**	1				
Cu_pH	-.519**	-0.306	1			
Mixed_pH	-0.065	0.138	0.543**	1		
Pb_pH	0.472**	0.577**	-0.039	0.088	1	
Zn_pH	-0.344*	-0.044	0.786**	0.560**	0.131	1
	As_Eh	Cr_Eh	Cu_Eh	Mixed_Eh	Pb_Eh	Zn_Eh
As_Eh	1					
Cr_Eh	.788**	1				
Cu_Eh	-0.292	-0.145	1			
Mixed_Eh	0.159	0.240	0.658**	1		
Pb_Eh	.546**	0.582**	0.322	0.461**	1	
Zn_Eh	-0.276	0.068	0.779**	0.676**	0.327	1
	As_EC	Cr_EC	Cu_EC	Mixed_EC	Pb_EC	Zn_EC
As_EC	1					
Cr_EC	-0.081	1				
Cu_EC	0.174	0.838**	1			
Mixed_EC	0.077	0.917**	0.787**	1		
Pb_EC	0.958**	-0.169	0.093	0.072	1	
Zn_EC	0.169	0.956**	0.837**	0.909**	0.060	1
	As_SOC	Cr_SOC	Cu_SOC	Mixed_SOC	Pb_SOC	Zn_SOC
As_SOC	1					
Cr_SOC	0.185	1				
Cu_SOC	.660**	0.314	1			
Mixed_SOC	.615**	0.243	.846**	1		
Pb_SOC	.637**	0.228	.486**	.548**	1	
Zn_SOC	.734**	0.082	.650**	.650**	.600**	1
	As_MC	Cr_MC	Cu_MC	Mixed_MC	Pb_MC	Zn_MC
As_MC	1					
Cr_MC	.912**	1				
Cu_MC	.935**	.827**	1			
Mixed_MC	.860**	.888**	.801**	1		
Pb_MC	.821**	.829**	.763**	.904**	1	
Zn_MC	.962**	.903**	.908**	.912**	.883**	1
N	36	36	36	36	36	36

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); _ = Under heavy metal contamination; MC = Moisture content; Mixed= combination of arsenic, chromium, copper, lead, and zinc.

6.6 Discussion

6.6.1 Effects of heavy metal contamination on soil properties

There may be effects of heavy metal contamination on soil chemical properties and mobility of trace element during the decomposition of soil organic matter. These effects in the present study were influenced by several factors soil pH, redox potential, electrical conductivity, heavy metal concentration, soil moisture content, types of heavy metal treatment, incubation periods, interaction between heavy metal treatment and incubation period (Tables 6.5, 6.6, 6.7, 6.8, 6.9, and 6.10). The present study shows that the concentration of arsenic, chromium, copper, and lead changes the soil pH from slightly alkaline to strongly alkaline while zinc concentration changes the pH from slightly alkaline to mildly acidic after 200 days incubation (Table 6.2 and Appendix 23). This may have effects of on the soil organism functioning, metal mobility, bioavailability, and forms with which metal can exist in the soil matrix as detailed in Chapter 4. According to Sauvé, McBride, Norvell & Hendershot (1997) copper solubility and speciation depends on the soil pH, Eh and organic matter variation while zinc mobilisation depends on soil pH and mobile soil content (Kalbitz & Wennrich, 1998). The distribution of redox potential (Eh) after the soil that was treated with heavy metals follows the pattern of the original Eh from the soil collected from the Upper Mersey estuarine floodplain showing negative redox potential (Tables 4.1, 6.3, Appendix 24 and Figure 5.5). The present study shows that increased concentration of heavy metal resulted in high values of electrical conductivity after 200 days incubation (Table 6.4 and Appendix 25), this could lead to high salinity and inactivity of soil microorganism. The values of pH, Eh and EC may affect the decomposition process of soil organic matter due to their effects on microbial activities and heavy metal retention. According to Williams et al., (1994), salinity affects flocculation-coagulation of colloidal particles, and adsorption-desorption reactions with organic matter. Generally, the concentration of heavy metal during the decomposition of soil organic matter changes the status of soil pH from slightly alkaline to mildly acidic and strongly alkaline while the soil electrical conductivity increased with increased concentration of heavy metal. This may have effects on the adsorption and mobility of heavy metal in the soil.

Heavy metal adsorbed in soil may be an important factor to examine the metal mobilty and availability due to the changes in soil chemical properties. In the present study, the amount of heavy metals adsorbed depends on the concentration of the heavy metal spiked into the soil (Figure 6.9 and Appendix 28), indicating that the adsorption potential of heavy metal in soil increased with increased concentration of heavy metal added. This may be due to the fact that the soil active site was not saturated allowing As^{3+}, Cr^{6+}, Cu^{2+} and Zn^{2+} to further react with the soil solution and as such the metals are easily adsorbed by the negatively charged soil surfaces. The redox potential-pH diagram has been used by (Masscheleyn, Delaune & Patrick, 1991; Otomo, Srivastava, Zakir, Mohiuddin & Shikazono, 2009; Navarro, Cardellach & Corbella, 2011) to determine the forms in which metals exist in solution. According to Lukman, Bukhari, Al-Malack, Mu'azu and Essa (2014), there is a redox state which controls the mobility of elements at different oxidation states. This is because of the generation of oxygen and hydrogen gases at the electrodes, in addition to the possible presence of iron (reducing agent), manganese (oxidising agent), or microorganism in the soil (Lukman et al., 2014). For example, most of the trivalent chromium species like the one used in the present study are less mobile because of their low solubility over a wide pH range (<12) and may be readily adsorbed by the negatively charged clay surfaces (Lukman et al., 2014). The presence of high concentrations of organic matter and ion exchange materials, such as clays, will greatly affect the ability of the saltmarsh sediments to retain metal ions via adsorption, chelation and ion exchange mechanisms (Williams et al., 1994). Therefore, the use of compost is an option for arsenic immobilisation in soils of low adsorption, taking into consideration the soil composition (Arco-Lázaro, Agudo, Clemente, & Bernal, 2016). The changes in soil pH, redox potential, and electrical conductivity in the present study resulted in more heavy metals being immobilised in the soil because the metals were easily adsorbed by the negatively charged soil surface.

6.6.2 Effects of heavy metal comtamination on soil carbon storage

Soil spiked with heavy metal may have effect on the amount of soil organic matter accumulation and enhanced soil carbon storage. The present study shows that the amount soil organic carbon was high in arsenic, chromium, copper, lead, and zinc contamination in soil after 200 days incubation (Figures 6.3, 6.4, 6.5, 6.6, 6.7, and 6.8, and Appendix 26). This may be due to incomplete carbon mineralisation to carbon dioxide in spiked treatments. The

rates of carbon mineralisation were complete in the control treatment leading to a greater amount of carbon loss in the form of carbon dioxide than the spiked soil (Figures 6.10, 6.11, and Appendix 27). According to Anderson (1982), mineralisation of organic carbon to carbon dioxide is a good index of total activity of microflora involved in organic matter decomposition while Carbon mineralisation decreased as the composting time lengthened (Bernal, Sanchez-Monedero, Paredes, & Roig, 1998). According to Hayes, Spurgeon, Lofts, & Jones (2018) contamination of soil by copper have potential effects on the following earthworm abundance, altered microbial activity and diversity, reduced rhizobacterial abundance leading to reduced N fixation, reduced plant cover/growth, reduced litter decomposition, and increased copper concentrations in plants and soil fauna. The reduction in litter decomposition due to impacts on detritivores could potentially lead to surface litter accumulation (Spurgeon, Ricketts, Svendsen, Morgan, & Kille, 2005; Hayes et al., 2018), leading to increased soil carbon stock (Kirschbaum, 1995; Nannipieri et al., 2013; van der Wal, Geydan, Kuyper, & De Boer, 2013). Microbial biomass and enzyme activities decreased with increasing heavy metal pollution, but the amount of decrease differed among the enzymes (Kuperman & Carreiro, 1997; Kunito et al., 2001; Hinojosa, García-Ruíz, Viñegla, & Carreira, 2004): enzymes involved in the carbon cycling were least affected. However, the effects of heavy metal on soil microbial diversity using DNA sequencing from the soil used for the present study is detailedin Chapter 7 of this thesis. Therefore, the enhanced soil organic carbon in the present study may be due to the effects of heavy metal on microbial activities responsible for the decomposition of soil organic matter, leading to incomplete mineralisation of carbon and reduced carbon dioxide emission.

There may be several other possible explanations for the high soil organic carbon storage from soils contaminated by heavy metal. For example, addition of maple leaves and alfalfa biomass to polycyclic aromatic hydrocarbon (PAH)-contaminated soil greatly increased its organic carbon content and the content of humic matter (Haderlein, Legros, & Ramsay, 2001). A similar observation was recorded in the present study with the used of freshly ground *Elytrigia repens* biomass collected from the Upper Mersey estuarine floodplain (Figures 6.3, 6.4, 6.5, 6.6, 6.7, 6.8 and 6.10). Litter additions in a soil incubation experiment were reported by Creamer et al. (2015) to have significant positive effects on bacterial communities (both gram-positive and gram-negative) degrading soil carbon, thereby affecting soil carbon

respiration. According to Landi, Renella, Moreno, Falchini & Nannipieri (2000), heavy metals may reduce the substrate availability for soil respiration by forming complexes with the substrates or by killing the microorganisms. Several enzyme activities related to the cycling of nitrogen, phosphorous and sulphur showed a considerable reduction in activity after heavy metal contamination in soil (Kandeler, Kampichle, & Horak, 1996). Heavy metal contaminated soils lose very common biochemical properties which are necessary for the functioning of the ecosystem (Kandeler et al., 1996). According to Cotrufo, De Santo, Alfani, Bartoli, & De Cristofaro (1995) metabolic activity (both as soil respiration and degree of mineralisation) was lower in the soil polluted with chromium, lead, and zinc. Therefore, the nature and quality of litter added during the incubation process could increased soil organic matter accumulation and carbon storage because of the reduction in enzyme activities and soil respiration.

6.6.3 Effects of heavy metal contamination on humic substances

Heavy metal contamination may have effects on the humic acids and fulvic acids fractions after soil incubation. In the present study, the effects of heavy metal contamination after the 200 days soil incubation indicates that differences exist in humic substances between the spiked soil and uncontaminated soil (Figure 6.10). The amount of organic carbon in the humic acids and fulvic acids was high in the control treatment compared to the spike soil (Figures 6.10, 6.11 and Appendices 29 to 44). This may be an indication of high microbial activities taking place within the uncontaminated soil compared to the contaminated treatment, leading to complete mineralisation of carbon. According to Stevenson (1994), fulvic acids have higher COOH groups than humic acids, indicating a high amount of carbohydrates in fulvic acids. The uncontaminated soil from the present study was higher in alkyl carbon across the humic acids and fulvic acids fractions. This is in corroboration with Baldock et al. (1997) findings which reported that the relative contribution of alkyl carbon tends to increase with increasing degrees of decomposition of the organic matter. Stevenson (1994) reported that ^{13}C-NMR spectra were higher in proportion of O-alkyl structures (carbohydrates) in soil organic matter than can be accounted for by conventional analyses for monosaccharides using acid hydrolysis, whereas the high content of aliphatic carbon in the fine clay was thought to be due to accumulation of recalcitrant plant waxes strongly associated with clay soil (Baldock, Currie, Oades, & Wilson, 1991). The ratio of A-alkyl to O-alkyl carbon has been reported to provide an indication to the decomposition of soil organic

matter. According to Baldock et al. (1997) and Shiau, Chen, Chung, Tian, & Chiu (2017) alkyl carbon will increased with increased degree of decomposition of organic matter. In the present study, the alkyl carbon in the humic substance within the control treatment was higher compared to the contaminated soils. According to Shiau et al. (2017) research on soil organic matter quality, O-alkyl carbon content (carbohydrate-derived structures) was higher in the grassland than the forest soils while alkyl carbon content (recalcitrant substances) was higher in forest than grassland soils. The higher the alkyl carbon the higher the humification (Shiau et al., 2017). This implies that alkyl carbon is more stable and difficult to decomposed by microorganism compare to O-alkyl carbon. The easily decomposable litter found in O-alkyl carbon may potentially remediate the impact of wildfire and may worth studying (Shiau et al., 2017). There is an indication that the rate of decomposition of organic matter in the present study was faster in the uncontaminated soils than the contaminated soils and this may be responsible for the variation in carbon content within the humic acids and fulvic acids fractions.

In summary, the fate of heavy metal contamination on soil carbon storage and humic substances characterisation is poorly understood. This is not only vital in predicting the soil carbon status but also useful in identifying the different carbon species or functional groups during the decomposition of organic matter to determine the transformation rates as well as making useful management decisions. The present study shows that the effects of heavy metal contamination on soil organic carbon content was controlled by several factors including, soil pH, redox potential, electrical conductivity, incubation period, soil moisture content, concentration of the heavy metal, soil microbial activities, adsorption capacity of the heavy metal, speciation of the heavy metal, soil types, and methods of analysis used. The soil spiked with arsenic, chromium, copper, lead, and zinc enhances the amount of soil organic storage in a controlled soil moisture regime after 200 days incubation period. This is because the decomposition of soil organic matter is reduced due to the effects of the heavy metal on soil microbial activities leading to reduced carbon dioxide emission or incomplete mineralisation of soil organic carbon. The humic substances characterisation shows three carbon species such as carbonyl carbon, O-alkyl carbon and A-alkyl carbon. This is an indication of the different stage of the decomposition processes. The control treatment shows high rate of decomposition of organic matter with high amount carbon in the

functional group, indicating complete mineralisation of carbon. Arsenic, chromium, copper, lead, and zinc contamination in soil changes the original chemical properties of the soil. The pH from soil spiked by arsenic was changed from slightly alkaline to moderately alkaline while the pH from soil spiked with chromium was changed from moderately to strongly alkaline. The pH from soil il contaminated by copper was changed from slightly alkaline to slightly acid while that of zinc contaminated soil was changed from slightly alkaline to moderately acid. This may lead to differences in metal adsorption and mobilty due to the different metal speciation. The redox potential and electrical conductivity were higher than those found in the original soil used, indicating high oxidation-reduction and salinity were taking place. This could have effect on the soil microbial activities and the the speciation of the metals used thereby leading to changes in oxidation state as well as the adsorption capacity of the metals. The amount of heavy metal adsorbed after 60 days incubation period was affected by the concentration; high concentrations resulted in high amounts of heavy metal adsorbed in the order 83% lead > 63% arsenic > 34% chromium > 25% copper > 14% zinc. The present study has shown that contamination of soil by heavy metal have both positive and negative effects to the environment.

7. Microbial Diversity in Soils Under Different Land uses/land covers

7.1 Introduction to the chapter

According to McGrath, Chaudri, & Giller (1995), heavy metal contaminated soil is known to affect soil microbial populations and their associated activities. Oliveira & Pampulha (2006) reported a decrease in microbial diversity due to soil heavy metal contamination. Asymbiotic nitrogen-fixers and heterotrophic bacteria were particularly sensitive to long-term contamination (Oliveira & Pampulha, 2006; Oliveira, Pampulha, Neto, & Almeida, 2010). According to Hiroki (1992) and Rajapaksha, Tobor-Kapłon, & Baath (2004) fungi, actinomycetes, and bacteria decreased significantly with increasing heavy metal content in soil. Other reports have shown that the effect of soil contamination is to reduce decomposition of soil organic matter which in turn will enhanced soil organic carbon storage as detailed in Section 6.6.

It is hypothesised that long-term contamination by heavy metals will affect the diversity of microbes due to the impact on soil and plant biomass (Pérez-de-Mora et al., 2006). According to Hartman, Richardson, Vilgalys, & Bruland (2008), microbial diversity could markedly affect the decomposition of soil organic matter. However, most previous studies on the effects of contamination on soil microbial communities have used laboratory cultural methods which are often biased due to the unculturability of many microorganisms and the lack of sensitivity of traditional microbiological methods (Martin-Laurent et al., 2001). DNA sequencing is being used in many studies as a powerful tool for gathering information about unculturable and culturable microorganisms and their environment (Sanger et al., 1977; Handelsman, Rondon, Brady, Clardy, & Goodman, 1998; Riesenfeld, Schloss & Handelsman, 2004; Zwolinski, 2007; Woo, Lau, Teng, Tse, & Yuen, 2008; Lui, Chou, Chen, & Kuo, 2015).

Until relatively recently most DNA sequencing was based on the traditional Sanger DNA-sequencing, which is only capable of sequencing up to approximately 1 kilo byte for 96 individual specimens (Schuster, 2007; Taberlet, Coissac, Pompanon, Brochmann & Willerslev, 2012). Therefore, it is inadequate for processing complex environmental samples, especially

for large-scale studies (Hajibabaei, Shokralla, Zhou, Singer, & Baird, 2011). Next-generation sequencing (NGS) platforms have made it possible to generate several hundred thousand to tens of millions of sequences for individual microorganisms and even collect whole-environment genome information (Sogin et al. 2006; Zwolinski, 2007; de Menezes et al., 2011; de Menezes, Clipson, & Doyle, 2012; Shokralla et al., 2012; Goodwin, McPherson, & McCombie, 2016; Edet et al., 2017). According to Hajibabaei et al. (2011), NGS has made it possible to explore biodiversity measures across time and space by annotating and clustering DNA sequences using a combination of assignment and phylogenetics techniques. The majority of the research to restore polluted soil and sediments through bioremediation using NGS has focused on organic pollutants such as oil-contaminated soil and hydrocarbon contaminants, like polycyclic aromatic hydrocarbons (PAHs). However, a few papers have reported on different bacterial communities and diversity in soils that are contaminated by heavy metal, like arsenic, chromium, copper, lead and zinc (Gołębiewski, Deja-Sikora, Cichosz, Tretyn, & Wróbel, 2014). According to Wang et al. (2007) polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis showed that heavy metals pollution has a significant impact on bacterial and actinomycetic community structures.

The objective of the research reported in this chapter is to use 16S rDNA (NGS) to characterise the microbial diversity based on heavy metal contamination history under the present land uses/land covers within the Upper Mersey estuarine floodplain. This will be done by analysing and evaluating the data collected from the DNA sequencing to understand the composition of bacterial communities and the relative contribution of each bacterial group to the community. This will allow certain relationships to be established between the microbial abundance and the soil properties of each site. Thereby, improving our understanding of bacterial identification, particularly those unculturable in the laboratory, and serve as baseline data for further research.

7.2 Material and methods

7.2.1 Sampling design, preparation and storage

The field sampling design is detailed in Chapter 3 while samples preparation is detailed in Chapter 4 and 5. Twelve composite soil samples collected from grazing, non-grazing and

reed bed land uses/land cover types within 0–15 cm and 15–30 cm depths at the lowlands part of Upper Mersey estuarine floodplain were used for the 16S DNA sequencing (that is, 2 replicates in 3 locations at 2 different depths). Selection of the soil samples was based on their significant level of heavy metals from the results of the preliminary studies detailed in Chapter 4. The soil sub-samples were collected from the prepared soil samples that was used for chemical analysis and were stored at 4 °C in the fridge for 3 weeks prior to DNA extraction while the extracted DNA samples were stored at -20 °C in the freezer prior to DNA sequencing.

7.2.2 Laboratory analysis

The laboratory analysis involves sample preparation/DNA extraction, library construction, sequencing, and data generation. The DNA extraction procedures were carried out in the University of Salford laboratory while the library construction, sequencing and data generation was carried out by the company Macrogen based in South Korea.

7.2.2.1 Sample preparation/DNA extraction

DNA extraction and polymerase chain reaction (PCR) amplification were determined using the MoBio Power soil kits protocol and gel electrophoresis procedures, respectively. During the DNA extraction, 0.25 g of soil sample was added to the powerbead tubes and was gently vortexed to mix. The procedures carried out was according to manufacturer's instructions. This involved the centrifuging at room temperature for 1 minute or 30 seconds (depending on the stage) at 10,000 x g, and collection of supernatants. At the final stage, spin filters were used to trap the DNA which was then dissolved by solution C6 into the Eppendorf tubes and the DNA was then ready for any downstream application (such as nucleic acid concentration, PCR amplification, and sequencing). Nucleic acid concentration was measured using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer.

PCR amplification was carried out with an initial denaturing step for 2 min at 95 °C, followed by 30 amplification cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s extension at 72 °C. The amplification cycle was followed by a final extension step for 7 minutes at 72 °C. Each PCR contained 1 µL of 10 picomol primer 515f (5'–TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG–3'), 1 µL of 10 picomol primer 806R (5'–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG–3'), 12.5 µL of 2.5x MyTaq™mastermix

(Bioline) containing dNTP solution, $MgCl_2$ and Taq polymerase, 1 µL of sample DNA and distilled water to 25 µL.

7.2.2.2 Library construction

The library construction was carried out by the company Macrogen based in South Korea after performing quality control from the DNA samples. This was done using, 'tagmentation' method which combines the fragmentation and ligation reactions into a single step that greatly increases the efficiency of the library preparation process. Adapter-ligated fragments are then PCR amplified and gel purified.

7.2.2.3 Sequencing

The DNA sequencing was carried out by the company Macrogen based in South Korea using Illumina sequencing by synthesis (SBS) technology which utilises a proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands. As all four reversible, terminator-bound dNTPs (A, C, T, G) are present during each sequencing cycle, natural competition minimises incorporation bias and greatly reduces raw error rates compared to other technologies. This makes the result highly accurate since the base-by-base sequencing virtually eliminates sequence-context-specific errors, even within repetitive sequence regions and homopolymers, whereas the sequencing procedures for cluster generation involve two steps: 1. the libraries are loaded into a flow cell where fragments are captured on a lawn of surface-bound oligos complementary to the library adapters; and 2. each fragment is then amplified into distinct, clonal clusters through bridge amplification and the templates are ready for sequencing.

7.3 Raw data and statistical analysis

The Illumina MiSeq generates raw images utilising MCS (MiSeq Control Software v2.2) for system control and base calling through an integrated primary analysis software called RTA (Real Time Analysis. v1.18). The BCL (base calls) binary is converted into FASTQ utilising the Illumina package bcl2fastq (v1.8.4). One-way ANOVA using IBM SPSS 24.0 version statistical tool was carried out to analyse the community diversity index based on the Shannon diversity index and the Simpson diversity index and was calculated using (Community Invasive Species Network, 2019) manual. A Post hoc multiple comparison for

observed means from the IBM SPSS 24.0 was carried out using Duncan Multiple Range Test in order to further separate the mean to test for significant differences using lower case alphabets (a, b, c, d etc), were the alphabets indicates different degree of significance (at $P < 0.05$).The heatmap process was carried out using the shiny heatmap described by Khomtchouk, Hennessy and Wahlestedt (2017). The top 30 genus and their reads from the various samples were used for the heatmap analysis; the read counts of the organisms were matched against their names in an Excel file and then used for plotting the heatmap. A Euclidean distance matrix was used and linkage algorithm kept at complete and clustering applied to both rows and columns. A relative abundance plot was created for the kingdom phyla using the various percentages, as described by Peng, Zi, & Wang (2015), as stacked bars.

7.4 Results

7.4.1 Sequencing quality, community richness and diversity

The data for sequencing quality, community richness and diversity are presented in Table 7.1. The results indicated that over 3,256,146 valid reads and total read bases of 77.1 million base pairs were produced. The mean values of the ratio of reads that have a Phred quality score of over 20 (Q20) and the ratio of reads that have a Phred quality score of over 30 (Q30) were 84.263% and 75.829% respectively. The operational taxonomic unit (OTU) and Chao1 across the sampling locations indicated that the genetic similarity score matching (GSM) was higher in the reed beds (RB) than for all the sampling depths. The Simpson index shows no significant difference (at $P < 0.05$) in GSM 15–30 cm and GSM-N 0–15 cm sampling locations (Table 7.1).

The Shannon diversity index was significantly different (at $P < 0.05$) across the sampling locations in the order GSM 0–15 cm > GSM-N 0–15 cm > GSM 15–30 cm > GSM-N 15–30 cm > RB 0–15 cm > RB 15–30 cm depths while the Simpson diversity index indicated that GSM was higher than the RB location and GSM-N 15–30 cm depth only. There was a high diversity of microbiomes across the three sampling locations with more diversity within 0–15 cm soil depth (Table 7.1). However, grazing and non-grazing locations appear to be more diverse compared to the reed bed location.

Table 7. 1 Microbial community richness and diversity in the soils under three different land uses/land covers

Sample location	OTUs	Chao1	Shannon	Simpson	Good's Coverage
RB 0–15 cm	840 ± 49.5c	1070 ± 70.6c	7.87 ± 0.003e	0.99 ± 0.000b	0.979 ± 0.002
RB 15–30 cm	814 ± 11.5c	1000 ± 2.39c	7.64 ± 0.037f	0.989 ± 0.000c	0.982 ± 0.002
GSM 0–15 cm	1280 ± 9a	1590 ± 48.1a	8.44 ± 0.037a	0.993 ± 0.000a	0.98 ± 0.001
GSM 15–30 cm	1140 ± 8b	1420 ± 47.5b	8.21 ± 0.049c	0.992 ± 0.001a	0.983 ± 0.001
GSM-N 0–15 cm	1170 ± 7b	1440 ± 4.5b	8.34 ± 7.75e-1b	0.993 ± 2.2e-1a	0.983 ± 0.000
GSM-N 15–30 cm	1160 ± 27.5b	1450 ± 13ab	8.07 ± 0.021d	0.99 ± 0.001bc	0.985 ± 0.000

RB= Reed Bed, GSM= Grazing saltmarsh, GSM-N= Non-grazing saltmarsh, OTUs= Operational taxonomic units, abc= Duncan multiple test at 0.05 level of confidence.

7.4.2 Relationships between bacterial communities among the different sampling sites

The 16S amplicon sequencing analyses identified the following taxonomic classification: 29 phyla, 74 classes, 122 orders, 219 families, 419 genus, and 693 species of bacteria. The dominant phyla across the study area were Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, Cynobacteria/Chloroplast, Verrucomicrobia, Planctomycetes, Gemmatimonadetes, Firmicutes and Nitrospirae, while Armatimonadetes, Deferribacteres, Deinococcus Thermus, Fusobacteria, Spirochaetes, Tenericutes, and Hydrogenedentes, amongst others, were less dominant (Appendix 47). Significant variations were observed generally across the sampling locations and within the sampling depths. The relative abundance of the top 12 phyla at 0–15 cm soil depth and 15–30 cm depth under different land use/land cover is shown in (Figure 7.1).

The results indicated that both the locations that were less contaminated (GSM and GSM-N) and the most contaminated (RB) site had different characteristic bacterial communities. Results from the relative abundance based on order level across the sampling locations indicated that the most abundant group were unassigned bacteria (Appendix 48). However, the nine top orders of assigned bacteria in order of decreasing abundance under

different sites are presented in Figure 7.2. Results indicated that relative abundance of the bacterial communities varies within the 0–15 cm and 15–30 cm depths (Figure 7.2).

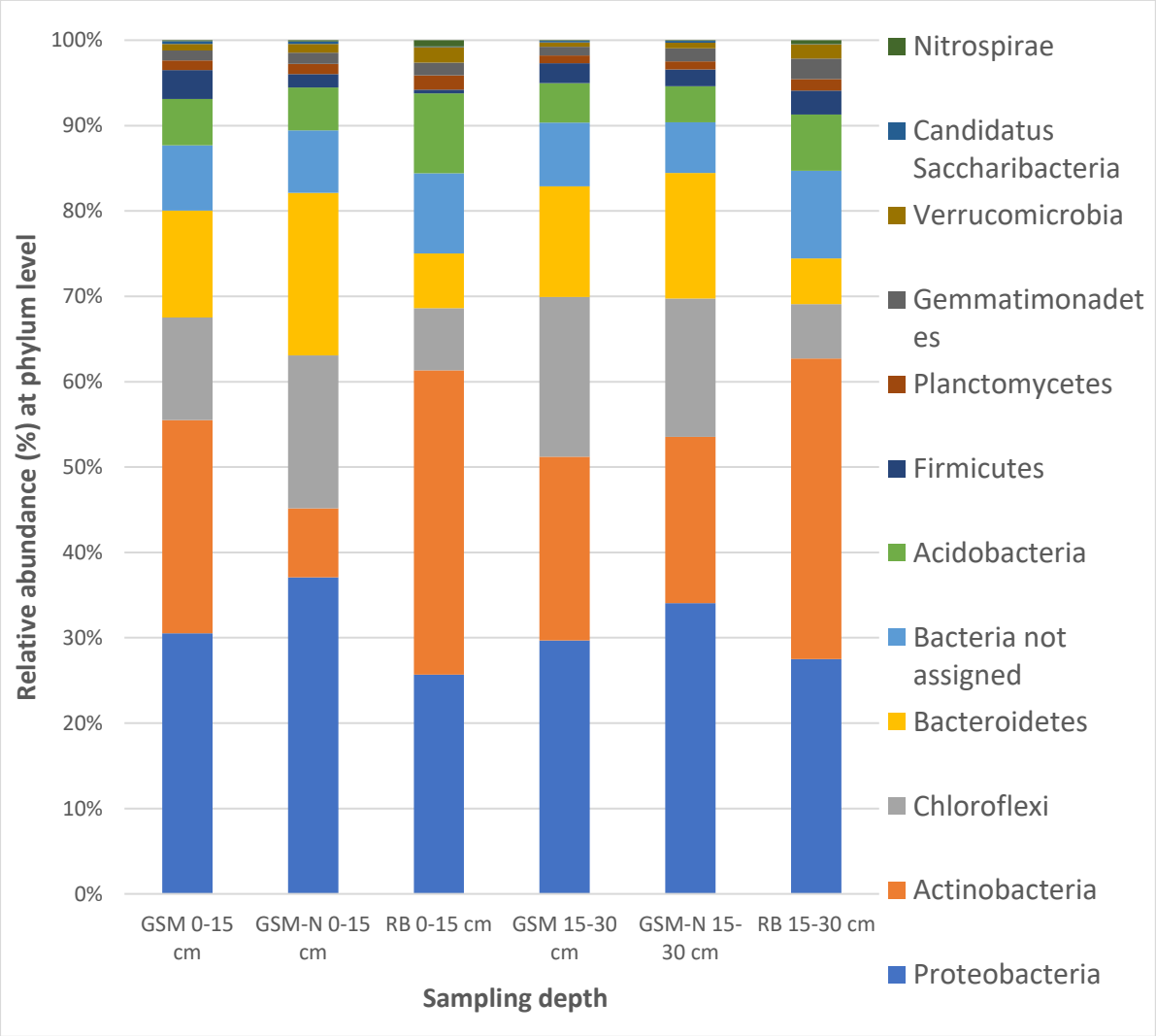


Figure 7. 1 Relative abundance of predominant bacterial composition at phylum level across Grazing Salt Marsh (GSM), Non-Grazing Salt Marsh (GSM-N) and Reed Bed (RB) at different depths

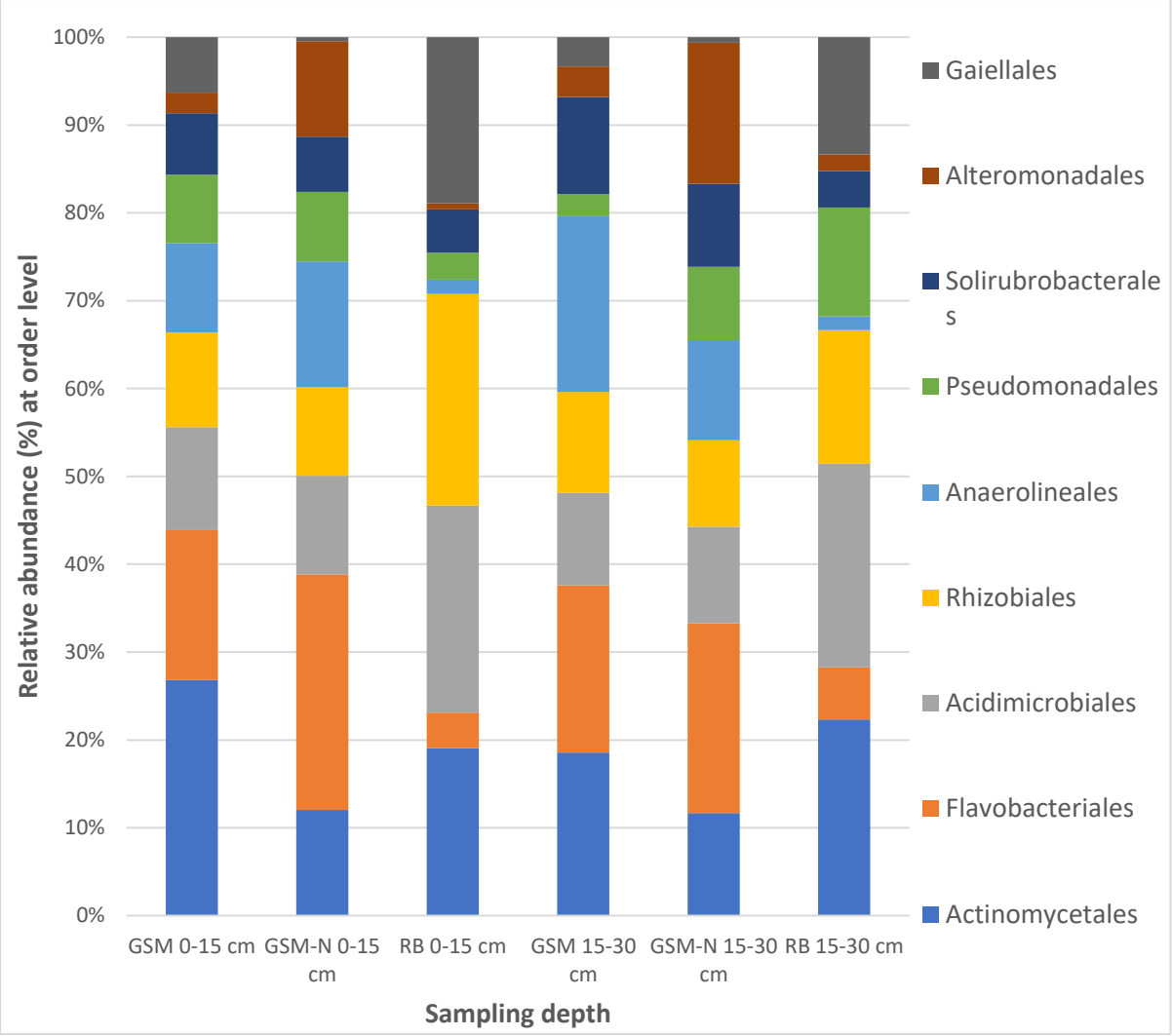


Figure 7. 2 Relative abundance of predominant bacterial composition at order level across Grazing Salt Marsh (GSM), Non-Grazing Salt Marsh (GSM-N) and Reed Bed (RB) at different depths

A hierarchically clustered heatmap of bacterial distribution of different communities from the different locations at the genus level was performed using the top 30 genus and their reads from the various samples to get a clearer view on the observed variations among the studied samples (Figure 7.3). The full names of the selected isolates and their reads counts are presented in Appendix 45. In the heatmap (Figure 7.3) the row represents the relative percentage of each of the selected bacterial genus while the column stands for different sampling locations. The relative abundance for each bacterial genus is shown by the different colour intensities using the legend at the top right-hand corner of the figure. The selected

isolates were designated as uncultured crenarchaeota, others (1 to 9), uncultured Acidobacteria bacterium 1, uncultured Chloroflexi bacterium, uncultured Firmicutes bacterium, uncultured Gemmatimonadetes bacterium, uncultured bacterium 1, uncultured candidate division WS6 bacterium, uncultured delta proteobacterium, uncultured marine bacterium, uncultured organism, uncultured planctomycete, uncultured soil bacterium, uncultured bacterium 2, uncultured Acidobacteria bacterium 2, uncultured bacterium 3, uncultured Acidobacteria bacterium 3, uncultured bacterium 4, unidentified bacterium, uncultured Acidobacteria bacterium 4 and uncultured bacterium 5.

The heatmap indicated that GSM libraries grouped together first and then clustered with GSM-N and were separated by different colour intensities before GSM-N clustered with RB. There was no clear pattern in the clustering across the three sampling locations, rather the clustering was clearer within the sampling locations. This may be due to variations in the bacterial diversity. It is observed that the majority of the sequence across the sampling location belonged to Uncultured bacterium 1. The sequences belonging to Uncultured Acidobacteria bacterium 1 and Uncultured Acidobacteria bacterium 4 were the most dominant bacterial genus within the RB samples. The sequences belonging to Uncultured Chloroflexi, Uncultured Planctomycete and Uncultured marine bacterium were the most dominant bacterial genus within GSM and GSM-N samples. The results indicated that GSM and GSM-N were more closely related compared to RB sampling location. The principal component analysis (PCA) shown in Figure 7.4 indicated that RB1 to RB4 were more closely related, forming a cluster, compared to GSM1 to GSM4 and GSM-N1 to GSM-N4, while GSM was closely related to GSM-N and as such clustered together. This is in line with the heatmap analysis, indicating that RB samples were more diverse therefore, the sampling locations had different characteristic bacterial communities.

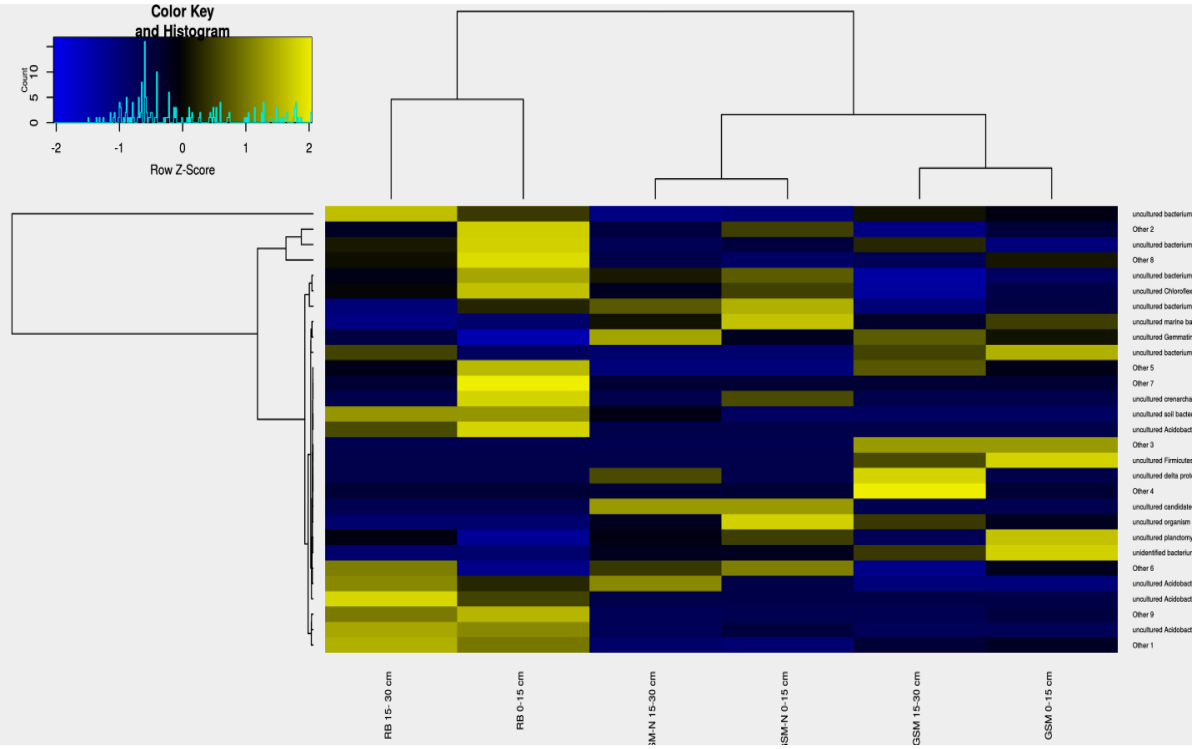


Figure 7. 3 Hierarchically clustered heatmap of the bacterial distribution of different communities from the different locations at the genus level.

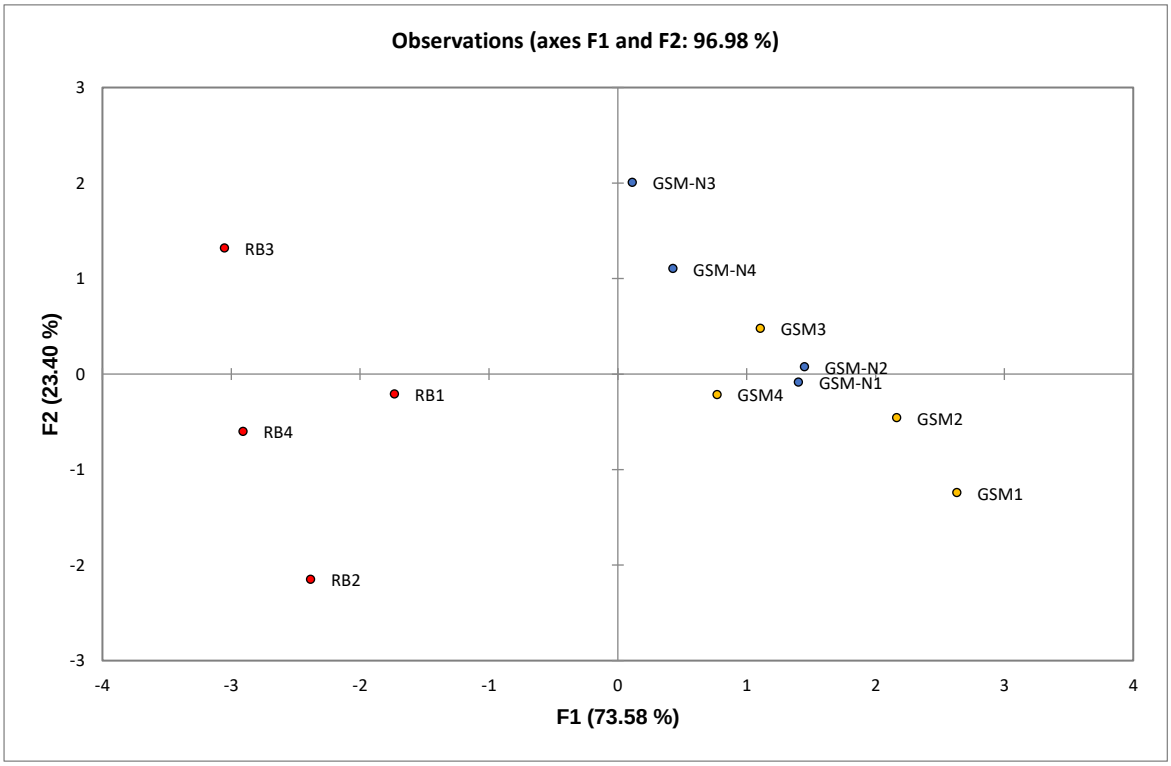


Figure 7. 4 Principal component analysis (RB1 to RB4= Reed Bed samples, GSM1 to GSM4= Grazing samples, GSM–N1 to GSM-N4 = Non-grazing samples)

7.5 Discussion

7.5.1 Bacterial diversity and community profiling

Historical contamination of inorganic pollutants could have effects on bacterial communities. This knowledge will be necessary to improve our understanding of the composition of the bacterial communities and their link with soil characteristics and different land uses/land covers due to long time inorganic pollution. The concentration of heavy metal in the present study affects the soil microbial diversity as shown in Table 7.1, indicating that grazing and non-grazing sampling locations were more diverse compared to the reed bed location. This may be due to the high concentration of heavy metals within the reed bed location (Figure 4.5), indicating that the reed bed sampling location was highly contaminated compared to the grazing and non-grazing locations. This is in corroboration with Abdu, Abdullahi, & Abdulkadir (2016) whose findings shows that a high level of heavy metals decreases microbial population, diversity and activities. According to Reddy & Delaune (2008) heavy metal contamination affect microbial activities, especially of methanogens, but to a lesser extent of heterotrophs in wetland ecosystems while Kelly & Tate III (2017) reported that viable plate counts and dehydrogenase activities were greatly reduced in zinc contaminated sites. According to Zhang et al. (2016) chromium and cadmium contamination were the major factors related to bacterial community structure changes. Therefore, the bacterial diversity observed in the present study using the next-generation sequencing tool may be due to historical contamination of the study area.

Differences in the microbial communities in the Upper Mersey Estuary were observed and it is important to seek an explanation for these differences. The present study identified 29 Phyla and 122 orders of bacteria (Appendices 47 and 48). This is showing over 12 dominant phyla and 9 dominant order with relative abundance of the bacterial communities found in the 0–15 cm sampling depth compared to 15–30 cm sampling depth across the study area (Figures 7.1 and 7.2). According to Feng et al. (2018) 25 dominant bacterial communities in soil that is contaminated with cadmium include, Gemmatimonadetes, Thermaarcheota, Firmicutes, Cyanobacteria, Planctomycetes, Chloroflexi, Crenarcheaota, Aminicenantes, Spirochaetes, Armatimonadetes, Others, Ignavibacteriae, Poribacteria, Chlorobi, Deinococcus-Thermus, Euryarcheota, Veruumicrobiota, Nitrpspirae, Bacteroidetes,

Actinobacteria, Acidobacteria, and Preoteobacteria while Jia et al. (2012) identified Tenericutes, Cyanobacteria, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria and others using the MG RAST pipeline. These phyla were also recorded in present study acrossing the sampled locations. The heatmap and principal component analysis shows that the samples are basically divided into three groups based on the three land uses/land covers, indicating that closer the samples are to each other the more similar is their microbial composition (Figures 7.3 and 7.4). The distribution of the bacteria within the study area follows past industrial activities. For example, the abundance of Acidimicrobiales has been reported by Peng et al. (2015) to be mainly due to saline-alkaline soil characteristics which the study area was known for from caustic soda production. Also, the presence of Actinobacteria and Planctomycetes identified in the present study (Figure 7.1) are very important for degrading contaminants in soil and sediments. There have been few reports on the isolation of Solirubrobacterales from the natural environment (Peng et al., 2015), but this was identified in the present study. This may be an indication that these bacteria participated in the degradation process of the contaminants. Therefore, the differences in the composition of bacterial communities may be associated with several factors such as historical contamination of organic and inorganic pollutants, land use/land cover, soil moisture content, grazing activities, and temperature.

Soil chemical properties in the present study could have control in the abundance of bacterial communities. The study area was known for high soil pH and electrical conductivity and cattle grazing activities and may be related to the Bacteroidetes found in the present study (Table 4.1). According to Peng et al. (2015) Bacteroidetes are related with increasing pH and electrical conductivity of the soil while Stewart, Flint, & Bryant (1997), Wallace (2008) and de Menezes et al. (2011) reported that Bacteroidetes and Firmicutes are associated with the rumen bacterial community. Actinobacteria are of great economic importance and help in the transformation of soil organic matter (Davinic et al., 2012) while Griffiths & Gupta (2007) identified *Deinococcus thermus* as species that are resistant to extreme environmental conditions, as well as several thermophiles. Actinobacteria were found to be dominant in the reed bed compared to other land uses/land covers (Figure 7.1) and the carbon storage within the reed bed was low compared to the grazing saltmarsh (Figures 5.9 and 5.10). This may be due to the rapid transformation of soil organic matter associated with the Actinobacteria,

leading to emission of carbon dioxide and reduced carbon storage. According to de Menezes et al. (2015) the carbon:nitrogen ratio drives soil actinobacterial cellobiohydrolase gene diversity compositions across different land uses due to substrate quality. Hence, there is a strong link between bacterial community, soil properties and soil organic carbon dynamics.

In conclusion, this present chapter provides baseline information that is useful to the understanding of bacterial community profiling linked to past industrial activities. The present study identified the presence of Acidomicrobiales and Solirubrobacterales that are associated with past caustic soda production within the study area which is an indication of how microorganism can response environmental changes. There is variation in microbial community profiling across the different land uses/land covers. The sampling location (reed bed) with a high concentration of arsenic, chromium, copper, lead, and zinc had lower microbial diversity compared to the locations (grazing and non-grazing saltmarsh) with a low concentration of heavy metals. Greater numbers of bacterial communities were found within 0–15 cm soil depth compared to the 15–30 cm depth. This could be due to the fact the concentration of heavy metal in the present study was higher in the subsoil compared to the topsoil. Dominant phyla within grazing and non-grazing saltmarsh were Proteobacteria (30–34%) > Actinobacteria (17–24%) > Bacteroidetes (12–17%) > Chloroflexi (11–18%) > Acidobacteria (4–5%), while at the reed bed location, Actinobacteria (35%) > Proteobacteria (25–27%) > Acidobacteria (6–9%) > Chloroflexi (6–7%) > Bacteroidetes (5–6%). This may be due to the variations in physical and chemical properties within the study area. Actinobacteria were found to be dominant in the reed bed compared to other land uses/land covers. This resulted in low amount of soil organic carbon storage in the reed bed sampling location compared the grazing and non-grazing locations because of the high rate of soil organic matter decomposition associated with the Actinobacteria. The bacterial composition was more similar within the grazing and non-grazing saltmarsh compared to the reed bed. This may be due to the differences in land uses/land covers and concentration of heavy metals. However, some microbes thrive in a contaminated environment because they are capable of elaborating genes with which they handle different contaminants. The present study has shown that past industrial activities controlled the abundance of bacterial communities. This should serve as baseline data to researchers with interest in bioremediation of contaminated soil and sediment.

8. General Discussion Including Conclusion

8.1 Integrating the findings from the four stands of research

Soil organic carbon is known to be the primary driver for all biogeochemical processes in wetland systems (Reddy & Delaune (2008). Saltmarsh ecosystems found in wetlands act as an important carbon sinks (Davidson et al., 1991; NEA, 2011; Hopkinson et at., 2012). In addition to acting as a carbon sink, saltmarshes also provide a wide array of benefits to coastal populations including immobilisation of pollutants (Burden et al., 2013) and wildlife habitat provision, (Wilson et al., 2005a, 2005b; Lamparter ert al., 2009; Hopkinson et al., 2012; Chen et al., 2015; Hansen & Reiss, 2015). While there have been several studies on soil carbon dynamics in estuarine floodplains, little attempt has been made to examine the effects of heavy metal contamination on these processes. This represents a knowledge gap that needs to be filled to better understand soil carbon decomposition in contaminated estuarine floodplains and its implications for carbon sequestration.

It is known that, because of heavy metal contamination in soils, there will be a decrease in plant biomass and the diversity and activities of soil microorganisms (Guimaraes et al., 2016). Both these factors are the major contributors to the input and output of organic carbon respectively. Therefore, the storage of organic carbon in a given soil reflects the balance between the input and output of soil organic carbon (He et al., 2013). Plant biomass are a major input of carbon to soil system and microbial activity can lead to major outputs from the system. Heavy metal-contaminated soil is known to affect soil microbial populations and their associated activities (McGrath et al., 1995). The presence of heavy metals in soils have been reported to have toxic effects on plant growth, resulting in reduced biomass (Steinbeiss et al., 2008; Nagajyoti, Lee, & Sreekanth, 2010). The general assumption in the present study is that if the soil is contaminated by heavy metals, the plant biomass and microbial activities will be affected, leading to reduced carbon dioxide emission and ehanced soil carbon accumulation. Other reports have shown that the effect of soil contamination is to reduce decomposition of organic matter which in turn will enhanced soil organic carbon storage as detailed in Section 6.6. Hence, it can be hypothesised that the functionality of saltmarshes will be affected by heavy metal contamination. In the study reported here the

multiple factors operating in this system are examined and from this an understanding of their individual and collective effects is obtained. These findings have significance in the context of global carbon budgets and the benefits associated with these habitats (Chapters 2 and 5).

The Upper Mersey estuarine floodplain was found to be contaminated by multiple heavy metals/metalloid of potential toxicity (Chapter 4) and this need explanations. The concentration of the heavy metals varied from location to location, depending on topographic position (Tables 4.4, 4.6, 4.8, 4.10, 4.12, 4.14 and Appendices 2 and 3). The low-lying saltmarshes tended to have high concentrations of soil-borne heavy metals, as compared to the woodlands with higher elevations (Figure 4.5). This suggests that low-lying areas could act as traps to receive heavy metal-laden fine sediments during flooding. There was a tendency that the concentration of heavy metals increased with increasing depth, reflecting the reduced level of contamination over time because of de-industrialisation in the region (Tables 4.4, 4.6, 4.8, 4.10, 4.12, 4.14 and Appendices 2 and 3). Due to the neutral to alkaline nature of soil pH, the soluble and exchangeable pools of heavy metals in the soils only accounted for a very small proportion of the total heavy metal pool (Tables 4.5, 4.7, 4.9, 4.11, 4.13, and 4.15). Uptake of heavy metals by plants caused accumulation of the heavy metals in the plant tissues, which poses a potential human health risk in the grasslands used for animal grazing (Wilkinson Hill & Philips, 2003 and Smith, Abrahams, Dagleish, & Steigmajer, 2009). In the present study, heavy metal concentration was higher in the plant roots compared to the plant shoots (Tables 4.16 and 4.17). This is in corroboration with Nworie, Qin, & Lin (2019) findings for plants growing in heavy metal-contaminated soils. It is found that different plant species had different bioaccumulation coefficient, translocation factor, and biological concentration factor (Table 4.18). This could be due to the differences in the potential for phytoextraction by the different plant species. Thus, it may be a good practice to remove the plant species with high translocation factor to minimise the health risk to grazing animals. The Upper Mersey estuarine floodplain is typical of many estuarine floodplains across the globe (Table 8.1), hence the findings here will be relevant to those working in other estuarine floodpains.

There was variation in soil properties in the present study and this need explanations. Field monitoring showed that the soil pH, EC, Eh, bulk density, soil porosity, and plant biomass varied seasonally (Figures 5.4, 5.5, 5.6, 5.7, 5.8, and 5.9). These, to a varying degree, affected

soil carbon dynamics in the investigated area. The soil carbon storage differed significantly under different land uses/land covers (Figures 5.11 and 5.12). The three monitoring plots selected in Chapter 5 represent three different land use/land cover types (non-grazing salt marsh, grazing salt marsh and reed bed). Cattle grazing land use resulted in an increased storage of soil carbon (Figure 5.11). This could be due to the enhancement of biomass production and rapid incorporation of partially decomposed organic matter from animal wastes into the soil layers. The reed bed tended to have lower soil salinity as compared to grazing and non-grazing land uses/land covers (Tables 4.1 and 5.2 and Figure 5.6). This is because the reed bed received less frequent tidal fooding compared to other land uses/land covers. According to Morrissey, Gillespie, Morina, & Franklin (2014) microbial decomposition rates of soil organic matter could be increased in low salinity wetlands. This suggests that reed bed ecosystems may experience decreased soil organic matter accumulation, accretion, and carbon sequestration rates even with modest levels of saltwater intrusion (Morrissey et al., 2014). For the saltmarsh, cattle grazing could markedly affect the plant biomass. On the one hand, cattle consume the grasses, resulting in the loss of plant biomass but on the other hand, cattle grazing could stimulate the growth of plant. The comparison in the mean plant biomass between the grazing land and non-grazing land is shown in Figure 5.8. The measured biomass was smaller in the grazing saltmarsh compared to the non-grazing land use (Figure 5.8). This may be an indication that the grazing practice had the effect on decreasing the measurable biomass. Although, the amount of grasses being consumed by the cattle was not measured during this present study but, it was likely that the actual plant biomass in the grazing saltmarsh (i.e. the measured biomass plus the consumed biomass) was greater than the plant biomass in the non-grazing saltmarsh. Therefore, the variations in soil physical and chemical properties observed in the present study may be due to seasonal and land use types differences.

There may be several other possible factors that will influence the soil carbon dynamics. In the investigated area, the major source of organic carbon entering the soils is the growing plants though inputs of organic carbon from tidal floodwater as shown in Figure 5.13 and atmospheric deposition are likely. According to Chambers, Reddy, & Osborne (2011) “pulses” of seawater may have a greater influence on the rate of carbon cycling in freshwater wetlands than a gradual sea level rise. The output of the organic carbon from the investigated

area could include removal of plant debris by tidal flooding (Figure 5.13) and wind. However, it is most likely that decomposition of plant debris and soil organic matter play a more important role in carbon loss in the investigated area. The seasonal monitoring results in Chapter 5 showed that the plant biomass was, to some extent, affected by the soil contamination (Figure 4.4), depending on the season when the biomass was collected and measured. However, despite that certain relationships between the plant biomass and the level of heavy metal contamination were observed, the R^2 was all less than 0.22 for all the seasons (Figure 5.10), suggesting that the plant biomass was largely affected by other factors, possibly the nutrient supply status, plant species, land use/land cover etc. According to Dotaniya et al. (2017) and Nannipieri et al. (2017), heavy metals may indirectly affect the growth of plants by inhibiting the activities of microbes that are responsible for nutrient transformation and thus reduce the capacity of soils to supply nutrients to the plants. Therefore, the impacts of heavy metals on plant biomass in the present study could not have a significant control on the amount of soil organic carbon storage. Rather, the variation in pH and redox potential across the different land uses/land covers at different sampling seasons as shown in Figure 5.10 and Table 5.3 played more important roles.

Several factors such as land use/land cover, elevation, and cow dung during cattle grazing have been reported to affect soil carbon dynamics in coastal wetlands (Davidson et al., 2002; Medina-Roldan et al., 2012; Hayes et al., 2017). The present study also identified land use/land cover types and seasonal variation as other factors that control the amount soil organic carbon stored in this historically heavy metal contaminated sites (Table 4.3 and Figure 5.9). According to Olsen et al. (2011), increased microbial biomass and soil respiration during cattle grazing were the major factors that influence the variation in soil carbon storage. This is because of the little effect grazing activities had on the rates of mineralisation of carbon–14 used as a respiratory substrate, but a larger proportion of carbon-14 was partitioned into microbial biomass and immobilised in long- and medium-term storage pools in the grazed land use (Olsen et al., 2011). The present study shows a significantly high amount of carbon storage within the grazing saltmarsh, compared to other land uses/land covers during the winter and summer sampling seasons (Figure 5.9). This may be due to the differences in microbial decomposition rate from one season to the other season. According to Aber et al. (1989), the decomposition rate of soil organic matter is assumed to be insignificant in the

annual cycling of carbon and nutrients during the winter periods compared to other seasons. Therefore, any model for soil carbon dynamics must consider these seasonal differences (Segoli et al., 2013) for effective soil carbon monitoring. Smith (2013) conducted research in the same study area and found that the grazed area had a higher carbon store in terms of tonnes per hectare to 15 cm than the non-grazed area. Many studies have shown that soil bulk density was independent of the soil organic carbon content (Gosselink, Hatton, & Hopkinson, 1984; Avnimelech, Ritvo, Meijer, & Kochba, 2001; Chaudhari, Ahire, Ahire, Chkravarty, & Maity, 2013). However, the soil bulk density in this study was not a major factor for soil carbon storage (Table 5.3). This is because the grazing location had higher carbon stock, as compared to the non-grazing land use (Table 5.6) even though the grazing locations had higher bulk densities. Hence, soil carbon dynamics in the present study was controlled by the land use types, seasonal types, and soil bulk density.

Globally, variation in soil organic carbon content is associated with factors such as climate, vegetation, topography and parent materials (Batjes, 1996; Fuchs, Simon, & Micheli, 2005; Kulawardhana et al., 2015). Table 8.1 contains data to allow a comparison of soil organic carbon content among some estuarine floodplains within the UK and around the world. The distribution of soil organic carbon depends on several factors such as sampling locations, sampling depths, and method of analyses (Table 8.1). Gwynne (2004) showed that most of the heavy metals were positively correlated with soil organic carbon, indicating that heavy metal contamination could markedly affect the accumulation of soil organic carbon in estuarine floodplains. The present study shows that heavy metal contamination, soil pH, redox potential and electrical conductivity played major roles in soil carbon storage (Figure 4.4, and Tables 4.3 and 4.21). This is in corroboration with Matsui, Meepol, & Chukwamdee, 2015 report (Table 8.1). According to Kulawardhana et al. (2015) the amount of soil organic carbon stored in Galveston Island, Texas, USA was independent to sea level rise and soil bulk density while in the present study soil bulk density did not play a major control over the the amount of soil organic carbon (Figures 4.2 and 5.7). Generally, the estuarine floodplains presented in Table 8.1 shows a similar trend in the amount of soil organic stored depending on the sampling depths and the sampling period. Therefore, the results obtained from this study can be transferred to other estuarine floodplains (Table 8.1).

Table 8. 1 A Comparison of soil organic carbon storage within some estuarine floodplains

Study area	Sampling period	Sampling depth (cm)	Carbon stored (%)	Major drivers of SOC storage	Method of Analysis	Land Uses/land covers	References
Colne Estuary, UK	X	0-35	8-12	High heavy metal	Loss on Ignition	Saltmarsh	Gwynne, 2004
Ribble Estuary UK	X	0-25	14-15	High heavy metal	Loss on Ignition	Saltmarsh	Gwynne, 2004
Loughor Estuary, UK	X	10-40	8-25	High heavy metal	Loss on Ignition	Saltmarsh	Gwynne, 2004
Dee Estuary, UK	X	10-40	2-45	High heavy metal	Loss on Ignition	Saltmarsh	Gwynne, 2004
Humber Estuary, UK	X	0-10	1-7	High heavy metal	Wet acid oxidation	Saltmarsh	Andrews, Samways, & Shimmield, 2008)
Upper Mersey Estuary, UK	Autumn 2012	0-15	6-10	Management duration	Loss on Ignition	Saltmarsh	Smith, 2013
Tungka Bay, Chumphon, Thailand	September, 2007	0-5	3-14	pH and EC	Loss on Ignition	Mangrove ecosystems	Matsui, Meepol, & Chukwamdee, 2015)
Galveston Island, Texas, USA	X	0-15	1-3	Bulk density, sea-level rise, soil depth	Loss on Ignition	Saltmarsh	Kulawardhana et al. (2015)
Upper Mersey Estuary, UK	Summer 2015	0-15	7-12	Heavy metal, season, pH, Eh, EC	Loss on Ignition	Saltmarsh	This Study (Appendix 1)
Upper Mersey Estuary, UK	Winter 2016	0-15	8-14	Heavy metal, season, pH, Eh, EC	Loss on Ignition	Saltmarsh	This Study (Appendix 19)
Upper Mersey Estuary, UK	Winter 2017	0-15	6-11	Heavy metal, season, pH, Eh, EC	Loss on Ignition	Saltmarsh	This Study (Appendix 19)
Upper Mersey Estuary, UK	Spring 2016	0-15	8-14	Heavy metal, season, pH, Eh, EC	Loss on Ignition	Saltmarsh	This Study (Appendix 20)
Upper Mersey Estuary, UK	Spring 2017	0-15	8-12	Heavy metal, season, pH, Eh, EC	Loss on Ignition	Saltmarsh	This Study (Appendix 20)
Upper Mersey Estuary, UK	Summer 2016	0-15	6-13	Heavy metal, season, pH, Eh, EC	Loss on Ignition	Saltmarsh	This Study (Appendix 21)
Upper Mersey Estuary, UK	Summer 2017	0-15	8-12	Heavy metal, season, pH, Eh, EC	Loss on Ignition	Saltmarsh	This Study (Appendix 21)
Upper Mersey Estuary, UK	Autumn 2017	0-15	7-10	Heavy metal, season, pH, Eh, EC	Loss on Ignition	Saltmarsh	This Study (Appendix 22)

X= not specified

According to Reddy & Delaune 2008, heavy metal contamination affects microbially mediated decomposition of organic matter in wetland soils. The laboratory experiment in Chapter 6 confirmed that soil contamination by heavy metals could impede microbially mediated decomposition of organic matter under water inundation conditions (Figures 6.10 and 6.11). The accumulation of soil organic carbon tended to be enhanced with increasing level of contamination by heavy metals though different heavy metal had different capacity to inhibit organic matter decomposition (Figures 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, and Appendix 27), leading to incomplete mineralisation of organic matter and enhanced organic carbon storage (6.10 and 6.11). Reduction in litter decomposition have been reported as an indicator for reduced microbial activities (Baath, 1989; Brookes, 1995; Aceves, Grace, Ansorena, Dendooven, & Brookes, 1999; Barajas-Aceves, 2005; Hayes et al., 2018). Hence, there will be a reduction in the rate of carbon dioxides emission (Nwachukwu & Pulford, 2011). In the present study, the amount of residual organic debris during and after the incubation periods were higher in the control soils than in the soils contaminated by heavy metals (Appendix 27). The diffeences in ratio of the A-alkyl carbon to O-alkyl carbon between the control soils and the soils contaminated by heavy metals (Figure 6.11) also supports this conclusion. Therefore, organic matter in the soils contaminated by heavy metal were less decomposed, as compared to the uncontaminated soils. The presence of heavy metals could also affect the functional group composition of humic substances. Under heavy metal stresses, the chemical shift range of ^{13}C-NMR spectra in the humic substances (humic acids and fulvic acids fractions) corresponded to alkyl-C (0–45 ppm), O-alkyl C (60–90 ppm) and carbonyl-C (110–190 ppm) with a higher amount of carbon in the carbonyl-C functional group (Appendices 29 to 44). However, the impacts of heavy metal contamination on soil carbon dynamics were mainly examined using laboratory experiment (Chapter 6). This needs to be validated in the field. It is suggested that field experiments be conducted to compare contrasting land plots with different levels of contamination. A wider range of parameters will be determined, including plant biomass (both below-ground portion and above-ground portion), plant tissue-borne heavy metals, microbial diversity and activities, soil organic carbon content and humic substances. This will allow a better evaluation of the carbon input and carbon output that determine the soil carbon storage.

Microorganisms can thrive in a contaminated soil because they are capable of elaborating genes with which they handle different contaminants (Ghosh & Singh, 2005 and Peng et al., 2015). The 16S rDNA next-generation sequencing results obtained from this study (Chapter 7) indicated that significant differences (at 95% confidence level) exist in microbial profiling across the different land uses/land covers and at different heavy metal concentrations (Figures 7.1, 7.2, and 7.4). The soils with a higher concentration of arsenic, chromium, copper, lead, and zinc had lower microbial diversity, as compared to the soils with a lower concentration of the heavy metals (Table 7.1). The abundance of bacterial communities was greater in the surface soil layer (0–15 cm) than in the subsoil layer (15–30 cm) (Figures 7.1). Dominant phyla within GSM and GSM-N locations were Proteobacteria (30–34%) > Actinobacteria (17–24%) > Bacteroidetes (12–17%) > Chloroflexi (11–18%) > Acidobacteria (4–5%), while at the RB location, Actinobacteria (35%) > Proteobacteria (25–27%) > Acidobacteria (6–9%) > Chloroflexi (6–7%) > Bacteroidetes (5–6%) (Figures 7.1 and 7.2). This shows that the abundance of the bacterial communities in the present study was controlled by the different level of contaminations and the types of land use.

There were effects of heavy metal contamination on soil carbon storage in the present study and this needs explanation. In Chapter 4 it was established that arsenic, chromium, copper, lead, and zinc concentrations in the surface soil layer (0-15 cm) were significantly higher at the reed bed sampling location than at the grazing and non-grazing saltmarsh locations, while the concentration of arsenic, chromium, copper, lead, and zinc at 15–30 cm depth follows the order: grazing saltmarsh > non-grazing saltmarsh > reed bed (Tables 4.4, 4.8, 4.10, 4.12, and 4.14). This may have some effects on the microbial community, leading to changes in its diversity. Heavy metal contamination has been reported to affect microbially mediated decomposition of organic matter (Reddy & Delaune 2008; Spurgeon et al., 2013; Hayes et al., 2018). A high level of heavy metals decreases microbial population, diversity, and activities (Abdu, Abdullahi, & Abdulkadir, 2016). Kelly & Tate III (2017) reported that viable plate counts and dehydrogenase activities were greatly reduced in zinc contaminated sites. According to Davinic et al. (2012), the abundance of Actinobacteria and Proteobacteria stored more soil organic carbon compared to other bacterial communities. This may be one of the reasons for the variation of soil organic carbon within the grazing saltmarsh and the non-grazing saltmarsh in Chapters 4 and 5. The percentage

abundance of Actinobacteria was 24.23% and 7.20% in the grazing and non-grazing sampling locations respectively (Figure 7.1 and Appendix 47). The inhibitory effects of heavy metals on decomposition of organic matter are clearly demonstrated from the laboratory experiments presented in Chapter 6. This resulted in a reduced soil carbon loss when heavy metals were present in the soils at a sufficiently high level. It is, therefore, assumed that the presence of heavy metals in the investigated soils could, to some extent, affect the decomposition of the plant debris and soil organic matter in the investigated area. Figure 4.4 shows a relationship between grazing saltmarsh and the heavy metal contamination within the study area, indicating that soil organic carbon increases with increasing level of heavy metal contamination. Within this land use/land cover, the soil characteristics except for the concentrations of heavy metals and plant biomass were relatively uniform (see Tables 4.1 and 5.4 and Figures 4.2, 4.3, 5.4, 5.5, 5.6, 5.7, 5.9, and 5.11). This gives an opportunity to examine the effects of heavy metal contamination on the soil carbon storage under field conditions.

There may be several possible explanations for the variation in soil organic carbon storage in the present study. When examining the whole investigated area, there was no clear relationship between the concentration of heavy metals and the soil organic content (Table 4.21). This is because other parameters such as pH, redox potential and electrical conductivity played more important roles in soil organic dynamics in the study area. The soil pH in the present study was alkaline and mildly acidic in nature across the lowland and upland soils respectively (Table 4.1). Fox et al. (1999) reported the production of sodium carbonate and caustic soda by chemical industries within the study area and this may have a great influence on the soil pH and microbial diversity within the lowland sampling locations. The present study identified these effects of the historical caustic soda production in the abundance of Acidimicrobiales and Pseudomonas within the study area (Figure 7.2). This is in corroboration with the findings by Peng et al. (2015), which stated that the presence of Acidimicrobiales is due to saline-alkaline soil properties. However, there have been several efforts towards using wetlands to treat acidic mine drainage and alkaline leachates resulting from caustic soda-rich industrial by-products by Mayes et al. (2009). According to Grybos, Davranche, Gruau, & Petitjean (2007), soil pH is a more crucial parameter than Eh for metal mobility in wetland soils since the process of soil organic matter release is mainly controlled by pH variations. This is in corroboration with the findings obtained in the present research (Table 4.21). The values

of the redox potential in the present study show a negative relationship with the soil organic carbon (Table 4.21). This may be an indication of the anaerobic condition of the study area due to frequent tidal inundation. This may also be responsible for the higher values of soil organic carbon obtained from the study area due to the negative values of redox potential. However, other studies have shown different reports on the effects of redox potential on heavy metal dynamics. According to Shaheen, Rinklebe, Rupp, & Meissner (2014) cadmium, copper, and zinc tended to be mobilised at high redox potential during short term cycles in a contaminated floodplain. Frohne, Rinklebe, Diaz-Bone, & Du Laing (2011) reported that the concentrations of cadmium, copper, copper, manganese, nickel, and zinc in solution were low at low redox potential and increased with rising redox potential, which might be attributed to the interaction with dissolved organic carbon and metals, leading to the precipitation of metal sulfides. Frohne, Rinklebe, and Diaz-Bone (2014) reported a decreasing arsenic concentration with increasing redox potential in soil solution at low pH. Therefore, soil pH and redox potential played major roles in the amount of soil carbon storage in the present study.

The soil electrical conductivity may have control in the amount of soil carbon storage in the present study. The electrical conductivity of the soil in the present study shows a positive correlation with soil organic carbon content (Table 4.21). High salinity may affect the soil microbial activities leading to enhanced soil carbon storage due to reduced carbon dioxide emission or incomplete mineralisation of organic carbon to carbon dioxide. High salinity was found to enhance the mobility of cadmium and its uptake by the plant in the intertidal sediments of the Scheldt estuary in Belgium by Du Laing et al. (2008) while Du Laing (2009) observed that copper, zinc, and cadmium are primarily released above the water table under high salinity conditions. According to Edmonds, Weston, Joye, Mou, & Moran (2009) and Neubauer, Franklin, & Berrier (2013) demonstrated that saltwater intrusion into tidal freshwater marshes affects the entire process of carbon mineralization, from the availability of organic carbon through its terminal metabolism to carbon dioxide and/or methane. This is an indication that long-term shifts in biogeochemical functioning are not necessarily consistent with short-term disturbance-type responses (Neubauer et al., 2013). Therefore, the present study indicates that high electrical conductivity in the soil influenced the mobility of cadmium and lead compared to other heavy metal. This is in corroboration with Acosta, Jansen, Kalbitz, Faz, & Martínez-Martínez (2011) report showing that salinity increases the

mobility of cadmium and lead. Therefore, soil electrical conductivity also played major role in the amount of soil organic carbon stored in the present study.

8.2 Conclusion

In summary, it has been established that soil carbon storage under heavy metal contamination is due to the effects of heavy metals on soil microbial activities leading to the accumulation of soil organic matter and enhanced soil organic carbon storage (Chapters 2, 4, and 6). The study reported here has extended that knowledge and indicates that heavy metal contamination, soil pH, redox potential, soil salinity, the forms in which heavy metal exist, incubation period, and seasonal variations control the amount of soil organic carbon in an estuarine floodplain that is inundated at least six times in the year (Figures 4.4, 5.11, 5.12, 5.14, 6.3 to 6.9 and Tables 4.19 to 4.21, and 5.3). The laboratory experiment in Chapter 6 indicates that more soil organic carbon was stored during the decomposition of soil organic matter as the concentration of heavy increased under different incubation period. On the other hand, it was found that soil salinity (because of high electrical conductivity), redox potential, and soil pH, played major roles in the amount of soil organic carbon stored under field monitoring condition. However, the reasons for the high amount of soil carbon storage due to the high concentration of heavy metals and high salinity under the laboratory and field conditions respectively are similar. Under different conditions, soil microbial activities, the main driver of the decomposition process of organic matter, will be decreased, leading to the accumulation of soil organic matter, reduced carbon dioxide emission, and enhanced soil carbon storage. This was borne out in the data presented on next-generation sequencing in Chapter 7 which show reduced bacterial communities as the concentration of heavy metals and salinity increase (Table 7.1 and Figures 7.1 and 7.2).

The decomposition rate was measured using the ratio of A-alkyl to O-alkyl carbon (Figure 6.11), indicating that uncontaminated soil decomposed faster compared to the contaminated soils (Appendix 27). Therefore, the amount of the soil organic carbon stored in the present study could be due to the incomplete mineralisation of organic carbon to inorganic carbon leading to accumulation of organic matter and enhanced carbon storage. The present study shows that the majority of the heavy metals exist in oxidisable or organic forms and as such account for the high amount of soil organic carbon storage within the

Upper Mersey estuarine floodplain. This could be because of the ability of the heavy metal to form a complex with organic carbon as detailed in Chapter 2. Other factors such as soil bulk density and plant biomass were reported in the literature reviewed in Chapter 5 to be independent of soil organic carbon storage but, this was found not to be the case in all the different land uses/land covers (Figures 5.7, 5.9, and 5.10). This is because the grazing saltmarsh had high soil bulk density and low plant biomass but stored high soil carbon compared to other sampling locations (Figures 5.7 and 5.9). This research provides evidence to show that contaminated estuarine floodplains are important carbon sinks.

8.3 Implications of the research

The findings in the present study are important to the progression of academic and practical work on heavy metal contamination and soil carbon dynamics. This is an issue of international concern as evidenced by the plethora of contiaminated sites around the globe. The findings obtained from this study have implications for better understanding the role of soil carbon in limiting heavy metal mobilization and the importance of microbial activity in soil carbon budgets, the management of saltmarsh under grazing regimes, national carbon budgets, and the design of future studies.

The present study has provided evidence to show that contaminated estuarine floodplains are important pollutant and carbon sinks (Tables 4.3 and Figures 4.4 and 4.5). Elevated concentrations of arsenic, chromium, copper, lead, and zinc were found in the low-lying saltmarshes of the Upper Mersey Estuary, as compared to the woodlands which were present at a higher elevation (Tables 4.4, 4.6, 4.8, 4.10, 4.12, and 4.14). This is an indication that saltmarshes in the present study act as a sink of pollutants due to the frequency of tidal inundation or sea level rise. This implies that saltmarshes should be continuously monitored, evaluated and protected as part of the environmental management for the Upper Mersey estuary and other estuaries with similar characteristics. This is especially the case if the saltmarsh is to be grazed commercially as comtaminants may enter the livestock and hence the food chain.

The data collected during the research reported here indicates that more soil organic carbon was stored during the decomposition of soil organic matter as the concentration of

heavy metals increased under different incubation periods (Figures 6.3 to 6.8). This could be due to the potential of heavy metal to form complexes with soil organic carbon, as reported by Stevenson (1994) and Reddy & Delaune (2008) and, hence, be immobilised in soil and plant roots thereby reducing the ease with which the heavy metal may be translocated from the soil to the plant roots and shoots and leached into the water system. Such binding of the heavy metals will lead to an improvement in water quality and associated aquatic life. The fact that this binding is due to soil carbon content indicates that carbon sequestration is an ecosystem service which reduces the amount of heavy metals being released into the environment.

The effects of heavy metal contamination on microbial activities were found to be similar under both laboratory and field conditions. Microbial activity was found to be a major driver of soil carbon storage and hence this implies that it is a factor that should be included in all future studies of this issue. High soil bulk density was found to be a second important driver. Where soils are compacted there is a reduction in the pore space and the microbial activities will be impeded due to a limited amount of air circulating within the soil system. This could have a negative effect on soil carbon storage. However, under esturine conditions, the frequency of tidal inundation could alter the soil system, resulting in anaerobic conditions which could have favoured the accumulation of soil organic matter leading to enhanced carbon storage. In this study differences were observed between the major controlling factors influencing the amount of soil organic carbon stored during heavy metal contamination in the laboratory experiment and under field condition. The implication of this is that results from laboratory investigations may not be readily transferable to field conditions. Sophisticated mirco- meso-cosms and fieldscale experiments will need to be developed and conducted to model esturine conditions better. This will further provide insights into the biogeochemical mechanisms that are responsible for the soil organic carbon dynamics in the contaminated estuarine floodplains.

The data obtained from this study are useful baseline information that can be used for the estimation of the national carbon budget. Recently, the UK government published 'A Green Future: Our 25 Year Plan to Improve the Environment'. Soil carbon sequestration features in several sections of the report through the restoration of lowland peat, by ensuring

that topsoil is retained as long as possible and greenhouse gas emissions are reduced. This research will also be beneficial to the landowners whose aims are to offer a mitigation platform to formulate, monitor, and implement ecosystem benefits to the Upper Mersey Estuary, by knowing which land use stored more carbon and the different level of heavy metal contamination to select which land use good for cattle grazing. The data presented in Table 8.1 and in Chapter 2 demonstrate that the Upper Mersey Estuary is typical of estuaries across the globe in terms of the amount of carbon sequestered and land use/land cover. Hence, the findings of the research reported in this thesis are applicable to all those other estuaries.

8.4 Recommendations for future research

In this research, questions have emerged regarding the status of the soil as a sink or source of carbon and the effect of microbial activity, as influenced by heavy metal contamination on this. To fill this gap, static chambers will be used according to the procedures used by previous researchers (Yuesi & Yinghong, 2003: Tang, Liu, Zhou, Zhang, & Zhou, 2006: Emran et al., 2012; Collier, Ruark, Oates, Jokela and Dell 2014). The carbon dioxide emission should be measured at different time intervals using a stopwatch, taking into consideration the time to reach the different chambers. Samples should be collected using syringe for gas chromatography analysis while in-situ measurement of carbon dioxide emission should also be taken using portable gas equipment with high resolution according to Emran, Gispert, & Pardini (2012) and Cheng & Chou (2016) procedures to compare results. According to Campbell, Collier, Ruark, & Powell (2015) and Krijnen, Richman, Lemke, & Farrell (2015), gas concentrations from gas chromatography (GC) and GasMet 4040 and Gasmet DX-4015 portable FTIR spectroscopy data were significantly similar for carbon dioxide data, however, methane and nitrous oxide concentrations were significantly different. Samples should be taken throughout the year so that details of year-round changes can be illucidated.

Also, the impacts of heavy metal contamination on soil carbon dynamics in the present study were mainly examined using laboratory experiments. The findings reported in this thesis should be validated in the field. It is suggested that field experiments could be conducted to compare contrasting land plots with different levels of contamination based on the European Community permissible metal concentration in soil directive 86/278/EEC. A wider range of parameters should be determined, including plant biomass (both below-

ground portion and above-ground portion), plant tissue-borne heavy metals, microbial diversity and activities, soil organic carbon content, and humic substances. This will allow a better evaluation of the carbon input and carbon output that determine the soil carbon storage.

In the current study, the amount of biomass that was grazed by the cattle was not determined. To assess the impacts of cattle grazing on carbon dynamics, the unaccounted plant biomass due to cattle consumption needs to be included. This can be done by artificially cutting out the grasses to simulate what was consumed by cattle. To evaluate the feasibility of using cattle grazing as a measure for enhancing soil carbon sequestration, the human health risk associated with heavy metal intake from consumption of beef derived from the cattle grazing the contaminated grasses needs to be assessed.

References

Abdu N, Abdullahi AA, & Abdulkadir A. (2016). Heavy metal and soil microbes. *Environmental Chemistry Letter*. 15(1):65–84.

Aber, J. D., Nadelhoffer, K. J., Steudler, P., & Melillo, J. M. (1989). Nitrogen saturation in northern forest ecosystems. *BioScience*, 39(6), 378–286.

Aceves, M. B., Grace, C., Ansorena, J., Dendooven, L., & Brookes, P. C. (1999). Soil microbial biomass and organic C in a gradient of zinc concentrations in soils around a mine spoil tip. *Soil Biology and Biochemistry*, *31*(6), 867–876.

Acosta, J. A., Jansen, B., Kalbitz, K., Faz, A., & Martínez-Martínez, S. (2011). Salinity increases mobility of heavy metals in soils. *Chemosphere*, 85(8), 1318–1324.

Ademoroti, C. M. A., Ukponmwan, D. O., & Omode, A. A. (1992). Studies of textile effluent discharges in Nigeria. *International journal of environmental studies*, *39*(4), 291–296.

Allison, S. D., Wallenstein, M. D., & Bradford, M. A. (2010). Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience, 3*(5), 336.

Alloway, B. (1995). Heavy metals in soils 2nd Ed. *Blackie Academic and Professional, London*

Al-Sheikh, A., Delgado, J. A., Barbarick, K., Sparks, R., Dillon, M., Qian, Y., & Cardon, G. (2005). Effects of potato–grain rotations on soil erosion, carbon dynamics and properties of rangeland sandy soils. *Soil and Tillage Research*, 81(2), 227–238.

Alvarez, E. A., Mochón, M. C., Sánchez, J. J., & Rodríguez, M. T. (2000). Determination of heavy metals in sewage sludge by microwave acid digestion and inductively coupled plasma atomic emission spectrometry. *Toxicological & Environmental Chemistry*, 75(3–4), 207–214.

Ander, E.L.; Cave, M.R.; Johnson, C.C.; Palumbo-Roe, B. (2012). Normal background concentrations of contaminants in the soils of England: results of the data exploration for Cd, Cu, Hg and Ni. Nottingham, UK, *British Geological Survey*, 74pp. (CR/12/041N) (Unpublished)

Anderson, J. P. (1982). Soil respiration. Methods of soil analysis. Part 2. *Chemical and microbiological properties*, (methodsofsoilan2), 831–871.

Anderson, T. H., & Domsch, K. H. (1993). The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil biology and biochemistry*, 25(3), 393–395.

Andersson, K. G., Brown, J., Mortimer, K., Jones, J. A., Charnock, T., Thykier-Nielsen, S., ... & Nielsen, S. P. (2008). New developments to support decision-making in contaminated inhabited areas following incidents involving a release of radioactivity to the environment. *Journal of environmental radioactivity*, 99(3), 439–454.

Andrews, J. E., Samways, G., & Shimmield, G. B. (2008). Historical storage budgets of organic carbon, nutrient and contaminant elements in saltmarsh sediments: biogeochemical context for managed realignment, Humber Estuary, UK. *Sci Total Environ*, 405(1–3), 1–13. doi: 10.1016/j.scitotenv.2008.07.044.

Anikwe, M. A. (2010). Carbon storage in soils of Southeastern Nigeria under different management practices. *Carbon Balance Manag*, 5, 5.

Annenkov, B. N. (1982). Mineral feeding of pigs. *Mineral nutrition of animals*, 355–389.

Anthony, W. H., Hutchinson, G. L., & Livingston, G. P. (1995). Chamber measurement of soil-atmosphere gas exchange: Linear vs. diffusion-based flux models. *Soil Science Society of America Journal*, 59(5), 1308–1310.

Arco-Lázaro, E., Agudo, I., Clemente, R., & Bernal, M. P. (2016). Arsenic (V) adsorption-desorption in agricultural and mine soils: effects of organic matter addition and phosphate competition. *Environmental pollution*, 216, 71–79.

Ashrafi, M., Mohamad, S., Yusoff, I., & Hamid, F. S. (2015)**.** Immobilization of Pb, Cd, and Zn in a contaminated soil using eggshell and banana stem amendments: metal leachability and a sequential extraction study. *Environmental Science and Pollution Research*, 22(1), 223–230.

ATSDR, 2008. Draft toxicological profile for cadmium. Atlanta: US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

Avnimelech, Y., Ritvo, G., Meijer, L. E., & Kochba, M. (2001). Water content, organic carbon and dry bulk density in flooded sediments. *Aquacultural engineering*, 25(1), 25–33.

Baath, E. (1989). Effects of heavy metals in soil on microbial processes and populations (a review). *Water, Air, and Soil Pollution*, *47*(3–4), 335–379.

Babich, H., & Stotzky, G. (1985). Heavy metal toxicity to microbe-mediated ecologic processes: a review and potential application to regulatory policies. *Environmental research*, 36(1), 111–137.

Bai, J., Xiao, R., Cui, B., Zhang, K., Wang, Q., Liu, X., . . . Huang, L. (2011). Assessment of heavy metal pollution in wetland soils from the young and old reclaimed regions in the Pearl River Estuary, South China. *Environ Pollut, 159*(3), 817-824

Baily, B., & Pearson, A. W. (2001). Change Detection Mapping of Saltmarsh Areas of South England from Hurst Castle to Pagham Harbour. Department of Geography, University of Portsmouth report to Posford Haskoning consultants. *English Nature and Environment Agency*.

Baldock, J. A., Currie, G. J., Oades, J. M., & Wilson, W. S. (1991). Organic matter as seen by solid state 13C NMR and pyrolysis tandem mass spectrometry. Advances in soil organic matter

research: the impact on agriculture and the environment. *Royal Society of Chemistry*, Cambridge, 45–60.

Baldock, J. A., Oades, J. M., Nelson, P. N., Skene, T. M., Golchin, A., & Clarke, P. (1997). Assessing the extent of decomposition of natural organic materials using solid-state 13C NMR spectroscopy. *Soil Research*, 35(5), 1061–1084.

Baldwin, D. S., & Mitchell, A. M. (2000). The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river–floodplain systems: a synthesis. *Regulated Rivers: Research & Management: An International Journal Devoted to River Research and Management*, *16*(5), 457–467.

Barajas-Aceves, M. (2005). Comparison of different microbial biomass and activity measurement methods in metal-contaminated soils. *Bioresource technology*, *96*(12), 1405–1414.

Barreto, R. C., Madari, B. E., Maddock, J. E. L., Machado, P. L. O. A., Torres, E., Franchini, J., & Costa, A. R. (2009). The impact of soil management on aggregation, carbon stabilization and carbon loss as CO_2 in the surface layer of a Rhodic Ferralsol in Southern Brazil. *Agriculture, Ecosystems & Environment*, 132(3–4), 243–251. doi: 10.1016/j.agee.2009.04.008.

Batjes, N. H. (1996). Total carbon and nitrogen in the soils of the world. *European journal of soil science*, *47*(2), 151-163.

Batty, L. C., Baker, A. J. M., Wheeler, B. D., & Curtis, C. D. (2000)**.** The effect of pH and plaque on the uptake of Cu and Mn in *Phragmites australis* (Cav.) Trin ex. Steudel. *Annals of Botany*, 86(3), 647–653.

Bazely, D. R., & Jefferies, R. L. (1986). Changes in the composition and standing crop of salt-marsh communities in response to the removal of a grazer. The *Journal of Ecology*, 74, 693–706.

Beaumont, N. J., Jones, L., Garbutt, A., Hansom, J. D., & Toberman, M. (2014). The value of carbon sequestration and storage in coastal habitats. *Estuarine, Coastal and Shelf Science*, 137, 32–40.

Beesley, L. (2010). Carbon in urban, brownfield and heavy metal contaminated soils (Doctoral dissertation, Liverpool John Moores University).

Bennett, C, Curtis, M S, & Fairhurst, C. 1995. Recent changes in the Mersey estuary 1958–1988. 12–23 in The Mersey estuary – naturally ours. Curtis, M S, Norman, D, and Walace, I D (editor). Warrington: Mersey Estuary Conservation Group.

Berhe, A. A. (2011). Decomposition of organic substrates at eroding vs. depositional landform positions. *Plant and Soil*, 350(1–2), 261–280. doi: 10.1007/s11104-011-0902-z.

Bernal, B., & Mitsch, W. J. (2012). Comparing carbon sequestration in temperate freshwater wetland communities. *Global Change Biology, 18*(5), 1636-1647.

Bernal, M. P., Sanchez-Monedero, M. A., Paredes, C., & Roig, A. (1998). Carbon mineralization from organic wastes at different composting stages during their incubation with soil. *Agriculture, Ecosystems & Environment*, 69(3), 175–189.

Bernard, L., Maron, P. A., Mougel, C., Nowak, V., Lévêque, J., Marol, C., ... & Ranjard, L. (2009). Contamination of soil by copper affects the dynamics, diversity, and activity of soil bacterial communities involved in wheat decomposition and carbon storage. *Applied and environmental microbiology*, 75(23), 7565–7569.

Bernhardt, J. (2015). Estimating Blue Carbon Storage in Texas Coastal Wetlands. https://www.openchannels.org/webinars/2015/estimating-blue-carbon-storage-texas-coastal-wetlands

Bigus, P., Tobiszewski, M., & Namieśnik, J. (2014). Historical records of organic pollutants in sediment cores. *Marine Pollution Bulletin*, *78*(1-2), 26-42.

Birge, H. E., Conant, R. T., Follett, R. F., Haddix, M. L., Morris, S. J., Snapp, S. S., ... & Paul, E. A. (2015). Soil respiration is not limited by reductions in microbial biomass during long-term soil incubations. *Soil Biology and Biochemistry*, 81, 304–310.

Bol, R., Bolger, T., Cully, R., & Little, D. (2003). Recalcitrant soil organic materials mineralize more efficiently at higher temperatures. *Journal of Plant Nutrition and Soil Science*, 166(3), 300–307.

Boon, P. I., Allen, T., Carr, G., Frood, D., Harty, C., Mcmahon, A., ... & Yugovic, J. (2015). Coastal wetlands of Victoria, south-eastern Australia: providing the inventory and condition information needed for their effective management and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25(4), 454–479.

Boorman, L. A., & Hazelden, J. (2017). Managed re-alignment; a salt marsh dilemma? *Wetlands Ecology and Management*, 25(4), 387–403.

Bouchard, V., Tessier, M., Digaire, F., Vivier, J. P., Valery, L., Gloaguen, J. C., & Lefeuvre, J. C. (2003). Sheep grazing as management tool in Western European saltmarshes. *Comptes Rendus Biologies*, 326, 148–157.

Boulton, A. J., & Boon, P. I. (1991). A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? *Marine and Freshwater Research*, 42(1), 1–43.

Bouyoucos, G. J. (1951). A Recalibration of the Hydrometer Method for Making Mechanical Analysis of Soils 1. *Agronomy journal*, 43(9), 434–438.

Brevik, E. C., & Homburg, J. A. (2004). A 5000-year record of carbon sequestration from a coastal lagoon and wetland complex, Southern California, USA. *Catena*, 57(3), 221–232. doi: 10.1016/j.catena.2003.12.001.

Brookes, P. C. (1995). The use of microbial parameters in monitoring soil pollution by heavy metals. *Biology and Fertility of soils*, 19(4), 269–279.

Bruland, G. L., Grunwald, S., Osborne, T. Z., Reddy, K. R., & Newman, S. (2006). Spatial distribution of soil properties in Water Conservation Area 3 of the Everglades. *Soil Science Society of America Journal*, 70(5), 1662–1676.

Bryan, G. W., & Langston, W. J. (1992). Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environmental pollution*, 76(2), 89–131.

Bryan, G. W., Langston, W. J., & Hummerstone, L. G. (1980). The use of biological indicators of heavy metal contamination in estuaries. Occasional Publication of the Marine Biological Association 1.

Buck, A. L. (1993). *An inventory of UK estuaries, vol. 4. North and east Scotland. Joint Nature Conservation Committee,* Peterborough, Cambs, 140.

Bull, I. D., Nott, C. J., van Bergen, P. F., Poulton, P. R., & Evershed, R. P. (2000). Organic geochemical studies of soils from the Rothamsted classical experiments—VI. The occurrence and source of organic acids in an experimental grassland soil. *Soil Biology and Biochemistry*, 32(10), 1367–1376.

Buol, S. W., Southard, R. J., Graham, R. C., & McDaniel, P. A. (2011). *Soil genesis and classification*. John Wiley & Sons: New York.

Burd, F. (1989). Saltmarsh survey of Great Britain: an *inventory of British Saltmarshes* (No. 17).

Burden, A., Garbutt, R. A., Evans, C. D., Jones, D. L., & Cooper, D. M. (2013). Carbon sequestration and biogeochemical cycling in a saltmarsh subject to coastal managed realignment. *Estuarine, Coastal and Shelf Science*, 120, 12–20.
doi: 10.1016/j.ecss.2013.01.014

Calace, N., Campisi, T., Iacondini, A., Leoni, M., Petronio, B. M., & Pietroletti, M. (2005). Metal-contaminated soil remediation by means of paper mill sludges addition: chemical and ecotoxicological evaluation. *Environmental Pollution*, 136(3), 485–492.

Campbell, C., Collier, S., Ruark, M.& Powell, J. (2015). Bringing a Needle to a Laser Fight: Comparing Greenhouse Gas Sampling Methods with Gas Chromatography and Fourier Transform Infrared Spectroscopy. Poster session presented at *the Annual Conference of the Soil Science Society of America, November 2015.*University of Wisconsin-Madison. USA

Castillo, M. A., Alonso, E. V., Cordero, M. S., Pavón, J. C., & de Torres, A. G. (2011). Fractionation of heavy metals in sediment by using microwave assisted sequential extraction procedure and determination by inductively coupled plasma mass spectrometry. *Microchemical Journal*, 98(2), 234–239.

Chambers, R. M., Meyerson, L. A., & Saltonstall, K. (1999)**.** Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic botany*, 64(3–4), 261–273.

Chambers, L. G., Reddy, K. R., & Osborne, T. Z. (2011). Short-term response of carbon cycling to salinity pulses in a freshwater wetland. *Soil Science Society of America Journal*, 75(5), 2000–2007.

Chao, A., & Chiu, C. H. (2014). Species richness: estimation and comparison. *Wiley StatsRef: Statistics Reference Online*, 1–26.

Chao, A., & Jost, L. (2012). Coverage-based rarefaction and extrapolation: Standardizing samples by completeness rather than size. *Ecology*, 93(12), 2533–2547.

Chaplot, V., & Cooper, M. (2015)**.** Soil aggregate stability to predict organic carbon outputs from soils. *Geoderma*, 243–244, 205–213.

Chaplot, V., Bernoux, M., Walter, C., Curmi, P., & Herpin, U. (2001). Soil carbon storage prediction in temperate hydromorphic soils using a morphologic index and digital elevation model. *Soil Science*, 166(1), 48–60.

Chatterjee, S., Datta, S., Mallick, P. H., Mitra, A., Veer, V., & Mukhopadhyay, S. K. (2013). Use of wetland plants in bioaccumulation of heavy metals. In *Plant-Based Remediation Processes* (pp. 117–139). Springer, Berlin, Heidelberg.

Chaudhari, P. R., Ahire, D. V., Ahire, V. D., Chkravarty, M., & Maity, S. (2013). Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. *International Journal of Scientific and Research Publications*, 3(2), 1–8.

Cheng, W. H., & Chou, M. S. (2016). Optimization of gas-water absorption equilibrium of carbon dioxide for algae liquors: selection of alkaline buffering chemicals. *International Journal of Photoenergy*, *2016*.

Chen, Y. P., Liu, Q., Liu, Y. J., Jia, F. A., & He, X. H. (2014). Responses of soil microbial activity to cadmium pollution and elevated CO 2. *Scientific reports*, 4, 4287.

Chen, Y., Chen, G., & Ye, Y. (2015). Coastal vegetation invasion increases greenhouse gas emission from wetland soils but also increases soil carbon accumulation. *Science of the Total Environment*, 526, 19–28.

Chenu, C., Rumpel, C., & Lehmann, J. (2015). Methods for studying soil organic matter: nature, dynamics, spatial accessibility, and interactions with minerals. *Soil microbiology, ecology and biochemistry*, 383–419.

Chirinda, N., Elsgaard, L., Thomsen, I. K., Heckrath, G., & Olesen, J. E. (2014). Carbon dynamics in topsoil and subsoil along a cultivated toposequence. *Catena*, 120, 20–28.

Clein, J. S., & Schimel, J. P. (1995). Nitrogen turnover and availability during succession from alder to poplar in Alaskan taiga forests. *Soil Biology and Biochemistry*, 27(6), 743–752.

Cole, D. W., Compton, J., Van Miegroet, H., & Homann, P. (1991). Changes in soil properties and site productivity caused by red alder. In *Management of Nutrition in Forests under Stress* (pp. 231–246). Springer, Dordrecht.

Collier, S. M., Ruark, M. D., Oates, L. G., Jokela, W. E., & Dell, C. J. (2014). Measurement of greenhouse gas flux from agricultural soils using static chambers. *Journal of visualized experiments: JoVE,* (90).

Collings, S. E., Johnson, M. S., & Leah, R. T. (1996). Metal contamination of angler-caught fish from the Mersey Estuary. *Mar Environ Res*, 41(3), 281–297.

Conte, P., Piccolo, A., Van Lagen, B., Buurman, P., & De Jager, P. A. (1997). Quantitative differences in evaluating soil humic substances by liquid-and solid-state 13C-NMR spectroscopy. *Geoderma*, 80(3–4), 339–352.

Coombs, J., Hall, D. O., & Long, S. P. (Eds.). (2014). Techniques in bioproductivity and photosynthesis: *pergamon international library of science, technology, engineering and social studies*. Oxford Elsevier

Community Invassive Species Network. (2019). How to Calculate Biodiversity. Retrieved from https://entnemdept.ifas.ufl.edu/hodges/protectus/lp_webfolder/9_12_grade/student_handout_1a.pdf

Cooper, N. J., Cooper, T., & Burd, F. (2001). 25 years of salt marsh erosion in Essex: Implications for coastal defence and nature conservation. *Journal of Coastal Conservation*, 7(1), 31–40.

Cotrufo, M. F., De Santo, A. V., Alfani, A., Bartoli, G., & De Cristofaro, A. (1995). Effects of urban heavy metal pollution on organic matter decomposition in Quercus ilex L. woods. *Environmental Pollution*, 89(1), 81–87.

Coxson, D. S., & Parkinson, D. (1987). The pattern of winter respiratory response to temperature, moisture, and freeze–thaw exposure in Bouteloua gracilis dominated grassland soils of southwestern Alberta. *Canadian journal of botany*, 65(8), 1716–1725.

Creamer, C. A., de Menezes, A. B., Krull, E. S., Sanderman, J., Newton-Walters, R., & Farrell, M. (2015). Microbial community structure mediates response of soil C decomposition to litter addition and warming. *Soil Biology and Biochemistry*, 80, 175–188.

Cronk, Q. C. B., & Fuller, J. L. (1995). Plant Invaders. *People and Plants Conservation Manual*.

Cundy, A. B., Croudace, I. W., Cearreta, A., & Irabien, M. J. (2003). Reconstructing historical trends in metal input in heavily-disturbed, contaminated estuaries: studies from Bilbao, Southampton Water and Sicily. *Applied Geochemistry*, *18*(2), 311-325.

da Silva Copertino, M. (2011). Add coastal vegetation to the climate critical list: forests are protected, but carbon sinks in mangroves, seagrasses and marshes are ignored. Margareth da Silva Copertino wants Brazil to change that. *Nature*, 473(7347), 255–256.

Dalias, P., Anderson, J. M., Bottner, P., & Coûteaux, M. M. (2001). Long-term effects of temperature on carbon mineralisation processes. *Soil Biology and Biochemistry*, 33(7–8), 1049–1057.

Davidson, C. M., Duncan, A. L., Littlejohn, D., Ure, A. M., & Garden, L. M. (1998). A critical evaluation of the three-stage BCR sequential extraction procedure to assess the potential mobility and toxicity of heavy metals in industrially-contaminated land. *Analytica Chimica Acta*, 363(1), 45–55.

Davidson, E. A., Savage, K. V. L. V., Verchot, L. V., & Navarro, R. (2002). Minimizing artifacts and biases in chamber-based measurements of soil respiration. *Agricultural and Forest Meteorology*, 113(1), 21–37.

Davidson, N. C., Laffoley, D. D. A., Doody, J. P., Way, L. S., Gordon, J., Key, R. E., ... & Duff, K. L. (1991). Nature conservation and estuaries in Great Britain. *Nature Conservancy Council*, Peterborough, 1–76.

Davinic, M., Fultz, L. M., Acosta-Martinez, V., Calderón, F. J., Cox, S. B., Dowd, S. E., ... & Moore-Kucera, J. (2012). Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. *Soil Biology and Biochemistry*, 46, 63–72.

Davis, A., Ruby, M. V., & Bergstrom, P. D. (1994). Factors controlling lead bioavailability in the Butte mining district, Montana, USA. *Environmental Geochemistry and Health*, 16(3), 147–157.

Deepali, K. K., & Gangwar, K. (2010). Metals concentration in textile and tannery effluents, associated soils and ground water. *NY Sci J*, 3(4), 82–9.

Delmont, T. O., Robe, P., Clark, I., Simonet, P., & Vogel, T. M. (2011). Metagenomic comparison of direct and indirect soil DNA extraction approaches. *Journal of microbiological methods*, 86(3), 397–400.

de Andrade Passos, E., Alves, J. C., dos Santos, I. S., Jose do Patrocínio, H. A., Garcia, C. A. B., & Costa, A. C. S. (2010). Assessment of trace metals contamination in estuarine sediments using a sequential extraction technique and principal component analysis. *Microchemical Journal*, *96*(1), 50-57.

de Menezes, A. B., Lewis, E., O'Donovan, M., O'Neill, B. F., Clipson, N., & Doyle, E. M. (2011). Microbiome analysis of dairy cows fed pasture or total mixed ration diets. *FEMS microbiology ecology*, 78(2), 256–265.

de Menezes, A. B., Prendergast-Miller, M. T., Poonpatana, P., Farrell, M., Bissett, A., Macdonald, L. M., ... & Thrall, P. H. (2015). C/N ratio drives soil actinobacterial cellobiohydrolase gene diversity. *Applied and environmental microbiology*, 81(9), 3016–3028.

de Menezes, A., Clipson, N., & Doyle, E. (2012). Comparative metatranscriptomics reveals widespread community responses during phenanthrene degradation in soil. *Environmental microbiology*, 14(9), 2577–2588.

De Nobili, M., Contin, M., Mahieu, N., Randall, E. W., & Brookes, P. C. (2008). Assessment of chemical and biochemical stabilization of organic C in soils from the long-term experiments at Rothamsted (UK). *Waste management*, 28(4), 723–733.

Derenne, S., & Quénéa, K. (2015). Analytical pyrolysis as a tool to probe soil organic matter. *Journal of Analytical and Applied Pyrolysis*, 111, 108–120.

Dorodnikov, M., Kuzyakov, Y., Fangmeier, A., & Wiesenberg, G. L. B. (2011). C and N in soil organic matter density fractions under elevated atmospheric CO_2: Turnover vs. stabilization. *Soil Biology and Biochemistry*, 43(3), 579–589.

Dotaniya, M. L., Rajendiran, S., Meena, V. D., Saha, J. K., Coumar, M. V., Kundu, S., & Patra, A. K. (2017). Influence of chromium contamination on carbon mineralization and enzymatic activities in Vertisol. *Agricultural Research*, 6(1), 91–96.

Dsikowitzky, L., & Schwarzbauer, J. (2014). Industrial organic contaminants: identification, toxicity and fate in the environment. *Environmental Chemistry Letters*, *12*(3), 371-386.

Du Laing, G., De Meyer, B., Meers, E., Lesage, E., Van de Moortel, A., Tack, F. M., & Verloo, M. G. (2008). Metal accumulation in intertidal marshes: role of sulphide precipitation. *Wetlands*, 28(3), 735–746.

Du Laing, G., Rinklebe, J., Vandecasteele, B., Meers, E., & Tack, F. M. (2009). Trace metal behaviour in estuarine and riverine floodplain soils and sediments: a review. *Science of the total environment*, 407(13), 3972–3985.

Du Laing, G., Tack, F., & Rinklebe, J. (2013). Factors Affecting Accumulation, Mobility and Availability of Trace Elements in Estuarine and Riverine Floodplain Soils and Sediments. In *12th International conference on the Biogeochemistry of Trace Elements* (ICOBTE 2013) (pp. 210–210). University of Georgia.

Du Laing, G., De Vos, R., Vandecasteele, B., Lesage, E., Tack, F. M., & Verloo, M. G. (2008). Effect of salinity on heavy metal mobility and availability in intertidal sediments of the Scheldt estuary. *Estuarine, Coastal and Shelf Science*, 77(4), 589–602.

Du Laing, G., Meers, E., Dewispelaere, M., Rinklebe, J., Vandecasteele, B., Verloo, M. G., & Tack, F. M. (2009). Effect of water table level on metal mobility at different depths in wetland soils of the Scheldt Estuary (Belgium). *Water, air, and soil pollution*, 202(1–4), 353–367.

Dudka, S., & Miller, W. P. (1999). Permissible concentrations of arsenic and lead in soils based on risk assessment. *Water, Air, and Soil Pollution*, 113(1–4), 127–132.

Dungait, J. A., Bol, R., Lopez-Capel, E., Bull, I. D., Chadwick, D., Amelung, W., ... & Evershed, R. P. (2010). Applications of stable isotope ratio mass spectrometry in cattle dung carbon cycling studies. *Rapid Communications in Mass Spectrometry*, 24(5), 495–500.

Dyer, K. R. (2002)**.** FutureCoast estuary assessment. *FutureCoast*, Halcrow, Swindon.

Edmonds, J. W., Weston, N. B., Joye, S. B., Mou, X., & Moran, M. A. (2009). Microbial community response to seawater amendment in low-salinity tidal sediments. *Microbial Ecology*, 58(3), 558–568.

Edmondson, J. L., Davies, Z. G., McCormack, S. A., Gaston, K. J., & Leake, J. R. (2014)**.** Land-cover effects on soil organic carbon stocks in a European city. *Sci Total Environ*, 472, 444–453.

Emran, M., Gispert, M., & Pardini, G. (2012). Comparing measurements methods of carbon dioxide fluxes in a soil sequence under land use and cover change in North Eastern Spain. *Geoderma*, 170, 176–185.

Enya, O. O., Omueti, J. A. I., & Akinbola, G. E. (2011). Particle size and free iron oxide distribution along two toposequence in South Western Nigeria. *Continental Journal of Agromony*, 5(2), 22–31.

Enya, O., Lin, C., & Qin, J. (2019). Heavy metal contamination status in soil-plant system in the Upper Mersey Estuarine Floodplain, Northwest England. *Marine Pollution Bulletin*, *146*, 292-304.

Erfanzadeh, R., Bahrami, B., Motamedi, J., & Pétillon, J. (2014). Changes in soil organic matter driven by shifts in co-dominant plant species in a grassland. *Geoderma*, 213, 74–78.

Esselink, P., Fresco, L. F., & Dijkema, K. S. (2002). Vegetation change in a man-made salt marsh affected by a reduction in both grazing and drainage. *Applied Vegetation Science*, 5(1), 17–32.

Fang, C., Smith, P., Moncrieff, J. B., & Smith, J. U. (2005). Similar response of labile and resistant soil organic matter pools to changes in temperature. *Nature*, *433*(7021), 57.

FAO, I. F. A. D., & UNICEF. (2017). WFP, WHO (2017). The state of food security and nutrition in the world 2017. *Building Resilience for Peace and Food Security (Food and Agriculture Organization, Rome)*.

Feijtel, T. C., DeLaune, R. D., & Patrick, W. H. (1988). Biogeochemical control on metal distribution and accumulation in Louisiana sediments. *Journal of Environmental Quality*, *17*(1), 88-94.

Feng, G., Xie, T., Wang, X., Bai, J., Tang, L., Zhao, H., ... & Zhao, Y. (2018). Metagenomic analysis of microbial community and function involved in cd-contaminated soil. *BMC microbiology*, 18(1), 11.

Fisk, M. C., Fahey, T. J., & Groffman, P. M. (2010). Carbon resources, soil organisms, and nitrogen availability: Landscape patterns in a northern hardwood forest. *Forest Ecology and Management*, 260(7), 1175–1183.

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., ... & Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature geoscience*, 5(7), 505.

Fox, W. M., Johnson, M. S., Jones, S. R., Leah, R. T., & Copplestone, D. (1999). The use of sediment cores from stable and developing salt marshes to reconstruct historical contamination profiles in the Mersey Estuary, UK. *Mar Environ Res, 47*(4), 311-329.

Fox, W. M., Connor, L., Copplestone, D., Johnson, M. S., & Leah, R. T. (2001). The organochlorine contamination history of the Mersey estuary, UK, revealed by analysis of sediment cores from salt marshes. *Mar Environ Res*, 51(3), 213–227.

French, P.W. (1997). Coastal and Estuarine Management (*Routledge Environmental Management Series*). Routledge, London, p. 251.

Friedman, S. P. (2005). Soil properties influencing apparent electrical conductivity: a review. *Computers and Electronics in Agriculture*, 46(1–3), 45–70.

Frohne, T., Rinklebe, J., & Diaz-Bone, R. A. (2014). Contamination of floodplain soils along the Wupper River, Germany, with As, Co, Cu, Ni, Sb, and Zn and the impact of pre-definite redox variations on the mobility of these elements. Soil and Sediment Contamination: *An International Journal*, 23(7), 779–799.

Frohne, T., Rinklebe, J., Diaz-Bone, R. A., & Du Laing, G. (2011). Controlled variation of redox conditions in a floodplain soil: impact on metal mobilization and biomethylation of arsenic and antimony. *Geoderma*, 160(3–4), 414–424.

Fuchs, M., Simon, B., & Micheli, E. (2005). Soil organic matter as a criteria in soil classification systems. *Cereal Research Communications*, *33*(1), 365-368.

Gambrell, R. P., & Patrick Jr, W. H. (1991). Handbook—*Remediation of contaminated sediments*. EPA/625/6–91/028.

Gao, Y., Zhu, B., Yu, G., Chen, W., He, N., Wang, T., & Miao, C. (2014). Coupled effects of biogeochemical and hydrological processes on C, N, and P export during extreme rainfall events in a purple soil watershed in southwestern China. *Journal of Hydrology*, 511, 692–702.

Garrard, S. L., & Beaumont, N. J. (2014)**.** The effect of ocean acidification on carbon storage and sequestration in seagrass beds; a global and UK context. *Mar Pollut Bull*, 86(1–2), 138–146.

Gerla, P. J. (2013). Can pH and electrical conductivity monitoring reveal spatial and temporal patterns in wetland geochemical processes? *Hydrology and Earth System Sciences Discussions*, 10(1), 699–728.

Ghosh, M., & Singh, S. P. (2005). A review on phytoremediation of heavy metals and utilization of it's by products. *Asian J Energy Environ*, 6(4), 18.

Giller, K. E., Witter, E., & Mcgrath, S. P. (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil biology and biochemistry, 30(10–11), 1389–1414.

Giller, K. E., Witter, E., & McGrath, S. P. (2009). Heavy metals and soil microbes. *Soil Biology and Biochemistry*, 41(10), 2031–2037.

Gołębiewski, M., Deja-Sikora, E., Cichosz, M., Tretyn, A., & Wróbel, B. (2014). 16S rDNA pyrosequencing analysis of bacterial community in heavy metals polluted soils. *Microbial ecology*, 67(3), 635–647.

Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nature Reviews Genetics*, 17(6), 333.

González-Alcaraz, M. N., Egea, C., Jiménez-Cárceles, F. J., Párraga, I., María-Cervantes, A., Delgado, M. J., & Álvarez-Rogel, J. (2012). Storage of organic carbon, nitrogen and phosphorus in the soil–plant system of *Phragmites australis* stands from a eutrophicated Mediterranean salt marsh. *Geoderma*, 185–186, 61–72.

Gosselink, J. G., Hatton, R., & Hopkinson, C. S. (1984). Relationship of organic carbon and mineral content to bulk density in Louisiana marsh soils. *Soil Science*, 137(3), 177–180.

Good, I. J. (1953). The population frequencies of species and the estimation of population parameters. *Biometrika*, 40(3–4), 237–264.

Grogan, P., & Chapin Iii, F. S. (1999). Arctic soil respiration: effects of climate and vegetation depend on season. *Ecosystems*, 2(5), 451–459.

Granberg, M. E., & Selck, H. (2007)**.** Effects of sediment organic matter quality on bioaccumulation, degradation, and distribution of pyrene in two macrofaunal species and their surrounding sediment. *Mar Environ Res*, 64(3), 313–335.

Gregory, A. S., Kirk, G. J. D., Keay, C. A., Rawlins, B. G., Wallace, P., & Whitmore, A. P. (2014). An assessment of subsoil organic carbon stocks in England and Wales. *Soil use and management*, 30(1), 10–22.

Griffiths, E., & Gupta, R. S. (2007). Identification of signature proteins that are distinctive of the Deinococcus-Thermus phylum. *International Microbiology*, 10(3), 201.

Griffiths, K., Shand, P., & Ingram, J. (2003). The Permo-Triassic Sandstones of Manchester And East Cheshire. Environment Agency: British Geological Survey, UK. Baseline report series. 8.

Grybos, M., Davranche, M., Gruau, G., & Petitjean, P. (2007). Is trace metal release in wetland soils controlled by organic matter mobility or Fe-oxyhydroxides reduction? *Journal of colloid and interface science*, 314(2), 490–501.

Guimarães, P. R., Ambrosini, V. G., Miotto, A., Ceretta, C. A., Simão, D. G., & Brunetto, G. (2016). Black oat (Avena strigosa Schreb.) growth and root anatomical changes in sandy soil with different copper and phosphorus concentrations. *Water, Air, & Soil Pollution*, 227(6), 192.

Gunina, A., & Kuzyakov, Y. (2014). Pathways of litter C by formation of aggregates and SOM density fractions: Implications from 13C natural abundance. *Soil Biology and Biochemistry*, 71, 95–104.

Guo, T., DeLaune, R. D., & Patrick Jr, W. H. (1997). The influence of sediment redox chemistry on chemically active forms of arsenic, cadmium, chromium, and zinc in estuarine sediment. *Environment International*, 23(3), 305–316.

Gwynne, B. J (2004). Heavy metal pollution of saltmarsh sediments. (Unpublished PhD Thesis), University of Salford, Salford.

Haderlein, A., Legros, R., & Ramsay, B. (2001). Enhancing pyrene mineralization in contaminated soil by the addition of humic acids or composted contaminated soil. *Applied microbiology and biotechnology*, 56(3–4), 555–559.

Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. A., & Baird, D. J. (2011). Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. *PLoS one*, 6(4), e17497.

Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J., & Goodman, R. M. (1998). Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chemistry & biology*, 5(10), R245–R249.

Hansen, V. D., & Reiss, K. C. (2015). Chapter 16 – Threats to Marsh Resources and Mitigation. In J. F. Shroder, J. T. Ellis, & D. J. Sherman (Eds.), *Coastal and Marine Hazards, Risks, and Disasters* (pp. 467–494). Boston: Elsevier.

Harland, B. J., Taylor, D., & Wither, A. (2000). The distribution of mercury and other trace metals in the sediments of the Mersey Estuary over 25 years 1974–1998. *Science of the Total Environment*, 253(1–3), 45–62.

Harrison-Kirk, T., Beare, M. H., Meenken, E. D., & Condron, L. M. (2014). Soil organic matter and texture affect responses to dry/wet cycles: Changes in soil organic matter fractions and relationships with C and N mineralisation. *Soil Biology and Biochemistry*, 74, 50–60.

Hartman, W. H., Richardson, C. J., Vilgalys, R., & Bruland, G. L. (2008). Environmental and anthropogenic controls over bacterial communities in wetland soils. *Proceedings of the National Academy of Sciences*, 105(46), 17842–17847.

Hattori, H. (1996). Decomposition of organic matter with previous cadmium adsorption in soils. *Soil science and plant nutrition*, 42(4), 745–752.

Hawley, J. K. (1985). Assessment of health risk from exposure to contaminated soil. *Risk analysis*, 5(4), 289–302.

Hayes, F., Spurgeon, D. J., Lofts, S., & Jones, L. (2018). Evidence-based logic chains demonstrate multiple impacts of trace metals on ecosystem services. *Journal of environmental management*, 223, 150–164.

Hayes, M. A., Jesse, A., Hawke, B., Baldock, J., Tabet, B., Lockington, D., & Lovelock, C. E. (2017). Dynamics of sediment carbon stocks across intertidal wetland habitats of Moreton Bay, Australia. *Global change biology*, 23(10), 4222–4234.

Hoggart, S., Hawkins, S. J., Bohn, K., Airoldi, L., van Belzen, J., Bichot, A., . . . Thompson, R. C. (2015). *Ecological Approaches to Coastal Risk Mitigation*. 171–236.

He, Y., Qin, L., Li, Z., Liang, X., Shao, M., & Tan, L. (2013). Carbon storage capacity of monoculture and mixed-species plantations in subtropical China. *Forest Ecology and Management*, 295, 193–198.

Heim, S., & Schwarzbauer, J. (2013). Pollution history revealed by sedimentary records: a review. *Environmental chemistry letters*, *11*(3), 255-270.

Heim, S., Schwarzbauer, J., Kronimus, A., Littke, R., Woda, C., & Mangini, A. (2004). Geochronology of anthropogenic pollutants in riparian wetland sediments of the Lippe River (Germany). *Organic Geochemistry*, *35*(11-12), 1409-1425.

Hill, A. R., & Shackleton, M. (1989). Soil N mineralization and nitrification in relation to nitrogen solution chemistry in a small forested watershed. *Biogeochemistry*, 8(2), 167–184.

Hill, G. T., Mitkowski, N. A., Aldrich-Wolfe, L., Emele, L. R., Jurkonie, D. D., Ficke, A., ... & Nelson, E. B. (2000). Methods for assessing the composition and diversity of soil microbial communities. *Applied soil ecology*, 15(1), 25–36.

Hinojosa, M. B., García-Ruíz, R., Viñegla, B., & Carreira, J. A. (2004). Microbiological rates and enzyme activities as indicators of functionality in soils affected by the Aznalcóllar toxic spill. *Soil Biology and Biochemistry*, 36(10), 1637–1644.

Hiroki, M. (1992). Effects of heavy metal contamination on soil microbial population. *Soil Science and Plant Nutrition*, 38(1), 141–147.

Hook, P. B., & Burke, I. C. (2000). Biogeochemistry in a shortgrass landscape: control by topography, soil texture, and microclimate. *Ecology*, 81(10), 2686–2703.

Hopkinson, C. S., Cai, W.-J., & Hu, X. (2012). Carbon sequestration in wetland dominated coastal systems—a global sink of rapidly diminishing magnitude. *Current Opinion in Environmental Sustainability*, 4(2), 186–194.

House of Commons. (2018). Carbon Price floor (CPF) and the price support mechanism. London: Stationery Office.

Hsu, J. H., & Lo, S. L. (1999). Chemical and spectroscopic analysis of organic matter transformations during composting of pig manure. *Environmental Pollution*, *104*(2), 189–196.

Huang, C.-Y., Hendrix, P. F., Fahey, T. J., Bohlen, P. J., & Groffman, P. M. (2010). A simulation model to evaluate the impacts of invasive earthworms on soil carbon dynamics. *Ecological Modelling*, 221(20), 2447–2457.

Hugenholtz, P., Goebel, B. M., & Pace, N. R. (1998). Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of bacteriology*, 180(18), 4765–4774.

Hurley, R. R., Rothwell, J. J., & Woodward, J. C. (2017). Metal contamination of bed sediments in the Irwell and Upper Mersey catchments, northwest England: exploring the legacy of industry and urban growth. *Journal of soils and sediments*, 17(11), 2648–2665.

Imentai, A., Thevs, N., Schmidt, S., Nurtazin, S., & Salmurzauli, R. (2015). Vegetation, fauna, and biodiversity of the Ile delta and southern Lake Balkhash—A review. *Journal of Great Lakes Research*, 41(3), 688–696.

Imperato, M., Adamo, P., Naimo, D., Arienzo, M., Stanzione, D., & Violante, P. (2003). Spatial distribution of heavy metals in urban soils of Naples city (Italy). *Environmental pollution*, 124(2), 247–256.

Jemmett, A. W. L. (1991). An investigation into the heavy metals, sediment and vegetation of a Mersey Estuary salt marsh (Unpublished PhD Thesis), University of Liverpool.

Jensen, A. (1985). The effect of cattle and sheep grazing on salt-marsh vegetation at Skallingen, Denmark. V*egetatio*, 60(1), 37–48.

Jenny, H. (1941). Factors of soil formation; a sytem of quantitative pedology (No. 631.4 J45).

Jia S, Wang Z, Zhang XX, Liu B, Li W, Cheng S. 2012. Metagenomics analysis of cadmium and copper resistance genes in activated sludge of a tannery wastewater treatment plant. *Journal of Environmental Biology*. 34, 375–380.

Jones, P. D. (2006). Water quality and fisheries in the Mersey estuary, England: A historical perspective. *Mar Pollut Bull*, 53(1–4), 144–154.

Jones, P.D., 2000. The Mersey Estuary–Back from the Dead? Solving a 150-Year Old Problem. *Water and Environment Journal*, 14(2), pp.124–130.

Jones, B., & Turki, A. (1997). Distribution and speciation of heavy metals in surficial sediments from the Tees Estuary, north-east England. *Marine Pollution Bulletin*, *34*(10), 768-779.

Kabatia-Pendias, A., & Pendias, H. (1992). Trace elements in soils and plants, CRC Press, Boca. Raton, FL, USA.

Kabata-Pendias, A., & Mukherjee, A. B. (2007). Trace elements from soil to human. Springer Science & Business Media.

Kalbitz, K., & Wennrich, R. (1998). Mobilization of heavy metals and arsenic in polluted wetland soils and its dependence on dissolved organic matter. *Science of the Total Environment*, 209(1), 27–39.

Kandeler, F., Kampichler, C., & Horak, O. (1996). Influence of heavy metals on the functional diversity of soil microbial communities. *Biology and fertility of soils*, 23(3), 299–306.

Kashem, M. A., & Singh, B. R. (2001). Metal availability in contaminated soils: I. Effects of floodingand organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn. *Nutrient Cycling in Agroecosystems*, 61(3), 247–255.

Kayranli, B., Scholz, M., Mustafa, A., & Hedmark, Å. (2010). Carbon storage and fluxes within freshwater wetlands: a critical review. *Wetlands*, 30(1), 111–124.

Kelly JJ, Tate III, RL. (2017). Effects of Heavy Metal Contamination and Remediation on Soil Microbial Communities in the Vicinity of a Zinc Smelter. *Journal of Environmental Quality*. 27(3):609–617.

Khomtchouk BB, Hennessy JR, Wahlestedt C (2017) shiny heatmap: Ultra fast low memory heatmap web interface for big data genomics. *PLoS ONE*. 12(5): e0176334.

Kirschbaum, M. U. (1995). The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biology and biochemistry*, 27(6), 753–760.

Kögel-Knabner, I. (2000). Analytical approaches for characterizing soil organic matter. *Organic Geochemistry*, 31(7), 609–625.

Köhler, H. R., Wein, C., Reiss, S., Storch, V., & Alberti, G. (1995). Impact of heavy metals on mass and energy flux within the decomposition process in deciduous forests. *Ecotoxicology*, 4(2), 114–137.

Krijnen, F., Richman, D., Lemke, R. & Farrell, R. (2015). Evaluation of a Closed-Path Fourier Transform Infra-Red (FTIR) Multi-Component Gas Analyzer for the Simultaneous Measurement of Nitrous Oxide, Carbon Dioxide and Methane. Poster session presented at the *Annual Conference of the Soil Science Society of America*.

Kron, W. (2013). Coasts: the high-risk areas of the world. *Natural hazards*, *66*(3), 1363-1382.

Kühn, P., Aguilar, J., & Miedema, R. (2010). Textural pedofeatures and related horizons. In *Interpretation of micromorphological features of soils and regoliths* (pp. 217–250).

Kulawardhana, R. W., Feagin, R. A., Popescu, S. C., Boutton, T. W., Yeager, K. M., & Bianchi, T. S. (2015). The role of elevation, relative sea-level history and vegetation transition in determining carbon distribution in Spartina alterniflora dominated salt marshes. *Estuarine, Coastal and Shelf Science*, 154, 48–57.

Kumpiene, J., Lagerkvist, A., & Maurice, C. (2008). Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments–a review. *Waste management*, 28(1), 215–225.

Kumpiene, J. (2010). Trace element immobilization in soil using amendments. *Trace Element in Soils. Wiley-Blackwell, Chichester, UK*, 353-380.

Kunito, T., Saeki, K., Goto, S., Hayashi, H., Oyaizu, H., & Matsumoto, S. (2001). Copper and zinc fractions affecting microorganisms in long-term sludge-amended soils. *Bioresource technology*, 79(2), 135–146.

Kuperman, R. G., & Carreiro, M. M. (1997). Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. *Soil Biology and Biochemistry*, 29(2), 179–190.

Lafleur, P. M., Moore, T. R., Roulet, N. T., & Frolking, S. (2005). Ecosystem respiration in a cool temperate bog depends on peat temperature but not water table. *Ecosystems*, 8(6), 619–629.

Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *science*, 304(5677), 1623–1627.

Lal, R. (2005). Forest soils and carbon sequestration. *Forest Ecology and Management*, 220(1–3), 242–258.

Lamparter, A., Bachmann, J., Goebel, M. O., & Woche, S. K. (2009). Carbon mineralization in soil: Impact of wetting–drying, aggregation and water repellency. *Geoderma*, 150(3–4), 324–333.

Landi, L., Renella, G., Moreno, J. L., Falchini, L., & Nannipieri, P. (2000). Influence of cadmium on the metabolic quotient, L-: D-glutamic acid respiration ratio and enzyme activity: microbial biomass ratio under laboratory conditions. *Biology and Fertility of Soils*, 32(1), 8–16.

Lavelle, P., Rodríguez, N., Arguello, O., Bernal, J., Botero, C., Chaparro, P., . . . Fonte, S. J. (2014). Soil ecosystem services and land use in the rapidly changing Orinoco River Basin of Colombia. *Agriculture, Ecosystems & Environment*, 185, 106–117.

Leifeld, J., Franko, U., & Schulz, E. (2006). Thermal stability responses of soil organic matter to long-term fertilization practices. *Biogeosciences*, 3(3), 371–374.

Lejon, D. P., Martins, J. M., Lévêque, J., Spadini, L., Pascault, N., Landry, D., ... & Ranjard, L. (2008). Copper dynamics and impact on microbial communities in soils of variable organic status. *Environmental science & technology*, 42(8), 2819–2825.

Lenart-Boroń, A., & Wolny-Koładka, K. (2015). Heavy metal concentration and the occurrence of selected microorganisms in soils of a steelworks area in Poland. *Plant Soil Environ*, 61(6), 273–278.

Li, M.S., Y.P. Luo and Z.Y. Su. (2007). Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. *Environmental Pollution*, 147: 168–175.

Li, Z., & Shuman, L. M. (1996). Heavy metal movement in metal-contaminated soil profiles. *Soil Science*, 161(10), 656–666.

Lindbo, D. L., Stolt, M. H., & Vepraskas, M. J. (2010). Redoximorphic features. In *Interpretation of micromorphological features of soils and regoliths* (pp. 129–147).

Lissner, J., & Schierup, H. H. (1997)**.** Effects of salinity on the growth of *Phragmites australis*. *Aquatic Botany*, 55(4), 247–260.

Liu, A. C., Chou, C. Y., Chen, L. L., & Kuo, C. H. (2015). Bacterial community dynamics in a swine wastewater anaerobic reactor revealed by 16S rDNA sequence analysis. *Journal of biotechnology*, 194, 124–131.

Lorenz, K., Lal, R., & Jiménez, J. J. (2009). Soil organic carbon stabilization in dry tropical forests of Costa Rica. *Geoderma*, 152(1–2), 95–103.

Lu, X. & Cheng, G. (2009). Climate change effects on soil carbon dynamics and greenhouse gas emissions in Abies fabri forest of subalpine, southwest China. *Soil Biology and Biochemistry*, 41(5), 1015–1021.

Luisetti, T., Jackson, E. L., & Turner, R. K. (2013). Valuing the European 'coastal blue carbon' storage benefit. *Mar Pollut Bull*, 71(1–2), 101–106.

Luizão, R. C., Luizão, F. J., Paiva, R. Q., Monteiro, T. F., Sousa, L. S., & Kruijt, B. (2004). Variation of carbon and nitrogen cycling processes along a topographic gradient in a central Amazonian forest. *Global Change Biology*, 10(5), 592–600.

Lukman, S., Bukhari, A., Al-Malack, M. H., Mu'azu, N. D., & Essa, M. H. (2014). Geochemical modeling of trivalent chromium migration in saline-sodic soil during Lasagna process: impact on soil physicochemical properties. *The Scientific World Journal*, 2014.

Maillard, É., Angers, D. A., Chantigny, M., Bittman, S., Rochette, P., Lévesque, G., Parent, L.-É. (2015). Carbon accumulates in organo-mineral complexes after long-term liquid dairy manure application. *Agriculture, Ecosystems & Environment*, 202, 108–119.

Malik, R. N., Husain, S. Z., & Nazir, I. (2010). Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. *Pak J Bot*, 42(1), 291–301.

Maniatis, T. (1989). Molecular cloning. *Decontamination of Dilute Solutions of Ethidium Bromide*.

Marchand, C., Allenbach, M., & Lallier-Vergès, E. (2011). Relationships between heavy metals distribution and organic matter cycling in mangrove sediments (Conception Bay, New Caledonia). *Geoderma*, 160(3–4), 444–456.

Margenot, A. J., Calderón, F. J., Bowles, T. M., Parikh, S. J., & Jackson, L. E. (2015). Soil organic matter functional group composition in relation to organic carbon, nitrogen, and phosphorus fractions in organically managed tomato fields. *Soil Science Society of America Journal*, 79(3), 772–782.

Marriott, E. E., & Wander, M. M. (2006). Total and labile soil organic matter in organic and conventional farming systems. *Soil Science Society of America Journal*, 70(3), 950–959.

Masscheleyn, P. H., Delaune, R. D., & Patrick Jr, W. H. (1991). Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environmental science & technology*, 25(8), 1414–1419.

Matejovic, I. (1997). Determination of carbon and nitrogen in samples of various soils by the dry combustion. *Communications in Soil Science and Plant Analysis*, *28*(17-18), 1499-1511.

Martin, C. W. (2000). Heavy metal trends in floodplain sediments and valley fill, River Lahn, Germany. *Catena*, *39*(1), 53-68.

Martin-Laurent, F., Philippot, L., Hallet, S., Chaussod, R., Germon, J. C., Soulas, G., & Catroux, G. (2001). DNA extraction from soils: old bias for new microbial diversity analysis methods. *Applied and environmental microbiology*, 67(5), 2354–2359.

Martinez, M.L., Maun, M.A., Psuty, N.P., 2004. The fragility and conservation of the world's coastal dunes: geomorphological, ecological and socioeconomic per-spectives. In: Martinez, M.L., Psuty, N.P. (Eds.), Coastal Dunes, *Ecology and Conservation*, *Ecological Studies*, vol. 171. Springer-Verlag, Berlin, pp. 355e369.

Matsui, N., Meepol, W., & Chukwamdee, J. (2015). Soil organic carbon in mangrove ecosystems with different vegetation and sedimentological conditions. *Journal of Marine Science and Engineering*, 3(4), 1404–1424.

Mauchamp, A., Blanch, S., & Grillas, P. (2001). Effects of submergence on the growth of *Phragmites australis* seedlings. *Aquatic botany*, 69(2–4), 147–164.

Mayes, W. M., Batty, L. C., Younger, P. L., Jarvis, A. P., Kõiv, M., Vohla, C., & Mander, U. (2009). Wetland treatment at extremes of pH: a review. *Science of the total environment*, 407(13), 3944–3957.

McGill, W. B. (1996). Review and classification of ten soil organic matter (SOM) models. In *Evaluation of soil organic matter models* (pp. 111–132). Springer, Berlin, Heidelberg.

McGrath, S. P., Chaudri, A. M., & Giller, K. E. (1995). Long-term effects of metals in sewage sludge on soils, microorganisms and plants. *Journal of industrial microbiology*, 14(2), 94–104.

Mcleod, E., Chmura, G. L., Bouillon, S., Salm, R., Björk, M., Duarte, C. M., ... & Silliman, B. R. (2011). A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. *Frontiers in Ecology and the Environment*, 9(10), 552–560.

McLusky, D. S. (1987). Intertidal habitats and benthic macrofauna of the Forth estuary, Scotland. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences, 93*(3-4), 389-399.

Medina-Roldán, E., Paz-Ferreiro, J., & Bardgett, R. D. (2012). Grazing exclusion affects soil and plant communities but has no impact on soil carbon storage in an upland grassland. *Agriculture, Ecosystems & Environment*, 149, 118–123.

Mendoza-Vega, J., Karltun, E., & Olsson, M. (2003). Estimations of amounts of soil organic carbon and fine root carbon in land use and land cover classes, and soil types of Chiapas highlands, Mexico. *Forest Ecology and Management*, 177(1–3), 191–206.

Mersey Gateway Delivery Phase Project (2011): Contamination of soils, sediments and groundwater. Widnes, UK: Mersey Gateway Environmental Trust (MGET)

Mersey Gateway Environmental Trust (MGET). (2012). Intertidal, Saltmarsh and Reedbed Habitats. Widnes, UK: Mersey Gateway Environmental Trust (MGET)

Mersey Gateway Environmental Trust (MGET). (2017). Intertidal, Saltmarsh and Reedbed Habitats. Widnes, UK: Mersey Gateway Environmental Trust (MGET)

Mikan, C. J., Schimel, J. P., & Doyle, A. P. (2002). Temperature controls of microbial respiration in arctic tundra soils above and below freezing. *Soil Biology and Biochemistry*, 34(11), 1785–1795.

Min, X. B., Xie, X. D., Chai, L. Y., Liang, Y. J., Mi, L. I., & Yong, K. E. (2013). Environmental availability and ecological risk assessment of heavy metals in zinc leaching residue. *Transactions of Nonferrous Metals Society of China*, *23*(1), 208-218.

Mishra, G. S., Zakerinia, S., Yeh, S., Teter, J., & Morrison, G. (2014). Mitigating climate change: Decomposing the relative roles of energy conservation, technological change, and structural shift. *Energy Economics*, 44, 448–455.

Mitra, S., Wassmann, R., & Vlek, P. L. (2005). An appraisal of global wetland area and its organic carbon stock. *Current Science*, 88(1), 25–35.

Möller, A., Müller, H. W., Abdullah, A., Abdelgawad, G., & Utermann, J. (2005). Urban soil pollution in Damascus, Syria: concentrations and patterns of heavy metals in the soils of the Damascus Ghouta. *Geoderma*, 124(1–2), 63–71.

Mollicone, D., Freibauer, A., Schulze, E. D., Braatz, S., Grassi, G., & Federici, S. (2007). Elements for the expected mechanisms on 'reduced emissions from deforestation and degradation, REDD' under UNFCCC. *Environmental Research Letters*, 2(4), 045024.

Morris, J., & Camino, M. (2011). Economic Assessment of Freshwater, Wetland and Floodplain (FWF) Ecosystem Services. NEA, Cambridge, UK: School of Applied Sciences, Cranfield University

Morrissey, E. M., Gillespie, J. L., Morina, J. C., & Franklin, R. B. (2014). Salinity affects microbial activity and soil organic matter content in tidal wetlands. *Global change biology*, 20(4), 1351–1362.

Mossman, H. L., Davy, A. J., & Grant, A. (2012). Does managed coastal realignment create saltmarshes with 'equivalent biological characteristics' to natural reference sites? *Journal of Applied Ecology*, 49(6), 1446–1456.

Mukwaturi, M., & Lin, C. (2015). Mobilization of heavy metals from urban contaminated soils under water inundation conditions. *J Hazard Mater*, 285, 445–452.

Munoz-Rojas, M., De la Rosa, D., Zavala, L. M., Jordan, A., & Anaya-Romero, M. (2011). Changes in land cover and vegetation carbon stocks in Andalusia, Southern Spain (1956–2007). *Sci Total Environ*, 409(14), 2796–2806.

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental chemistry letters*, 8(3), 199–216.

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., & Renella, G. (2003). Microbial diversity and soil functions. *European journal of soil science*, *54*(4), 655–670.

Nannipieri, P., Ascher-Jenull, J., Ceccherini, M., Giagnoni, L., Pietramellara, G., & Renella, G. (2017). Microbial diversity and soil functions. Reflections by P. Nannipieri, J. Ascher-Jenull, MT Ceccherini, L. Giagnoni, G. Pietramellara & G. Renella (2003). *European Journal of Soil Science, 68*(1), 2-5.

National Research Council (1980). Lead in the Human Environment. Washington, DC: National Academy of Sciences

Natural Environment Research Council (1975). Estuaries research. *Natural Environment Research Council* publication series 'B', No. 9.

Navarro, A., Cardellach, E., & Corbella, M. (2011). Immobilization of Cu, Pb and Zn in mine-contaminated soils using reactive materials. *Journal of hazardous materials*, 186(2–3), 1576–1585.

Nwachukwu, O. I., & Pulford, I. D. (2011). Microbial respiration as an indication of metal toxicity in contaminated organic materials and soil. *Journal of hazardous materials*, 185(2–3), 1140–1147.

Negrin, V. L., Spetter, C. V., Asteasuain, R. O., Perillo, G. M. E., & Marcovecchio, J. E. (2011). Influence of flooding and vegetation on carbon, nitrogen, and phosphorus dynamics in the pore water of a Spartina alterniflora salt marsh. *Journal of Environmental Sciences*, 23(2), 212–221.

Nelson, D. W., & Sommers, L. (1982). Total carbon, organic carbon, and organic matter1. Methods of soil analysis. Part 2. *Chemical and microbiological properties*, (methodsofsoilan2), 539–579.

NEA, 2011. UK National Ecosystem Assessment (2011) The UK National Ecosystem Assessment: *Synthesis of the Key Findings*. UNEP-WCMC, Cambridge.

Neubauer, S. C., Franklin, R. B., & Berrier, D. J. (2013). Saltwater intrusion into tidal freshwater marshes alters the biogeochemical processing of organic carbon. *Biogeosciences*, *10*(12), 8171–8183.

Neumann-Cosel, L., Zimmermann, B., Hall, J. S., van Breugel, M., & Elsenbeer, H. (2011). Soil carbon dynamics under young tropical secondary forests on former pastures—A case study from Panama. *Forest Ecology and Management*, 261(10), 1625–1633.

NRA (1995). The Mersey Estuary: A Report on Environmental Quality. National Rivers

Nworie, O. E., Qin, J., & Lin, C. (2017). Differential effects of low-molecular-weight organic acids on the mobilization of soil-borne arsenic and trace metals. *Toxics*, 5(3), 18.

Nworie, O. E., Qin, J., & Lin, C. (2019). Trace element uptake by herbaceous plants from the soils at a multiple trace element-contaminated site. *Toxics*, 7(1), 3.

Obbard, J. P. (2001). Ecotoxicological assessment of heavy metals in sewage sludge amended soils. *Applied Geochemistry*, 16(11–12), 1405–1411.

Ohya, H., Fujiwara, S., Komai, Y., & Yamaguchi, M. (1988). Microbial biomass and activity in urban soils contaminated with Zn and Pb. *Biology and fertility of soils*, 6(1), 9–13.

Olafisoye, O. B., Adefioye, T., & Osibote, O. A. (2013). Heavy metals contamination of water, soil, and plants around an electronic waste dumpsite. *Pol. J. Environ. Stud*, *22*(5), 1431-1439.

Oliveira, A., & Pampulha, M. E. (2006). Effects of long-term heavy metal contamination on soil microbial characteristics. *Journal of bioscience and bioengineering*, 102(3), 157–161.

Oliveira, A., Pampulha, M. E., Neto, M. M., & Almeida, A. C. (2010). Mercury tolerant diazotrophic bacteria in a long-term contaminated soil. *Geoderma*, 154(3–4), 359–363.

Olsen, Y. S., Dausse, A., Garbutt, A., Ford, H., Thomas, D. N., & Jones, D. L. (2011)**.** Cattle grazing drives nitrogen and carbon cycling in a temperate salt marsh. *Soil Biology and Biochemistry*, 43(3), 531–541.

Olson, G. W. (1981). Soil profile descriptions. In *Soils and the Environment* (pp. 7–40). Springer, Dordrecht.

Otomo, K., Srivastava, J., Zakir, H. M., Mohiuddin, K. M., & Shikazono, N. (2009). Factors controlling chromium release from chromate containing soils. *Int. J. of Appl. Env. Sci*. 4(2), 113–128.

Oorts, K., Bronckaers, H., & Smolders, E. (2006). Discrepancy of the microbial response to elevated copper between freshly spiked and long-term contaminated soils. *Environmental Toxicology and Chemistry*, 25(3), 845–853.

Ordóñez, J. A. B., de Jong, B. H. J., García-Oliva, F., Aviña, F. L., Pérez, J. V., Guerrero, G., . . . Masera, O. (2008). Carbon content in vegetation, litter, and soil under 10 different land-use and land-cover classes in the Central Highlands of Michoacan, Mexico. *Forest Ecology and Management*, 255(7), 2074–2084.

OSPAR. 1998. OSPAR strategy with regard to hazardous substances. Summary Record of the Ministerial Meeting of the OSPAR Commission, Sintra, Portugal, Oslo and Paris Commissions, Vol. OSPAR 98/14/1, Annex 34, 20 pp

Ostle, N. J., Levy, P. E., Evans, C. D., & Smith, P. (2009). UK land use and soil carbon sequestration. *Land Use Policy*, 26, S274–S283.

Pal, D., Broadbent, F. E., & Mikkelsen, D. S. (1975). Influence of temperature on the kinetics of rice straw decomposition in soils. *Soil Science*, 120(6), 442–449.

Panagos, P., Van Liedekerke, M., Yigini, Y., & Montanarella, L. (2013). Contaminated sites in Europe: review of the current situation based on data collected through a European network. *Journal of Environmental and Public Health*, 2013 Article ID 158764, 11 pages http://dx.doi.org/10.1155/2013/158764

Payton, R. W. (1993). Fragipan formation in argillic brown earths (Fragiudalfs) of the Milfield Plain, north-east England. II. Post Devensian developmental processes and the origin of fragipan consistence. *European Journal of Soil Science*, 44(4), 703–723.

Pendergrass, A., & Butcher, D. J. (2006). Uptake of lead and arsenic in food plants grown in contaminated soil from Barber Orchard, NC. *Microchemical Journal*, 83(1), 14–16.

Peng, M., Zi, X., & Wang, Q. (2015). Bacterial community diversity of oil-contaminated soils assessed by high throughput sequencing of 16S rRNA genes. *International journal of environmental research and public health*, 12(10), 12002–12015.

Pezeshki, S. R., & DeLaune, R. D. (2012). Soil oxidation-reduction in wetlands and its impact on plant functioning. *Biology* (Basel), 1(2), 196–221.

Pérez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaeckel, P., & Schloter, M. (2006). Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. *Soil Biology and Biochemistry*, 38(2), 327–341.

Plante, A. F., & Parton, W. J. (2007). The dynamics of soil organic matter and nutrient cycling. In *Soil Microbiology, Ecology and Biochemistry (Third Edition)* (pp. 433–467).

Poirier, N., Sohi, S. P., Gaunt, J. L., Mahieu, N., Randall, E. W., Powlson, D. S., & Evershed, R. P. (2005). The chemical composition of measurable soil organic matter pools. *Organic geochemistry*, 36(8), 1174–1189.

Porter, E., 1973. Pollution in Four Industrialised Estuaries. Four case studies undertaken for the Royal Commission on Environmental Pollution. Her Majesty's Stationery Office, London.

Post, R. D., & Beeby, A. N. (1996). Activity of the microbial decomposer community in metal-contaminated roadside soils. *Journal of Applied Ecology*, 33, 703–709.

Powlson, D. S., Whitmore, A. P., & Goulding, K. W. T. (2011). Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. *European Journal of Soil Science*, 62(1), 42–55.

Prescott, C. E. (2005). Do rates of litter decomposition tell us anything we really need to know? *Forest Ecology and Management*, 220(1–3), 66–74.

Ptistišek, N., Milačič, R., & Veber, M. (2001). Use of the BCR three-step sequential extraction procedure for the study of the partitioning of Cd, Pb and Zn in various soil samples. *Journal of Soils and Sediments*, 1(1), 25–29.

Pumpanen, J., Kolari, P., Ilvesniemi, H., Minkkinen, K., Vesala, T., Niinistö, S., ... & Janssens, I. (2004). Comparison of different chamber techniques for measuring soil CO_2 efflux. *Agricultural and Forest Meteorology*, 123(3), 159–176.

Pye, K., & Blott, S. J. (2014). The geomorphology of UK estuaries: the role of geological controls, antecedent conditions and human activities. *Estuarine, Coastal and Shelf Science*, 150, 196–214.

Quenea, K., Lamy, I., Winterton, P., Bermond, A., & Dumat, C. (2009). Interactions between metals and soil organic matter in various particle size fractions of soil contaminated with waste water. *Geoderma*, 149(3–4), 217–223.

Querol, X., Fernández-Turiel, J., & López-Soler, A. (1995). Trace elements in coal and their behaviour during combustion in a large power station. *Fuel*, 74(3), 331–343.

Rajapaksha, R. M. C. P., Tobor-Kapłon, M. A., & Bååth, E. (2004). Metal toxicity affects fungal and bacterial activities in soil differently. *Appl. Environ. Microbiol.*, 70(5), 2966–2973.

Ranjard, L., Nowak, V., Echairi, A., Faloya, V., & Chaussod, R. (2008). The dynamics of soil bacterial community structure in response to yearly repeated agricultural copper treatments. *Research in microbiology*, 159(4), 251–254.

Rawlins, B., O'Donnell, K., & Ingham, M. (2003). Geochemical survey of the Tamar catchment (south-west England). UK: Environment Agency: British Geological Survey.

Rawlins, A. J., Bull, I. D., Ineson, P., & Evershed, R. P. (2007). Stabilisation of soil organic matter in invertebrate faecal pellets through leaf litter grazing. *Soil Biology and Biochemistry*, 39(5), 1202–1205.

Reddy, K. R., & DeLaune, R. D. (2008). Biogeochemistry of wetlands: science and applications. CRC press.

Ridgway, J., & Shimmield, G. (2002). Estuaries as repositories of historical contamination and their impact on shelf seas. *Estuarine, Coastal and Shelf Science*, 55(6), 903–928.

Ridgway, J., Breward, N., Langston, W. J., Lister, R., Rees, J. G., & Rowlatt, S. M. (2003). Distinguishing between natural and anthropogenic sources of metals entering the Irish Sea. *Applied Geochemistry*, *18*(2), 283-309.

Ridgway, J., Bee, E., Breward, N., Cave, M., Chenery, S., Gowing, C., ... & Jarrow, A. (2012). The Mersey Estuary: sediment geochemistry. Nottingham, UK. *British Geological Survey, Report RR/10/002, 206pp*.

Riesenfeld, C. S., Schloss, P. D., & Handelsman, J. (2004). Metagenomics: genomic analysis of microbial communities. *Annu. Rev. Genet.*, 38, 525–552.

Robson, T. M., Baptist, F., Clément, J. C., & Lavorel, S. (2010). Land use in subalpine grasslands affects nitrogen cycling via changes in plant community and soil microbial uptake dynamics. *Journal of Ecology*, 98(1), 62–73.

Rowlatt, S. M., & Lovell, D. R. (1994). Lead, zinc and chromium in sediments around England and Wales. *Marine Pollution Bulletin*, 28(5), 324–329.

Rühling, Å., & Tyler, G. (1973). Heavy metal pollution and decomposition of spruce needle litter. *Oikos*, 24, 402–416.

Sagova-Mareckova, M., Cermak, L., Novotna, J., Plhackova, K., Forstova, J., & Kopecky, J. (2008). Innovative methods for soil DNA purification tested in soils with widely differing characteristics. *Applied and Environmental Microbiology*, 74(9), 2902–2907.

Salomons, W., De Rooij, N. M., Kerdijk, H., & Bril, J. (1987). Sediments as a source for contaminants? In *Ecological Effects of in situ Sediment Contaminants* (pp. 13–30). Springer, Dordrecht.

Salomons, W., & Förstner, U. (2012). *Metals in the Hydrocycle.* Springer Science & Business Media.

Saltonstall, K. (2002). Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences*, 99(4), 2445–2449.

Sandhu, H. S., Wratten, S. D., & Cullen, R. (2010). Organic agriculture and ecosystem services. *Environmental Science & Policy*, 13(1), 1–7.

Sandroni, V., Smith, C. M., & Donovan, A. (2003). Microwave digestion of sediment, soils and urban particulate matter for trace metal analysis. *Talanta*, 60(4), 715–723.

Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., ... & Smith, M. (1977). Nucleotide sequence of bacteriophage ϕX174 DNA. nature, 265(5596), 687.

Santonen, T., Zitting, A., Riihimäki, V., & Howe, P. D. (2009). Inorganic chromium (III) compounds (Vol. 76). *World Health Organization, Geneva, Switzerland*.

Sauvé, S., McBride, M. B., Norvell, W. A., & Hendershot, W. H. (1997). Copper solubility and speciation of in situ contaminated soils: effects of copper level, pH and organic matter. *Water, Air, and Soil Pollution*, 100(1–2), 133–149.

Scheiter, S., & Higgins, S. I. (2009). Impacts of climate change on the vegetation of Africa: an adaptive dynamic vegetation modelling approach. *Global Change Biology*, 15(9), 2224–2246.

Schnitzer, M., & Preston, C. M. (1983). Effects of acid hydrolysis on the 13 C NMR spectra of humic substances. *Plant and soil*, 75(2), 201–211.

Schuster, S. C. (2007). Next-generation sequencing transforms today's biology. *Nature methods*, 5(1), 16.

Segoli, M., De Gryze, S., Dou, F., Lee, J., Post, W. M., Denef, K., & Six, J. (2013). AggModel: A soil organic matter model with measurable pools for use in incubation studies. *Ecological modelling*, 263, 1–9.

Shaheen, S. M., Rinklebe, J., Rupp, H., & Meissner, R. (2014). Temporal dynamics of pore water concentrations of Cd, Co, Cu, Ni, and Zn and their controlling factors in a contaminated floodplain soil assessed by undisturbed groundwater lysimeters. *Environmental pollution*, 191, 223–231.

Sharp, R., Tallis, H., Ricketts, T., Guerry, A., Wood, S., Chaplin-Kramer, R., . . . Olwero, N. (2014). InVEST user's guide. CA, USA: The Natural Capital Project: Stanford, CA, USA.

Shiau, Y. J., Chen, J. S., Chung, T. L., Tian, G., & Chiu, C. Y. (2017). 13 C NMR spectroscopy characterization of particle-size fractionated soil organic carbon in subalpine forest and grassland ecosystems. *Botanical studies*, 58(1), 23.

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. Molecular ecology, 21(8), 1794–1805.
Change Biology, 10(11), 1878–1883.

Skelcher, G. (2003). *Mersey Estuary saltmarsh vegetation survey 2002*. Carnforth, Lancashire LA5 9PP: English Nature Cheshire to Lancashire Team.

Slater, J., & Capone, D. G. (1984). Effects of metals on nitrogen fixation and denitrification in slurries of anoxic saltmarsh sediment. *Marine ecology progress series. Oldendorf*, 18(1), 89–95.

Smith, P., Smith, J. U., Powlson, D. S., McGill, W. B., Arah, J. R. M., Chertov, O. G., ... & Jensen, L. S. (1997). A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. *Geoderma*, 81(1–2), 153–225.

Smith, K. M., Abrahams, P. W., Dagleish, M. P., & Steigmajer, J. (2009). The intake of lead and associated metals by sheep grazing mining-contaminated floodplain pastures in mid-Wales, UK: I. Soil ingestion, soil–metal partitioning and potential availability to pasture herbage and livestock. *Science of the Total Environment*, 407(12), 3731–3739.

Smith, D. J. (2013). *Changes in Perspectives of the Values and Benefits of Nature* (Unpublished PhD Thesis), University of Salford, Salford.

Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., ... & Herndl, G. J. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences*, 103(32), 12115–12120.

Sneddon, J., Hardaway, C., Bobbadi, K. K., & Reddy, A. K. (2006). Sample preparation of solid samples for metal determination by atomic spectroscopy—an overview and selected recent applications. *Applied Spectroscopy Reviews*, 41(1), 1–14.

Spencer, K. L., & Harvey, G. L. (2012). Understanding system disturbance and ecosystem services in restored saltmarshes: Integrating physical and biogeochemical processes. *Estuarine, Coastal and Shelf Science*, 106, 23–32.

Spurgeon, D. J., Keith, A. M., Schmidt, O., Lammertsma, D. R., & Faber, J. H. (2013). Land-use and land-management change: relationships with earthworm and fungi communities and soil structural properties. *BMC ecology*, 13(1), 46.

Spurgeon, D. J., Ricketts, H., Svendsen, C., Morgan, A. J., & Kille, P. (2005). Hierarchical responses of soil invertebrates (earthworms) to toxic metal stress. *Environmental science & technology*, 39(14), 5327–5334.

Statutory Instrument (1995) The Feeding Stuffs Regulations 1995. Statutory Instrument no. 1412. London: H. M. Stationery Office.

Steinbeiss, S., BEßLER, H. O. L. G. E. R., Engels, C., Temperton, V. M., Buchmann, N., Roscher, C., ... & Gleixner, G. (2008). Plant diversity positively affects short-term soil carbon storage in experimental grasslands. *Global Change Biology*, 14(12), 2937–2949.

Steinbeiss, S., Gleixner, G., & Antonietti, M. (2009). Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology and Biochemistry*, 41(6), 1301–1310.

Stevenson, F. J. (1982). Extraction, fractionation, and general chemical composition of soil organic matter. Humus chemistry. In: F.J. Stevenson *Genesis, Composition, Reactions*, 26–54. John Wiley & Sons: New York.

Stevenson, F. J. (1994). Humus chemistry: Genesis, Composition, Reactions. John Wiley & Sons: New York.

Stevenson, F. J. & Cole M. A. (1999). Cycles of soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrient. John Wiley & Sons: New York.

Stewart, C. E., Paustian, K., Conant, R. T., Plante, A. F., & Six, J. (2009). Soil carbon saturation: Implications for measurable carbon pool dynamics in long-term incubations. *Soil Biology and Biochemistry*, 41(2), 357–366.

Stewart, C. S., Flint, H. J., & Bryant, M. P. (1997). The rumen bacteria. In *The rumen microbial ecosystem* (pp. 10–72). Springer, Dordrecht.

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchi, N., Jenkins, M., . . . Zimmermann, M. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems & Environment*, 164, 80–99.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular ecology*, 21(8), 2045–2050.

Tallis, H. T., et al. "InVEST 2.1 beta user's guide." *Integrated Valuation of Ecosystem Services and Tradeoffs* (2011).

Tang, X., Liu, S., Zhou, G., Zhang, D., & Zhou, C. (2006). Soil-atmospheric exchange of CO_2, CH_4, and N_2O in three subtropical forest ecosystems in southern China. *Global Change Biology*, 12(3), 546–560.

Teixeira, R. F. M., Domingos, T., Costa, A. P. S. V., Oliveira, R., Farropas, L., Calouro, F., . . . Carneiro, J. P. B. G. (2011). Soil organic matter dynamics in Portuguese natural and sown rainfed grasslands. *Ecological Modelling*, 222(4), 993–1001.

Tessier, A., Campbell, P. G., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical chemistry*, 51(7), 844–851.

Thomas, A. D., Dougill, A. J., Elliott, D. R., & Mairs, H. (2014). Seasonal differences in soil CO_2 efflux and carbon storage in Ntwetwe Pan, Makgadikgadi Basin, Botswana. *Geoderma*, 219, 72–81.

Tsai, Y. L., & Olson, B. H. (1991). Rapid method for direct extraction of DNA from soil and sediments. *Applied and environmental microbiology*, 57(4), 1070–1074.

U. O. Edet, S. P. Antai, A. A. Brooks, A. D. Asitok, O. Enya and F. H. Japhet (2017)**:** An Overview of Cultural, Molecular and Metagenomic Techniques in Description of Microbial Diversity. *Journal of Advances in Microbiology* 7(2): 1–19.

Vaccari, F. P., Lugato, E., Gioli, B., D'Acqui, L., Genesio, L., Toscano, P. Miglietta, F. (2012). Land use change and soil organic carbon dynamics in Mediterranean agro-ecosystems: The case study of Pianosa Island. *Geoderma*, 175–176, 29–36.

Van der Eijk, W. (1977). Standards. The Community Bureau of Reference (BCR). In *Proceedings of the Analytical Division of the Chemical Society* (Vol. 14, No. 3, pp. 53–54). Royal Society of Chemistry.

Van der Putten, W. H. (1997)**.** Die-back of *Phragmites australis* in European wetlands: an overview of the European research programme on reed die-back and progression (1993–1994). *Aquatic Botany*, 59(3–4), 263–275.

van der Wal, A., Geydan, T. D., Kuyper, T. W., & De Boer, W. (2013). A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS microbiology reviews*, 37(4), 477–494.

Vane, C. H., Jones, D. G., & Lister, T. R. (2009). Mercury contamination in surface sediments and sediment cores of the Mersey Estuary, UK. *Marine pollution bulletin*, 58(6), 940–946.

Verchot, L. V., Dutaur, L., Shepherd, K. D., & Albrecht, A. (2011). Organic matter stabilization in soil aggregates: understanding the biogeochemical mechanisms that determine the fate of carbon inputs in soils. *Geoderma*, 161(3–4), 182–193.

Vig, K., Megharaj, M., Sethunathan, N., & Naidu, R. (2003). Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. *Advances in Environmental Research*, 8(1), 121–135.

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. G., & Pigna, M. (2010). Mobility and bioavailability of heavy metals and metalloids in soil environments. *Journal of soil science and plant nutrition*, 10(3), 268–292.

von Luetzow, M., Koegel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., & Marschner, B. (2007). SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology & Biochemistry*, 39(9), 2183–2207.

Walker, S. M., & Desanker, P. V. (2004). The impact of land use on soil carbon in Miombo Woodlands of Malawi. *Forest Ecology and Management*, 203(1–3), 345–360.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science*, 37(1), 29–38.

Wallace, R. J. (2008). Gut microbiology–broad genetic diversity, yet specific metabolic niches. Animal, 2(5), 661–668.

Wang, C., Li, W., Guo, M., & Ji, J. (2017). Ecological risk assessment on heavy metals in soils: Use of soil diffuse reflectance mid-infrared Fourier-transform spectroscopy. *Scientific Reports*, 7.

Wang, Y., Shi, J., Wang, H., Lin, Q., Chen, X., & Chen, Y. (2007). The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. *Ecotoxicology and environmental safety*, 67(1), 75–81.

Webster, J. R., & Benfield, E. F. (1986). Vascular plant breakdown in freshwater ecosystems. *Annual review of ecology and systematics*, 17(1), 567–594.

Wedd, C. B., Smith, B., Simmons, W. C., & Wray, D. A. (1923). *The Geology of Liverpool: With Wirral and Part of the Flintshire Coalfield* (No. 96). HM Stationery Office.

West, L. J., Stewart, D. I., Duxbury, J. R., & Johnston, S. R. (1999). Toxic metal mobility and retention at industrially contaminated sites. *Geological Society, London, Special Publications*, 157(1), 241–264.

Whiting, G. J., & Chanton, J. P. (2001). Greenhouse carbon balance of wetlands: methane emission versus carbon sequestration. Tellus B: *Chemical and Physical Meteorology*, 53(5), 521–528.

Wiesenberg, G. L. B., Dorodnikov, M., & Kuzyakov, Y. (2010). Source determination of lipids in bulk soil and soil density fractions after four years of wheat cropping. *Geoderma*, 156(3–4), 267–277.

Wiesmeier, M., Hübner, R., Barthold, F., Spörlein, P., Geuß, U., Hangen, E., . . . Kögel-Knabner, I. (2013). Amount, distribution and driving factors of soil organic carbon and nitrogen in cropland and grassland soils of southeast Germany (Bavaria). *Agriculture, Ecosystems & Environment*, 176, 39–52.

Wiesmeier, M., Lützow, M. v., Spörlein, P., Geuß, U., Hangen, E., Reischl, A., . . . Kögel-Knabner, I. (2015). Land use effects on organic carbon storage in soils of Bavaria: The importance of soil types. *Soil and Tillage Research*, 146, 296–302.

Wiesmeier, M., Prietzel, J., Barthold, F., Spörlein, P., Geuß, U., Hangen, E., . . . Kögel-Knabner, I. (2013). Storage and drivers of organic carbon in forest soils of southeast Germany (Bavaria) – Implications for carbon sequestration. *Forest Ecology and Management*, 295, 162–172.

Wiesmeier, M., Schad, P., von Lützow, M., Poeplau, C., Spörlein, P., Geuß, U., . . . Kögel-Knabner, I. (2014). Quantification of functional soil organic carbon pools for major soil units and land uses in southeast Germany (Bavaria). *Agriculture, Ecosystems & Environment*, 185, 208–220.

Wickings, K., Grandy, A. S., Reed, S. C., & Cleveland, C. C. (2012). The origin of litter chemical complexity during decomposition. *Ecology letters*, 15(10), 1180–1188.

Wilfinger, W. W., Mackey, K., & Chomczynski, P. (1997). PCR-based method for isolation of full-length clones and splice variants from cDNA libraries. *BioTechniques*, 22(3), 481–486.

Williams, T. P., Bubb, J. M., & Lester, J. N. (1994). Metal accumulation within salt marsh environments: a review. *Marine pollution bulletin*, 28(5), 277–290.

Wilkinson, J. M., Hill, J., & Phillips, C. J. C. (2003). The accumulation of potentially-toxic metals by grazing ruminants. *Proceedings of the Nutrition Society*, *62*(2), 267–277.

Wilson, G. P., Lamb, A. L., Leng, M. J., Gonzalez, S., & Huddart, D. (2005). Variability of organic δ13C and C/N in the Mersey Estuary, UK and its implications for sea-level reconstruction studies. *Estuarine, Coastal and Shelf Science*, 64(4), 685–698.

Wilson, G. P., Lamb, A. L., Leng, M. J., Gonzalez, S., & Huddart, D. (2005). δ13C and C/N as potential coastal palaeoenvironmental indicators in the Mersey Estuary, UK. *Quaternary Science Reviews*, 24(18–19), 2015–2029.

Windham, L., & Lathrop, R. G. (1999)**.** Effects of *Phragmites australis* (common reed) invasion on aboveground biomass and soil properties in brackish tidal marsh of the Mullica River, New Jersey. *Estuaries*, 22(4), 927–935.

World reference base for soil resources 2014, update 2015: International soil classification system for naming soils and creating legends for soil maps. *World Soil Resources Reports No. 106*, 192. Rome.

Woo, P. C. Y., Lau, S. K. P., Teng, J. L. L., Tse, H., & Yuen, K. Y. (2008). Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clinical Microbiology and Infection*, 14(10), 908–934.

Wu, C.-Y., Chen, Y., Wang, J.-Y., & Wang, S.-J. (2006). Estimation of Turnover and Equilibrium of Soil Organic Matter Using a Mathematical Approach. *Pedosphere*, 16(5), 634–645.

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *Isrn Ecology*, 2011. 1–20 http://dx.doi.org/10.5402/2011/402647

Xiong, X., Grunwald, S., Myers, D. B., Ross, C. W., Harris, W. G., & Comerford, N. B. (2014). Interaction effects of climate and land use/land cover change on soil organic carbon sequestration. *Science of the Total Environment*, 493, 974–982.

Yadav, V., & Malanson, G. (2008). Spatially explicit historical land use land cover and soil organic carbon transformations in Southern Illinois. *Agriculture, Ecosystems & Environment*, 123(4), 280–292.

Yan, J., Quan, G., & Ding, C. (2013). Effects of the combined pollution of lead and cadmium on soil urease activity and nitrification. *Procedia Environmental Sciences*, 18, 78–83.

Yang, Z. H., Singh, B. R., & Sitaula, B. K. (2004). Soil organic carbon fractions under different land uses in Mardi watershed of Nepal. *Communications in soil science and plant analysis*, 35(5–6), 615–629.

Yanqun, Z., Yuan, L., Schvartz, C., Langlade, L., & Fan, L. (2004). Accumulation of Pb, Cd, Cu and Zn in plants and hyperaccumulator choice in Lanping lead–zinc mine area, China. *Environment International*, 30(4), 567–576.

Yeates, G. W., Orchard, V. A., Speir, T. W., Hunt, J. L., & Hermans, M. C. C. (1994). Impact of pasture contamination by copper, chromium, arsenic timber preservative on soil biological activity. *Biology and Fertility of soils*, 18(3), 200–208.

Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the total environment*, 368(2–3), 456–464.

Yuesi, W., & Yinghong, W. (2003). Quick measurement of CH_4, CO_2 and N_2O emissions from a short-plant ecosystem. *Advances in Atmospheric Sciences*, 20(5), 842–844.

Zedler, J. B., & Kercher, S. (2005). Wetland resources: status, trends, ecosystem services, and restorability. *Annu. Rev. Environ. Resour.*, *30*, 39-74.

Zhang, C., Yu, Z. G., Zeng, G. M., Jiang, M., Yang, Z. Z., Cui, F., . . . Hu, L. (2014). Effects of sediment geochemical properties on heavy metal bioavailability. *Environ Int*, 73, 270–281.

Zhang, W., Ricketts, T. H., Kremen, C., Carney, K., & Swinton, S. M. (2007)**.** Ecosystem services and dis-services to agriculture. *Ecological Economics*, 64(2), 253–260.

Zhang, C., Nie, S., Liang, J., Zeng, G., Wu, H., Hua, S., ... & Xiang, H. (2016). Effects of heavy metals and soil physicochemical properties on wetland soil microbial biomass and bacterial community structure. *Science of the Total Environment*, 557, 785–790.

Zwoliński, J. (1994). Rates of organic matter decomposition in forests polluted with heavy metals. *Ecological Engineering*, 3(1), 17–26.

Zwolinski, M. D. (2007). DNA sequencing: strategies for soil microbiology. *Soil Science Society of America Journal*, *71*(2), 592–600.

Appendices

Appendix 1: Selected physical and chemical properties under different land uses/land covers

		0–15 cm	0–15 cm	0–15 cm	15–30 cm	15–30 cm	15–30 cm	0–15 cm	15–30 cm	
Location	Samples collected	pH	Eh(mV)	EC (mS/cm)	pH	Eh(mV)	EC (mS/cm)	SOC (%)	SOC (%)	Bulk Density g/cm3
GSM1	1	7.6	-37	7.04	7.7	-47.5	3.35	7.34	6.51	0.37
GSM2	1	7.7	-47.6	4.60	7.5	-35.4	3.59	7.64	6.71	0.87
GSM3	1	8.3	-78.4	2.12	8.1	-67.4	1.62	6.54	3.92	1.07
GSM-N1	2	7.4	-31.4	6.64	7.1	-6.0	3.82	12.43	8.17	1.01
GSM-N2	2	7.7	-45.2	3.21	7.6	-33.9	2.09	7.77	4.31	1.02
GSM-N3	2	7.7	-45.2	3.21	7.6	-33.9	2.09	7.77	4.31	1.02
NG1	3	7.9	-59.3	0.141	8.1	-75.7	0.16	3.89	4.96	1.21
NG2	3	8.2	-74.2	0.175	8.4	-89.1	0.15	10.90	4.61	0.89
NG3	3	5.3	103.1	0.06	5.9	82.9	0.03	6.90	5.79	0.78
RB 1	4	6.0	57.8	0.20	7.2	-13.1	0.24	8.31	3.64	0.23
RB 2	4	7.7	-44.5	1.50	8.2	-72.1	1.24	6.54	3.17	0.63
RB 3	4	7.9	-54.4	0.99	7.9	-52.8	1.01	8.27	3.52	0.61
RG1	5	7.1	1.7	0.08	7.2	-10.8	0.08	5.55	2.44	0.53
RG2	5	6.7	23.8	0.04	6.99	7.5	0.03	3.44	4.16	0.31
RG3	5	7.0	1.1	0.06	7.2	3.3	0.05	2.91	4.07	0.25
SM1 1	6	5.5	61.3	7.20	6.9	10.0	5.14	10.14	6.19	-
SM1 2	6	7.7	-40.1	3.78	7.7	-41.6	2.76	7.25	3.72	-
SM1 3	6	6.7	16.8	7.34	6.8	9.8	4.25	10.77	8.84	-
SM2 1	7	8.1	-65.1	0.91	7.9	-56.0	1.21	7.61	1.49	-
SM2 2	7	8.0	-64.2	2.65	8.0	-60.1	2.52	6.86	0.95	-
SM2 3	7	8.3	-80.5	2.32	8.2	-78.0	2.30	10.85	8.92	-
WD1 1	8	7.2	-6.3	96.40	7.5	-23.4	0.15	8.21	2.69	-
WD1 2	8	8.1	-69.6	0.20	7.7	-65.2	0.19	6.40	3.45	-
WD1 3	8	7.9	-50.1	0.28	8.3	-82.9	0.19	6.32	1.70	-
WD2 1	9	7.2	-8.3	0.04	8.0	-60.3	0.06	11.14	5.67	-
WD2 2	9	5.5	90.5	0.39	6.5	34.8	0.13	8.58	5.29	-
WD2 3	9	4.6	141.1	0.07	4.4	151.8	0.08	4.10	2.92	-

Appendix 2: Heavy metal distribution at 0-15 cm depth under different land uses/land covers

		Arsenic	Cadmium	Chromium	Copper	Lead	Zinc
Soil Samples		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
GSM1	1	44.25	0.00	93.60	80.75	112.10	367.60
GSM2	1	52.05	31.50	126.10	87.15	126.10	413.40
GSM3	1	4.60	2.28	0.55	94.00	110.20	11.90
GSM-N1	2	37.55	0.00	77.40	80.40	76.35	334.80
GSM-N2	2	33.55	0.00	83.35	46.10	75.70	292.40
GSM-N3	2	33.55	0.00	83.35	46.10	75.70	292.40
NGD1	3	27.90	8.11	47.60	47.25	125.90	127.90
NGD2	3	40.65	0.00	63.40	75.35	194.75	219.70
NGD3	3	44.30	0.00	52.50	48.25	70.95	150.90
RB1	4	63.85	0.00	67.35	180.75	212.30	172.75
RB2	4	94.40	0.00	198.30	243.05	303.50	829.35
RB3	4	49.10	0.00	147.20	119.10	193.60	524.10
RGD1	5	28.80	0.00	21.70	26.10	34.30	102.70
RGD2	5	50.75	0.00	39.30	51.20	96.15	108.00
RGD3	5	50.60	0.00	37.55	84.25	112.30	141.15
SM1 1	6	40.50	0.00	148.75	109.95	143.55	1975.15
SM1 2	6	40.70	17.15	122.70	122.75	131.00	422.55
SM1 3	6	23.05	0.00	78.05	49.80	63.00	212.85
SM2 1	7	34.40	0.00	87.40	70.95	114.60	1179.90
SM2 2	7	49.55	0.00	191.05	81.75	146.15	392.85
SM2 3	7	34.20	0.00	70.60	38.45	59.15	282.90
WD1 1	8	39.05	0.00	66.50	52.60	62.80	167.90
WD1 2	8	135.90	0.00	202.55	147.10	305.50	263.35
WD1 3	8	48.45	0.00	46.50	59.30	145.05	168.40
WD2 1	9	28.90	0.00	29.35	11.40	14.40	93.90
WD2 2	9	25.55	0.00	30.50	38.05	616.40	110.50
WD2 3	9	43.75	0.00	14.30	23.95	62.80	94.20

0.00= under detection limit

Appendix 3: Heavy metal distribution at 15-30 cm under different land uses/land covers

		Arsenic	Cadmium	Chromium	Copper	Lead	Zinc
Soil Samples		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
GSM1	1	48.75	0.00	122.70	170.25	180.45	547.60
GSM2	1	63.45	0.00	144.60	296.60	282.75	694.80
GSM3	1	103.55	0.00	221.65	356.00	363.70	975.65
GSM-N1	2	104.00	0.00	306.95	267.90	370.05	1029.40
GSM-N2	2	38.90	0.00	83.20	64.70	101.35	358.55
GSM-N3	2	38.90	0.00	83.20	64.70	101.35	358.55
NGD1	3	48.60	0.00	77.05	70.45	155.10	213.60
NGD2	3	26.05	27.41	43.35	52.15	87.85	135.85
NGD3	3	40.60	0.00	35.60	31.15	48.40	123.05
RB1	4	7.40	0.00	0.00	0.00	0.00	377.05
RB2	4	82.15	0.00	175.40	225.45	240.70	844.75
RB3	4	36.20	0.59	89.25	104.35	123.20	389.90
RGD1	5	28.55	0.00	53.30	33.45	38.75	90.25
RGD2	5	41.90	0.00	43.55	61.30	100.50	99.95
RGD3	5	42.65	0.00	28.80	69.95	83.15	125.25
SM1 1	6	46.65	0.00	96.75	146.85	173.30	535.35
SM1 2	6	59.60	0.63	187.35	189.95	207.80	652.80
SM1 3	6	44.25	3.54	89.35	151.40	154.70	453.65
SM2 1	7	104.25	0.00	193.15	242.30	305.30	1897.10
SM2 2	7	59.35	0.00	118.95	121.70	161.80	425.15
SM2 3	7	27.60	0.00	54.45	30.95	45.40	223.00
WD1 1	8	46.70	0.00	61.20	75.75	89.95	198.10
WD1 2	8	55.10	0.00	81.90	158.05	445.50	304.25
WD1 3	8	89.40	0.00	67.15	81.50	211.85	254.30
WD2 1	9	35.50	0.00	21.25	16.25	10.95	87.05
WD2 2	9	66.95	0.00	50.75	58.55	120.60	157.25
WD2 3	9	55.70	0.00	39.70	140.50	145.25	103.25

0.00= under detection limit

Appendix 4: Heavy metal distribution in roots portion under different land uses/land covers

Roots									
Samples		Arsenic	Cadmium	Chromium	Copper	Iron	Manganese	Lead	Zinc
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/g
GSM1	1	25.54	6.03	27.29	65.59	7572.80	365.37	65.68	219.29
GSM2	1	15.95	4.87	9.99	33.69	5841.21	439.06	31.82	126.07
GSM3	1	5.89	3.99	6.63	20.73	562.77	115.56	9.31	75.71
GSM-N1	2	8.07	9.3	6.42	33.36	779.37	49.94	13.92	219.03
GSM-N2	2	4.90	4.43	5.54	19.67	249.56	40.22	6.33	92.77
GSM-N3	2	4.90	4.43	5.54	19.67	249.56	40.22	6.33	92.77
NGD1	3	5.48	4.07	7.31	25.17	1006.10	99.86	40.55	54.09
NGD2	3	5.01	4.5	4.78	22.00	208.21	31.86	10.57	54.52
NGD3	3	5.93	5.08	6.33	14.92	655.80	348.08	21.77	65.33
RB1	4	4.67	4.26	4.54	8.68	68.46	33.64	6.3	16.99
RB2	4	5.07	4.97	5.84	18.18	213.10	50.60	6.62	116.2
RB3	4	4.72	4.97	5.28	14.36	117.31	57.95	5.29	112.19
RGD1	5	6.13	4.41	5.78	16.94	1454.20	89.86	14.48	37.77
RGD2	5	6.47	4.78	8.12	20.19	559.71	82.75	21.46	36.99
RGD3	5	5.62	4.15	4.74	11.83	103.64	12.97	8.03	22.14
SM1 1	6	11.54	5.03	11.37	36.02	4254.81	429.28	31.91	162.49
SM1 2	6	4.90	4.70	4.97	18.87	166.77	32.15	5.7	72.41
SM1 3	6	44.04	5.12	16.25	54.43	17048.45	377.21	120.73	202.97
SM2 1	7	4.93	5.15	5.36	14.41	206.51	39.37	6.01	280.41
SM2 2	7	4.85	4.33	5.46	15.15	275.19	52.44	6.86	63.02
SM2 3	7	5.29	3.97	8.00	26.26	506.50	64.44	8.94	83.25
WD1	8	4.65	4.30	4.51	10.00	123.12	27.61	6.51	37.52
WD2	8	0	0	0	0	0	0	0	0
WD3	8	0	0	0	0	0	0	0	0
WD2 1	9	5.14	3.87	5.03	13.33	384.89	69.71	8.95	36.13
WD2 2	9	0	0	0	0	0	0	0	0
WD2 3	9	0	0	0	0	0	0	0	0

Appendix 5: Heavy metal distribution in shoots portion under different land uses/land covers

Shoots								
Samples	Arsenic	Cadmium	Chromium	Copper	Iron	Manganese	Lead	Zinc
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/g
GSM1	5.52	3.96	10.07	12.95	1314.11	290.91	10.89	56.79
GSM2	4.96	3.82	7.21	8.81	689.97	284.36	7.46	27.25
GSM3	6.28	3.92	15.39	18.00	2876.42	273.25	20.71	104.18
GSM-N1	8.42	4.22	27.92	31.22	6645.30	583.71	47.39	159.38
GSM-N2	4.83	3.97	8.81	9.38	515.04	44.10	7.49	35.03
GSM-N3	4.83	3.97	8.81	9.38	515.04	44.10	7.49	35.03
NGD1	4.76	4.25	5.78	12.10	330.86	43.24	12.79	43.30
NGD2	4.45	4.16	4.85	12.57	72.42	16.20	5.40	37.85
NGD3	4.4	3.96	5.28	12.94	141.87	41.27	5.77	25.08
RB1	4.51	4.21	4.49	8.17	57.80	92.04	4.72	27.46
RB2	4.49	4.34	4.54	11.60	71.48	79.83	4.64	103.61
RB3	4.69	4.20	5.63	13.45	96.42	88.20	5.08	87.64
RGD1	4.82	4.46	5.36	9.14	174.44	63.86	6.46	30.24
RGD2	4.6	4.45	4.35	9.47	52.10	12.35	5.13	17.18
RGD3	4.59	4.34	4.45	7.48	63.67	47.97	5.41	17.57
SM1 1	4.68	3.81	5.65	9.06	365.60	127.14	6.02	27.87
SM1 2	6.42	3.82	15.31	21.25	3323.20	373.335	25.99	130.50
SM1 3	4.85	4.15	8.08	10.27	377.31	76.76	7.03	27.34
SM2 1	4.44	4.49	4.90	9.01	77.49	21.24	4.93	128.33
SM2 2	4.37	4.27	4.38	7.36	59.49	14.21	4.62	28.36
SM2 3	4.50	4.47	5.62	18.82	84.34	43.10	4.90	122.65
WD1 1	4.58	4.38	4.66	10.32	151.42	35.73	5.44	47.90
WD1 2	6.65	5.26	6.15	32.13	1013.65	221.54	26.72	245.32
WD1 3	5.71	4.38	5.45	20.11	389.09	52.60	15.16	68.70
WD2 1	4.81	4.65	5.09	9.91	265.69	384.74	10.04	43.38
WD2 2	5.90	8.12	5.16	20.31	337.11	574.29	16.40	421.68
WD2 3	4.72	4.35	4.64	16.44	197.45	455.43	13.56	101.26

Appendix 6: Showing soil profile characteristic at grazing land use

Elevation 10m

Described by: Osim Enya

Profile position: Valley bottom

Location: Upper Mersey estuary, Northwest England (SJ 52917 BNG 85058)

Drainage: poorly drain

Sandstone parent material

Horizon	Depth (cm)	Colour (moist)	Description
A	0-25	2.5Y 4/2	sandy, dark grayish brown, medium sub-angular blocky, friable, slightly sticky (moist) and slightly plastic (wet), no inclusion and many fine roots with clearly smooth boundary
AB	25-30	2.5Y 4/2	sandy, dark grayish brown, angular blocky, friable, slightly sticky (moist) and slightly plastic wet), no inclusion and many fine roots with clearly smooth boundary
Btg1	30-40	2.5Y 2.5/1	sandy, black, angular blocky, friable, sticky (moist) and slightly plastic wet), no inclusion and few fine roots with clearly Smooth boundary
Btg2	40-70	2.5Y 2.5/3	sandy, dark olive brown, angular blocky, very firm, slightly sticky (moist) and plastic (wet), Fe & Mn mottles, no roots clearly smooth boundary
BC	70-100	2.5Y 2.5/3	sandy, dark olive brown, angular blocky, very firm, slightly sticky (moist) and slightly plastic (wet), Fe & Mn mottles, clearly smooth boundary

Appendix 7: Showing soil profile characteristic at non-grazing land use

Elevation 1m

Described by: Osim Enya

Profile position: Valley bottom

Location: Upper Mersey estuary, Northwest England (SJ 52759 BNG 84975)

Drainage: poorly drain

Sandstone parent material

Horizon	Depth (cm)	Colour (moist)	Description
A	0-20	2.5Y 4/1	sandy, dark gray, medium sub-angular blocky, friable, slightly sticky (moist) and slightly plastic (wet), no inclusion and many fine roots with clearly smooth boundary
AB	20-39	2.5Y 2.5/1	sandy, black, angular blocky, friable, slightly sticky (moist) and slightly plastic wet), no inclusion and many fine roots with clearly smooth boundary
Btg1	39-55	2.5Y 5/2	sandy, grayish brown angular blocky, firm, slightly sticky (moist) and slightly plastic wet), mottles and few fine roots with clearly Smooth boundary
Btg2	55-70	2.5Y 5/2	loamy sand, grayish brown, angular blocky, very firm, slightly sticky (moist) and slightly plastic wet), mottles and few fine roots with clearly Smooth boundary
BC	70-100	2.5Y 5/2	sandy, grayish brown, angular blocky, very firm, slightly sticky (moist) and slightly plastic (wet), Fe & Mn mottles, clearly smooth boundary

Appendix 8: Showing soil profile characteristic of reed beds land cover

Elevation 8m

Described by: Osim Enya

Profile position: Valley bottom

Location: Upper Mersey estuary, Northwest England (SJ 52851 BNG 85046)

Drainage: poorly drain

Sandstone parent material

Horizon	Depth (cm)	Colour (moist)	Description
A	0-38	2.5Y 6/1	sandy, gray, medium sub-angular blocky, friable, slightly sticky (moist) and slightly plastic (wet) no inclusion and many fine roots with clearly smooth boundary
AB	38-46	2.5Y 6/1	sandy, black, angular blocky, friable, slightly sticky (moist) and slightly plastic wet), no inclusion and many fine roots with clearly smooth boundary
Btg1	46-54	2.5Y 6/4	sandy, grayish brown angular blocky, firm, slightly sticky (moist) and slightly plastic wet), and few fine roots with clearly Smooth boundary
Btg2	54-70	2.5Y 6/4	loamy sand, grayish brown, angular blocky, very firm, slightly sticky (moist) and slightly plastic wet), mottles and few fine roots with clearly Smooth boundary
BC	70-100	2.5Y 6/4	sandy, grayish brown, angular blocky, very firm, slightly sticky (moist) and slightly plastic (wet), Fe & Mn mottles, clearly smooth boundary

Appendix 9: Calculation steps to determine the textural classes from the Hydrometer method of particle size analysis

	H1 (A)	T1 20-19 (B)	H2 (C)	T2 20-20 (D)	CF T<20°C (E)	CF Wt 100/50 (F)	CF H1 (G) (A-E)	CF H2 (H)	Silt+Clay (I) (A+G) *C	Clay (J) (C+H) *F	Silt K (I-J)	Sand L (M-K-J)	K Constant	Texture
P1 0-20 cm	12	1	2	0	0.3	2	0.7	0.3	13.4	2.6	10.8	86.6	100	Sand
P1 20-39 cm	10	1	2	0	0.3	2	0.7	0.3	11.4	2.6	8.8	88.6	100	Sand
P1 39-55 cm	16	1	4	0	0.3	2	0.7	0.3	18.8	4.6	14.2	81.2	100	Sand
P1 55-70 cm	22	1	12	0	0.3	2	0.7	0.3	30.4	12.6	17.8	69.6	100	Loamy sand
P1 70-100 cm	18	1	6	0	0.3	2	0.7	0.3	22.2	6.6	15.6	77.8	100	Sand
P2 0-38 cm	14	1	3	0	0.3	2	0.7	0.3	16.1	3.6	12.5	83.9	100	Sand
P2 38-46 cm	14	1	3	0	0.3	2	0.7	0.3	16.1	3.6	12.5	83.9	100	Sand
P2 46-54 cm	20	1	6	0	0.3	2	0.7	0.3	24.2	6.6	17.6	75.8	100	Sand
P2 54-70 cm	22	1	6	0	0.3	2	0.7	0.3	26.2	6.6	19.6	73.8	100	Sand
P2 70-100 cm	22	1	6	0	0.3	2	0.7	0.3	26.2	6.6	19.6	73.8	100	Sand
P3 0-25 cm	14	1	2	0	0.3	2	0.7	0.3	15.4	2.6	12.8	84.6	100	Sand
P3 25-30 cm	8	1	2	0	0.3	2	0.7	0.3	9.4	2.6	6.8	90.6	100	Sand
P3 30-40 cm	10	1	3	0	0.3	2	0.7	0.3	12.1	3.6	8.5	87.9	100	Sand
P3 40-70 cm	18	1	4	0	0.3	2	0.7	0.3	20.8	4.6	16.2	79.2	100	Sand
P3 70-100 cm	18	1	6	0	0.3	2	0.7	0.3	22.2	6.6	15.6	77.8	100	Sand

Appendix 10: Showing soil chemical properties at the three profile pits (P1, P2 and P3)

		P1			P2			P3	
	pH	Eh mV	EC mS/cm	pH	Eh mV	EC mS/cm	pH	Eh mV	EC mS/cm
1	7.6	-29.2	3.56	7.5	-26.4	2.02	6.8	8.1	2.34
1	7.6	-29.0	3.66	7.5	-28.2	2.12	6.72	16.1	2.54
1	7.6	-29.1	3.6	7.6	-30.2	2.13	6.87	4.2	2.54
2	6.8	-10.7	3.36	7.1	-10.8	1.7	6.82	5.7	2.43
2	6.9	-11.4	3.42	7.0	-8.1	1.74	6.91	1.3	2.45
2	7.0	-24.0	3.42	7.09	-10.5	1.74	6.93	1.8	2.49
3	6.8	-1.7	1.96	7.09	-8.8	1.34	6.88	2.7	2.27
3	7.0	-9.7	2.00	7.09	-8.8	1.34	6.88	2.4	2.32
3	7.0	-19.7	2.00	7.10	-10.5	1.35	6.94	-0.4	2.32
4	7.3	-11.4	1.98	7.31	-21.5	1.41	6.86	2.5	1.65
4	7.2	-13.3	2.03	7.39	-23.4	1.43	6.90	1.6	1.64
4	7.2	-17.7	2.03	7.09	-9.3	1.43	6.97	-2.4	1.65
5	7.2	-12.4	2.27	6.95	-5.3	1.24	6.83	4.6	1.34
5	7.2	-14.2	2.31	6.95	-1.7	1.28	6.91	2.2	1.36
5	7.2	-14.7	2.31	6.85	-4.0	1.28	6.96	-1.8	1.36

Appendix 11: Seasonal pH data under different land uses/land covers

		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
		0–15	0–15	0–15	0–15	15–30	15–30	15–30	15–30
GSM1 1	1	7.5	7.1	6.9	7.7	7.7	7.6	8.1	8.0
GSM1 2	1	7.5	7.1	6.9	7.7	7.7	7.6	8.1	8.0
GSM1 3	1	7.5	7.1	6.9	7.7	7.7	7.6	8.1	8.0
GSM2 1	2	7.4	7.3	7.3	7.7	7.7	7.6	7.7	8.0
GSM2 2	2	7.4	7.3	7.3	7.7	7.7	7.6	7.7	8.0
GSM2 3	2	7.4	7.3	7.3	7.7	7.7	7.6	7.7	8.0
GSM3 1	3	7.1	7.3	7.3	7.8	7.1	7.7	7.7	8.1
GSM3 2	3	7.1	7.3	7.3	7.8	7.1	7.7	7.7	8.1
GSM3 3	3	7.1	7.3	7.3	7.8	7.1	7.7	7.7	8.1
GSM-N1 1	4	7.7	7.3	7.4	7.5	7.2	7.3	7.3	7.4
GSM-N1 2	4	7.7	7.3	7.4	7.5	7.2	7.3	7.3	7.4
GSM-N1 3	4	7.7	7.3	7.4	7.5	7.2	7.3	7.3	7.4
GSM-N2 1	5	7.7	7.3	7.6	8.0	7.4	7.4	7.7	8.1
GSM-N2 2	5	7.7	7.3	7.6	8.0	7.4	7.4	7.7	8.1
GSM-N2 3	5	7.7	7.3	7.6	8.0	7.4	7.4	7.7	8.1
GSM-N3 1	6	7.6	7.5	7.3	7.4	7.2	7.3	7.8	7.5
GSM-N3 2	6	7.6	7.5	7.3	7.4	7.2	7.3	7.8	7.5
GSM-N3 3	6	7.6	7.5	7.3	7.4	7.2	7.3	7.8	7.5
RB1 1	7	7.5	7.4	7.2	8.0	7.8	7.4	7.2	7.7
RB1 2	7	7.5	7.4	7.2	8.0	7.8	7.4	7.2	7.7
RB1 3	7	7.5	7.4	7.2	8.0	7.8	7.4	7.2	7.7
RB2 1	8	7.8	7.6	7.6	8.2	7.6	7.8	7.8	7.8
RB2 2	8	7.8	7.6	7.6	8.2	7.6	7.8	7.8	7.8
RB2 3	8	7.8	7.6	7.6	8.2	7.6	7.8	7.8	7.8
RB3 1	9	8.2	7.9	7.7	8.3	8.1	8.1	7.9	8.0
RB3 2	9	8.2	7.9	7.7	8.3	8.1	8.1	7.9	8.0
RB3 3	9	8.2	7.9	7.7	8.3	8.1	8.1	7.9	8.0

Appendix 12: Seasonal Eh (mV) data under different land uses/land covers

		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
		0–15	0–15	0–15	0–15	15–30	15–30	15–30	15–30
GSM1 1	1	-29.9	-9	-1.2	-41.4	-39.6	-36.8	-63.1	-56.6
GSM1 2	1	-30.4	-9	-1.2	-41.4	-39.6	-36.8	-63.1	-56.6
GSM1 3	1	-30.9	-9	-1.2	-41.4	-39.6	-36.8	-63.1	-56.6
GSM2 1	2	-2.9	-20.9	-21.8	-43.5	-39.8	-37.7	-41.4	-54.2
GSM2 2	2	-30.2	-20.9	-21.8	-43.5	-39.8	-37.7	-41.4	-54.2
GSM2 3	2	-31.7	-20.9	-21.8	-43.5	-39.8	-37.7	-41.4	-54.2
GSM3 1	3	-8.2	-18.9	-17.4	-500	-9.2	-40.5	-39.9	-64.4
GSM3 2	3	-9.6	-18.9	-17.4	-500	-9.2	-40.5	-39.9	-64.4
GSM3 3	3	10.2	-18.9	-17.4	-500	-9.2	-40.5	-39.9	-64.4
GSM-N1 1	4	-42.8	-17.9	-25.7	-31.9	-15.1	-19.4	-17.9	-24.6
GSM-N1 2	4	-42.8	-17.9	-25.7	-31.9	-15.1	-19.4	-17.9	-24.6
GSM-N1 3	4	-42.8	-17.9	-25.7	-31.9	-15.1	-19.4	-17.9	-24.6
GSM-N2 1	5	-43.8	-19.6	-37.2	-60.2	-25.9	-23.3	-43.7	-65.2
GSM-N2 2	5	-43.8	-19.6	-37.2	-60.2	-25.9	-23.3	-43.7	-65.2
GSM-N2 3	5	-43.8	-19.6	-37.2	-60.2	-25.9	-23.3	-43.7	-65.2
GSM-N3 1	6	-36.7	-29.9	-21.4	-25.6	-15.8	-24.1	-50.1	-29.3
GSM-N3 2	6	-36.7	-29.9	-21.4	-25.6	-15.8	-24.1	-50.1	-29.3
GSM-N3 3	6	-36.7	-29.9	-21.4	-25.6	-15.8	-24.1	-50.1	-29.3
RB1 1	7	-28.2	-25.2	-14.7	-57.4	-47.5	-23.4	-12.7	-39.9
RB1 2	7	-28.2	-25.2	-14.7	-57.4	-47.5	-23.4	-12.7	-39.9
RB1 3	7	-28.2	-25.2	-14.7	-57.4	-47.5	-23.4	-12.7	-39.9
RB2 1	8	-40.4	-35.2	-37.2	-68.7	-35.8	-47.8	-46.6	-47.9
RB2 2	8	-40.4	-35.2	-37.2	-68.7	-35.8	-47.8	-46.6	-47.9
RB2 3	8	-40.4	-35.2	-37.2	-68.7	-35.8	-47.8	-46.6	-47.9
RB3 1	9	-73.6	-54.8	-43.2	-73.6	-62.3	-62.1	-52.6	-58.8
RB3 2	9	-73.6	-54.8	-43.2	-73.6	-62.3	-62.1	-52.6	-58.8
RB3 3	9	-73.6	-54.8	-43.2	-73.6	-62.3	-62.1	-52.6	-58.8

Appendix 13: Seasonal EC (mS/cm) data under different land uses/land covers

		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
		0–15	0–15	0–15	0–15	15–30	15–30	15–30	15–30
GSM1 1	1	0.89	4.39	2.59	1.09	1.14	2.25	1.69	1.96
GSM1 2	1	0.90	4.41	2.59	1.10	1.14	2.26	1.70	1.95
GSM1 3	1	0.89	4.45	2.59	1.10	1.14	2.26	1.70	1.94
GSM2 1	2	2.29	4.60	4.23	3.11	3.04	2.93	2.67	2.83
GSM2 2	2	2.30	4.58	4.26	3.09	3.05	2.92	2.69	2.87
GSM2 3	2	2.30	4.56	4.23	3.09	3.02	2.91	2.67	2.87
GSM3 1	3	4.70	6.87	3.11	2.73	4.80	2.73	4.47	2.05
GSM3 2	3	4.80	6.88	3.12	2.74	4.87	2.79	4.44	2.07
GSM3 3	3	4.85	6.85	3.11	2.75	4.92	2.79	4.45	2.08
GSM-N1 1	4	0.78	5.48	3.12	1.69	1.84	3.20	3.06	2.30
GSM-N1 2	4	0.78	5.48	3.14	1.70	1.86	3.19	3.09	2.30
GSM-N1 3	4	0.78	5.44	3.15	1.69	1.85	3.18	3.14	2.31
GSM-N2 1	5	1.35	6.32	4.39	1.77	2.45	3.57	2.99	1.93
GSM-N2 2	5	1.35	6.35	4.40	1.77	2.60	3.51	2.99	1.92
GSM-N2 3	5	1.35	6.32	4.42	1.77	2.51	3.41	2.99	1.92
GSM-N3 1	6	1.59	6.05	3.08	2.44	1.80	3.09	3.57	2.97
GSM-N3 2	6	1.57	6.03	3.10	2.46	1.81	3.11	3.57	2.99
GSM-N3 3	6	1.57	6.03	3.12	2.48	1.80	3.15	3.56	3.00
RB1 1	7	0.88	3.27	3.15	0.94	0.58	2.00	3.01	1.40
RB1 2	7	0.88	3.27	3.17	0.95	0.58	1.99	3.02	1.41
RB1 3	7	0.88	3.27	3.18	0.96	0.59	1.99	3.00	1.42
RB2 1	8	1.77	2.63	2.14	0.95	2.23	1.61	1.83	1.66
RB2 2	8	1.77	2.63	2.17	0.96	2.22	1.60	1.84	1.68
RB2 3	8	1.77	2.62	2.18	0.96	2.22	1.60	1.84	1.67
RB3 1	9	0.51	1.66	2.03	0.89	1.01	1.28	2.15	1.09
RB3 2	9	0.51	1.66	2.05	0.89	1.00	1.33	2.17	1.10
RB3 3	9	0.50	1.65	2.07	0.90	1.00	1.30	2.17	1.09

Appendix 14: Calculation steps to determine soil organic carbon using Loss on ignition at 0-15 cm depth

		Soil Wt. (g) (A)	Wt. at 105^{o}C (g) (B)	Wt. at 550^{o}C (g) (C)	SOM % (D) (C-B)/A*100	SOC % (E) D*0.55
1	GSM1 1	5	18.450	17.590	17.20	9.46
1	GSM1 2	5	20.774	19.892	17.64	9.70
1	GSM1 3	5	21.479	20.598	17.62	9.69
2	GSM2 1	5	21.323	20.645	13.56	7.46
2	GSM2 2	5	20.686	20.006	13.60	7.48
2	GSM2 3	5	21.839	21.159	13.60	7.48
3	GSM3 1	5	22.335	21.665	13.40	7.37
3	GSM3 2	5	24.119	23.451	13.36	7.35
3	GSM3 3	5	21.632	20.947	13.70	7.54
4	GSM-N1 1	5	22.998	22.142	17.12	9.42
4	GSM-N1 2	5	21.415	20.543	17.44	9.59
4	GSM-N1 3	5	26.098	25.243	17.10	9.41
5	GSM-N2 1	5	20.437	19.812	12.50	6.88
5	GSM-N2 2	5	22.855	22.201	13.08	7.19
5	GSM-N2 3	5	23.856	23.206	13.00	7.15
6	GSM-N3 1	5	22.957	22.268	13.78	7.58
6	GSM-N3 2	5	23.981	23.281	14.00	7.70
6	GSM-N3 3	5	20.477	19.979	9.96	5.48
7	RB1 1	5	22.474	21.753	14.42	7.93
7	RB1 2	5	23.262	22.522	14.80	8.14
7	RB1 3	5	20.478	19.772	14.12	7.77
8	RB2 1	5	22.492	21.827	13.30	7.32
8	RB2 2	5	22.403	21.748	13.094	7.20
8	RB2 3	5	22.288	21.628	13.20	7.26
9	RB3 1	5	23.530	22.936	11.88	6.53
9	RB3 2	5	21.406	20.819	11.74	6.46
9	RB3 3	5	23.988	23.398	11.80	6.49

Appendix 15: Calculation steps to determine soil organic carbon using Loss on ignition at 15-30 cm depth

		Soil Wt. (g) (A)	Wt. at 105^{o}C (g) (B)	Wt. at 550^{o}C (g) (C)	SOM % (D) (C-B)/A*100	SOC % (E) D*0.55
1	GSM1 1	5	21.794	21.037	15.14	8.33
1	GSM1 2	5	22.099	21.330	15.38	8.46
1	GSM1 3	5	23.174	22.388	15.72	8.65
2	GSM2 1	5	21.596	20.975	12.42	6.83
2	GSM2 2	5	18.497	17.878	12.38	6.81
2	GSM2 3	5	22.861	22.251	12.20	6.71
3	GSM3 1	5	21.323	20.787	10.72	5.90
3	GSM3 2	5	20.872	20.326	10.92	6.01
3	GSM3 3	5	20.673	20.135	10.76	5.92
4	GSM-N1 1	5	26.980	26.177	16.06	8.83
4	GSM-N1 2	5	23.809	23.015	15.88	8.73
4	GSM-N1 3	5	22.921	22.128	15.86	8.72
5	GSM-N2 1	5	22.372	21.847	10.50	5.78
5	GSM-N2 2	5	23.661	23.142	10.38	5.71
5	GSM-N2 3	5	23.050	22.533	10.34	5.69
6	GSM-N3 1	5	21.007	20.291	14.32	7.88
6	GSM-N3 2	5	22.804	22.100	14.08	7.74
6	GSM-N3 3	5	21.591	20.885	14.12	7.77
7	RB1 1	5	22.299	21.682	12.34	6.79
7	RB1 2	5	21.924	21.314	12.20	6.71
7	RB1 3	5	21.563	20.956	12.14	6.68
8	RB2 1	5	23.178	22.535	12.86	7.07
8	RB2 2	5	24.553	23.884	13.38	7.36
8	RB2 3	5	25.061	24.415	12.92	7.11
9	RB3 1	5	21.425	20.818	12.14	6.68
9	RB3 2	5	23.190	22.728	9.24	5.08
9	RB3 3	5	20.457	19.995	9.24	5.08

Appendix 16: Seasonal bulk density (BD) g/cm^3 and porosity (P) data under different land uses/land covers

	BD Winter 2017	BD Spring 2017	BD Summer 2017	BD Autumn 2017	P Winter 2017	P Spring 2017	P Summer 2017	P Autumn 2017
GSM 1	0.95	0.88	1.02	1.02	0.64	0.67	0.62	0.62
GSM 2	0.99	0.88	0.98	0.81	0.63	0.67	0.63	0.69
GSM 3	0.69	0.82	1.02	1.00	0.74	0.69	0.62	0.62
GSM -N 1	0.59	0.70	0.52	0.73	0.78	0.73	0.80	0.73
GSM -N 2	0.87	0.81	0.54	0.97	0.67	0.69	0.80	0.63
GSM -N 3	0.76	0.71	0.62	0.85	0.71	0.73	0.76	0.68
RB 1	0.82	0.56	0.87	0.93	0.69	0.79	0.67	0.65
RB 2	0.93	0.88	0.86	0.86	0.65	0.67	0.68	0.68
RB 3	0.90	1.01	0.80	0.85	0.66	0.62	0.70	0.68

Appendix 17: Seasonal soil organic carbon (SOC) % data under different land uses/land covers

	SOC Winter 2017	SOC Spring 2017	SOC Summer 2017	SOC Autumn 2017
GSM 1	10.49	11.63	12.060	9.62
GSM 2	9.12	8.84	8.80	7.47
GSM 3	9.46	9.76	7.60	7.42
GSM-N 1	9.76	10.80	9.34	9.47
GSM-N 2	6.66	9.38	7.95	7.07
GSM-N 3	5.67	7.99	7.97	6.92
RB 1	10.51	9.42	7.68	7.95
RB 2	7.65	10.50	7.20	7.26
RB 3	6.82	7.78	7.50	6.49

Appendix 18: Seasonal Plant Biomass (kg/ha) data under different land uses/land covers

	Winter 2017	Spring 2017	Summer 2017	Autunm 2017
GSM 1	44,772.8	19,462.8	20,109.6	14,373.0
GSM 2	25,476.8	16,205.2	25,629.2	8,302.1
GSM 3	20,955.2	876.0	18,889.6	11,807.8
GSM-N 1	16,814.4	13,278.4	23,253.2	10,997.6
GSM-N 2	17,485.2	18,046.0	54,133.2	15,900.8
GSM-N 3	24,475.2	25,739.6	34,140.8	18,548.7
RB 1	15,041.6	9,666.4	33,869.2	8,345.3
RB 2	9,985.2	11,560.8	1,352.1	7,263.6
RB 3	8,494.8	7,094.4	16,812.4	14,757.3

Appendix 19: Calculation steps to determine the soil carbon stock at 15 cm depth (Winter 2016 and 2017)

Winter 2016	% C	g/kg C	BD g/cm3	BD g/m3	BD Kg/m2 to 1m	BD Kg/ha to 1m	BD Kg/ha to 15 cm	Soil C Stock Kg C to 15 cm	Soil C Stock t C/ha to 15 cm
GSM1	13.99	139.865	1.24	1241241.76	1241.24	12412417.60	1861862.64	260409.4182	260.41
GSM2	9.96	99.605	0.93	927029.60	927.03	9270296.01	1390544.40	138505.1751	138.51
GSM3	9.64	96.360	1.23	1234263.46	1234.26	12342634.59	1851395.19	178400.4404	178.40
GSM4	8.59	85.855	1.26	1261384.53	1261.38	12613845.33	1892076.80	162444.2536	162.44
GSM5	9.80	98.010	0.67	666408.91	666.41	6664089.10	999613.36	97972.1058	97.97
GSM-N1	10.61	106.095	0.66	664107.96	664.11	6641079.56	996161.93	105687.8004	105.69
GSM-N2	9.34	93.390	1.21	1206369.11	1206.37	12063691.14	1809553.67	168994.2174	168.99
GSM-N3	8.65	86.460	0.97	969700.97	969.70	9697009.70	1454551.46	125760.5188	125.76
GSM-N4	8.23	82.335	0.97	969700.97	969.70	9697009.70	1454551.46	119760.4941	119.76
GSM-N5	8.15	81.510	0.97	969700.97	969.70	9697009.70	1454551.46	118560.4891	118.56
RB1	13.33	133.265	0.39	386088.66	386.09	3860886.62	579132.99	77178.1583	77.18
RB2	8.03	80.300	0.99	992437.03	992.44	9924370.30	1488655.55	119539.0403	119.54
RB3	10.33	103.290	0.59	591297.87	591.30	5912978.70	886946.80	91612.7355	91.61
RB4	14.89	148.940	0.67	667729.13	667.73	6677291.29	1001593.69	149177.3647	149.18
RB5	12.40	123.970	0.82	820374.75	820.37	8203747.54	1230562.13	152552.7873	152.55
Winter 2017	% C	g/kg C	BD g/cm3	BD g/m3	BD Kg/m2 to 1m	BD Kg/ha to 1m	BD Kg/ha to 15 cm	Soil C Stock Kg C to 15 cm	Soil C Stock t C/ha to 15 cm
GSM 1	10.49	104.940	0.95	954857.93	954.86	9548579.35	1432286.90	150304.1875	150.30
GSM 2	9.12	91.153	0.99	990777.33	990.78	9907773.26	1486165.99	135468.4884	135.47
GSM 3	9.46	94.563	0.69	688399.99	688.40	6883999.89	1032599.98	97645.7522	97.65
GSM-N 1	9.76	97.570	0.59	591448.75	591.45	5914487.52	887173.13	86561.4821	86.56
GSM-N 2	6.66	66.623	0.87	868930.53	868.93	8689305.28	1303395.79	86836.1379	86.84
GSM-N 3	5.67	56.687	0.76	764623.78	764.62	7646237.85	1146935.68	65016.3427	65.02
RB 1	10.51	105.05	0.82	819337.44	819.34	8193374.39	1229006.16	129107.0969	129.11
RB 2	7.65	76.45	0.93	932433.07	932.43	9324330.70	1398649.60	106926.7623	106.93
RB 3	6.82	68.237	0.90	898390.28	898.39	8983902.76	1347585.41	91955.1859	91.96

C= Carbon, BD= Bulk Density

Appendix 20: Calculation steps to determine the soil carbon stock at 15 cm depth (Spring 2016 and 2017)

Spring 2016	% C	g/kg C	BD g/cm3	BD g/m3	BD Kg/m2 to 1m	BD Kg/ha to 1m	BD Kg/ha to 15 cm	Soil C Stock Kg C to 15 cm	Soil C Stock t C/ha to 15 cm
GSM1	7.75	77.51	1.09	1090199.26	1090.20	10901992.59	1635298.89	126745.4756	126.75
GSM2	13.74	137.43	0.89	894486.20	894.49	8944861.99	1341729.30	184399.2244	184.40
GSM3	11.63	116.323	0.67	672161.29	672.16	6721612.93	1008241.94	117283.7436	117.28
GSM4	7.98	79.79	1.06	1060569.77	1060.57	10605697.69	1590854.65	126940.6562	126.94
GSM5	9.64	96.36	1.10	1104382.18	1104.38	11043821.85	1656573.28	159627.401	159.63
GSM-N1	11.52	115.18	0.49	485114.53	485.11	4851145.29	727671.79	83813.9649	83.81
GSM-N2	10.06	100.62	0.72	720556.76	720.56	7205567.55	1080835.13	108750.3886	108.75
GSM-N3	9.08	90.85	0.72	720509.60	720.51	7205096.05	1080764.41	98186.3656	98.19
GSM-N4	7.59	75.92	0.88	877332.78	877.33	8773327.80	1315999.17	99913.2890	99.91
GSM-N5	9.03	90.33	0.86	859736.14	859.74	8597361.45	1289604.22	116492.5281	116.49
RB1	11.18	111.82	1.41	1413558.65	1413.56	14135586.51	2120337.98	237085.5908	237.09
RB2	8.70	87.03	0.63	633139.39	633.14	6331393.87	949709.08	82655.0807	82.66
RB3	8.63	86.26	0.60	595051.06	595.05	5950510.64	892576.60	76995.4424	77.00
RB4	6.85	68.51	0.57	572098.11	572.10	5720981.11	858147.17	58789.9461	58.79
RB5	7.49	74.90	0.78	777411.05	777.41	7774110.50	1166116.58	87340.9654	87.34
Spring 2017	% C	g/kg C	BD g/cm3	BD g/m3	BD Kg/m2 to 1m	BD Kg/ha to 1m	BD Kg/ha to 15 cm	Soil C Stock Kg C to 15 cm	Soil C Stock t C/ha to 15 cm
GSM 1	11.63	116.34	0.88	876531.22	876.53	8765312.18	1314796.83	152967.4073	152.97
GSM 2	8.84	88.40	0.88	883415.22	883.42	8834152.18	1325122.83	117144.8333	117.14
GSM 3	9.76	97.61	0.82	824665.47	824.67	8246654.66	1236998.20	120739.6832	120.74
GSM-N 1	10.79	107.95	0.70	703393.91	703.39	7033939.06	1055090.86	113893.8930	113.89
GSM-N 2	9.38	93.757	0.81	814009.41	814.01	8140094.11	1221014.12	114478.6206	114.48
GSM-N 3	7.99	79.897	0.71	706788.76	706.79	7067887.56	1060183.13	84705.4518	84.71
RB 1	9.42	94.197	0.556472	556472.37	556.47	5564723.74	834708.56	78627.0424	78.63
RB 2	10.50	105.013	0.877097	877097.03	877.10	8770970.27	1315645.54	138159.8851	138.16
RB 3	7.78	77.843	1.013551	1013551.11	1013.55	10135511.07	1520326.66	118346.7882	118.35

C= Carbon, BD= Bulk Density

Appendix 21: Calculation steps to determine the soil carbon stock at 15 cm depth (Summer 2016 and 2017)

Summer 2016	% C	g/kg C	BD g/cm3	BD g/m3	BD Kg/m2 to 1m	BD Kg/ha to 1m	BD Kg/ha to 15 cm	Soil C Stock Kg C to 15 cm	Soil C Stock t C/ha to 15 cm
GSM1	12.72	127.215	0.95	947200.66	947.20	9472006.64	1420801.00	180747.1987	180.75
GSM2	9.61	96.140	1.02	1015371.12	1015.37	10153711.23	1523056.68	146426.6697	146.43
GSM3	8.41	84.095	1.02	1017634.36	1017.63	10176343.56	1526451.53	128366.9417	128.37
GSM4	7.11	71.060	0.93	927897.17	927.90	9278971.74	1391845.76	98904.5598	98.90
GSM5	8.20	81.950	1.08	1075261.92	1075.26	10752619.22	1612892.88	132176.5718	132.18
GSM-N1	9.58	95.810	0.84	836019.35	836.02	8360193.51	1254029.03	120148.5210	120.15
GSM-N2	6.89	68.860	1.04	1041596.33	1041.60	10415963.34	1562394.50	107586.4853	107.59
GSM-N3	8.06	80.575	0.88	882717.39	882.72	8827173.88	1324076.08	106687.4303	106.69
GSM-N4	3.94	39.380	0.77	766943.60	766.94	7669435.98	1150415.40	45303.3584	45.30
GSM-N5	7.78	77.825	1.00	995765.87	995.77	9957658.69	1493648.80	116243.2181	116.24
RB1	7.39	73.865	1.10	1095018.06	1095.02	10950180.59	1642527.09	121325.2634	121.33
RB2	6.28	62.755	1.47	1470809.01	1470.81	14708090.11	2206213.52	138450.9293	138.45
RB3	10.60	106.040	0.52	522156.11	522.16	5221561.06	783234.16	83054.1503	83.05
RB4	10.49	104.885	0.68	678837.83	678.84	6788378.30	1018256.74	106799.8587	106.80
RB5	10.07	100.705	0.68	681648.01	681.65	6816480.11	1022472.02	102968.0444	102.97
Summer 2017	% C	g/kg C	BD g/cm3	BD g/m3	BD Kg/m2 to 1m	BD Kg/ha to 1m	BD Kg/ha to 15 cm	Soil C Stock Kg C to 15 cm	Soil C Stock t C/ha to 15 cm
GSM 1	12.06	120.597	1.02	1017134.56	1017.13	10171345.59	1525701.84	183995.0645	184.00
GSM 2	9.00	89.980	0.98	982243.05	982.24	9822430.52	1473364.58	132573.3448	132.57
GSM 3	7.60	76.010	1.02	1017606.07	1017.61	10176060.65	1526409.10	116022.3556	116.02
GSM-N 1	9.34	93.427	0.52	517054.40	517.05	5170544.02	775581.60	72460.2625	72.46
GSM-N 2	7.95	79.530	0.54	537800.70	537.80	5378007.03	806701.06	64156.9349	64.16
GSM-N 3	7.97	79.713	0.62	624557.96	624.56	6245579.62	936836.94	74678.0833	74.68
RB 1	7.68	76.817	0.87	874833.79	874.83	8748337.94	1312250.69	100803.1613	100.80
RB 2	7.20	71.977	0.86	860122.78	860.12	8601227.80	1290184.17	92863.5861	92.86
RB 3	7.50	74.947	0.80	801278.73	801.28	8012787.27	1201918.09	90080.1551	90.08

C= Carbon, BD= Bulk Density

Appendix 22: Calculation steps to determine the soil carbon stock at 15 cm depth (Autumn 2017)

Autumn 2017	% C	g/kg C	BD g/cm3	BD g/m3	BD Kg/m2 to 1m	BD Kg/ha to 1m	BD Kg/ha to 15 cm	Soil C Stock Kg C to 15 cm	Soil C Stock t C/ha to 15 cm
GSM 1	9.6177	96.177	1.01770037	1017700.37	1017.70	10177003.67	1526550.55	146819.0523	146.82
GSM 2	7.4727	74.727	0.81391511	813915.11	813.92	8139151.10	1220872.66	91232.1516	91.23
GSM 3	7.4177	74.177	1.00082042	1000820.42	1000.82	10008204.22	1501230.63	111356.7847	111.36
GSM-N 1	9.4710	94.710	0.72593193	725931.93	725.93	7259319.33	1088897.90	103129.5201	103.13
GSM-N 2	7.0730	70.730	0.97281292	972812.92	972.81	9728129.16	1459219.37	103210.5863	103.21
GSM-N 3	6.9190	69.190	0.84635478	846354.78	846.35	8463547.81	1269532.17	87838.9309	87.84
RB 1	7.9457	79.457	0.93103741	931037.41	931.04	9310374.09	1396556.11	110966.1592	110.97
RB 2	7.2589	72.589	0.85569062	855690.62	855.69	8556906.16	1283535.92	93170.5892	93.17
RB 3	6.4937	64.937	0.84776930	847769.30	847.77	8477693.01	1271653.95	82577.39266	82.58

C= Carbon, BD= Bulk Density

Appendix 23: Showing pH data after 200 days incubation

60 days incubation	pH					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	8.79	8.97	7.18	7.69	7.93	8.35
L2	8.67	9	7.23	7.65	7.93	8.37
L3	8.42	8.96	7.28	7.66	7.86	8.37
M1	8.85	8.63	6.92	7.61	7.81	6.55
M2	8.78	8.6	6.95	7.66	7.81	6.56
M3	8.81	8.58	7.03	7.66	7.88	6.57
H1	8.88	8.73	6.59	6.59	7.95	5.86
H2	8.98	8.57	6.65	6.61	7.95	5.9
H3	8.98	8.44	6.54	6.61	7.95	5.88
Control 1	7.66	7.66	7.66	7.66	7.66	7.66
Control 2	7.67	7.67	7.67	7.67	7.67	7.67
Control 3	7.65	7.65	7.65	7.65	7.65	7.65
120 days incubation	pH					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	8.8	9.77	7.03	7.28	7.99	7.65
L2	8.4	9.75	7.2	7.3	8.28	7.71
L3	9.13	9.63	7.1	7.38	7.37	7.81
M1	9.49	9.46	6.29	7.77	7.73	6.4
M2	8.09	9.04	7.07	8.2	7.55	6.39
M3	9.34	9.04	7.16	7.63	7.09	6.36
H1	8.35	8.75	6.36	6.96	7.64	5.63
H2	8.77	9.06	6.58	7.04	7.6	5.62
H3	8.6	8.99	6.96	7.1	7.57	5.69
Control 1	7.05	7.05	7.05	7.05	7.05	7.05
Control 2	6.99	6.99	6.99	6.99	6.99	6.99
Control 3	6.98	6.98	6.98	6.98	6.98	6.98
200 days incubation	pH					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	8.2	9.3	7.5	7.7	7.9	7.9
L2	8.2	9.3	7.5	7.7	7.9	7.9
L3	8.2	9.3	7.5	7.7	7.9	7.9
M1	8.5	8.9	6.7	7.8	7.8	6.8
M2	8.5	8.9	6.7	7.8	7.8	6.8
M3	8.5	8.9	6.7	7.8	7.8	6.8
H1	8.6	8.8	6.5	6.9	7.8	6.1
H2	8.6	8.8	6.5	6.9	7.8	6.1
H3	8.6	8.8	6.5	6.9	7.8	6.1
Control 1	7.7	7.7	7.7	7.7	7.7	7.7
Control 2	7.7	7.7	7.7	7.7	7.7	7.7
Control 3	7.7	7.7	7.7	7.7	7.7	7.7

(Mx = Combination of arsenic, chromium, copper, lead, and zinc)

Appendix 24: Showing Eh (mV) data after 200 days incubation

60 days incubation	Eh (mV)					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	-92.8	-120.5	-30.4	-62.8	-73.6	-95.2
L2	-82.3	-122.2	-31.4	-60.4	-73.9	-96.6
L3	-70.0	-120.0	-33.3	-60.6	-70.8	-96.1
M1	-116.6	-104.5	-12.5	-58.2	-68.5	-2.5
M2	-112.5	-102.1	-14.5	-59.8	-68.3	-3.2
M3	-112.2	-101.9	-18.5	-60.2	-71.2	-3.6
H1	-116.2	-107.5	5.5	-4.7	-75.2	37
H2	-120.8	-100.3	6.21	-5.5	-75.2	34.9
H3	-120.9	-95.6	7.1	-5.6	-75.1	35.9
Control 1	-60.7	-60.7	-60.7	-60.7	-60.7	-60.7
Control 2	-61.8	-61.8	-61.8	-61.8	-61.8	-61.8
Control 3	-60.1	-60.1	-60.1	-60.1	-60.1	-60.1
120 days incubation	Eh (mV)					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	-93.3	-130.5	1.3	-13.4	-47.2	-31.3
L2	-100.3	-126.7	-3.8	-14.4	-61.1	-37.1
L3	-102.3	-122	-2.1	-15.9	-16.7	-36.1
M1	-120.7	-113.3	42.3	-34	-34.2	33.1
M2	-118.8	-90.2	2.5	-57.8	-23.8	38.1
M3	-114.8	-94.2	-0.5	-30.4	-1.6	39.0
H1	-92.7	-90.2	7.6	7.2	-27.8	80.0
H2	-84.6	-96.8	5.5	1.0	-44.1	77.3
H3	-77.8	-94.5	5.8	-0.4	-25.9	74.6
Control 1	7.05	7.05	7.05	7.05	7.05	7.05
Control 2	6.99	6.99	6.99	6.99	6.99	6.99
Control 3	6.98	6.98	6.98	6.98	6.98	6.98
200 days incubation	Eh					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	-69.8	-134.2	-28	-42.1	-50.8	-52.4
L2	-69.8	-134.2	-28	-42.1	-50.8	-52.4
L3	-69.8	-134.2	-28	-42.1	-50.8	-52.4
M1	-85.7	-108.9	13.8	-46.4	-47.8	8.0
M2	-85.7	-108.9	13.8	-46.4	-47.8	8.0
M3	-85.7	-108.9	13.8	-46.4	-47.8	8.0
H1	-93.3	-104.2	25.2	5.0	-49.8	49.7
H2	-93.3	-104.2	25.2	5.0	-49.8	49.7
H3	-93.3	-104.2	25.2	5.0	-49.8	49.7
Control 1	-41.7	-41.7	-41.7	-41.7	-41.7	-41.7
Control 2	-41.7	-41.7	-41.7	-41.7	-41.7	-41.7
Control 3	-41.7	-41.7	-41.7	-41.7	-41.7	-41.7

(Mx = Combination of arsenic, chromium, copper, lead, and zinc)

Appendix 25: Showing EC (mS/cm) data after 200 days incubation

60 days incubation	EC (mS/cm)					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	2.09	6.35	5.18	7.60	3.41	3.11
L2	2.13	6.17	5.48	8.10	3.38	3.12
L3	2.14	6.31	5.51	7.63	3.37	3.11
M1	2.88	14.74	5.85	10.36	3.03	7.87
M2	2.85	14.78	5.9	10.26	3.02	7.78
M3	2.86	14.59	5.83	9.48	3.00	7.91
H1	3.03	23.2	7.84	14.38	3.21	14.92
H2	3.04	23.4	7.79	13.49	3.20	14.78
H3	3.02	23.9	7.74	14.20	3.19	14.74
Control 1	3.90	3.90	3.90	3.90	3.90	3.90
Control 2	4.01	4.01	4.01	4.01	4.01	4.01
Control 3	4.01	4.01	4.01	4.01	4.01	4.01
120 days incubation	EC (mS/cm)					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	2	4.88	3.57	3.69	2.26	2.53
L2	1.93	4.85	4.09	3.87	2.28	2.51
L3	1.92	4.86	4.21	3.95	2.26	2.33
M1	2.38	9.22	5.57	4.89	2.61	5.11
M2	2.36	10.15	5.53	5.22	2.56	5.31
M3	2.38	9.81	5.52	4.77	2.47	5.05
H1	2.52	16.67	8.38	8.30	2.20	8.92
H2	2.49	15.18	8.25	8.53	2.15	9.51
H3	2.4	15.52	8.20	8.54	2.13	9.34
Control 1	7.05	7.05	7.05	7.05	7.05	7.05
Control 2	6.99	6.99	6.99	6.99	6.99	6.99
Control 3	6.98	6.98	6.98	6.98	6.98	6.98
200 days incubation	EC (mS/cm)					
	Arsenic	Chromium	Copper	Mx	Lead	Zinc
L1	2.24	4.87	4.75	4.15	2.41	3.02
L2	2.26	4.91	4.74	4.22	2.43	3.03
L3	2.26	4.89	4.68	4.19	2.43	3.02
M1	2.23	10.62	6.27	6.05	2.58	5.62
M2	2.25	10.73	6.29	6.07	2.58	5.60
M3	2.26	10.72	6.28	6.05	2.60	5.63
H1	2.69	18.73	9.26	9.87	2.50	10.93
H2	2.72	18.64	9.24	9.95	2.50	10.96
H3	2.75	18.61	9.21	9.94	2.50	10.97
Control 1	2.56	2.56	2.56	2.56	2.56	2.56
Control 2	2.58	2.58	2.58	2.58	2.58	2.58
Control 3	2.57	2.57	2.57	2.57	2.57	2.57

(Mx = Combination of arsenic, chromium, copper, lead, and zinc)

Appendix 26: Showing soil organic carbon (%) data after 200 days incubation

	60 days	120 days	200 days	60 days	120 days	200 days	60 days	120 days	200 days
	Arsenic	Arsenic	Arsenic	Chromium	Chromium	Chromium	Copper	Copper	Copper
L1	9.41	8.76	8.62	10.10	10.21	9.99	9.35	9.28	9.35
L2	8.91	9.00	8.47	10.27	9.79	10.21	9.59	9.26	9.64
L3	9.26	8.76	8.49	10.21	9.61	10.03	9.50	9.37	9.50
M1	9.28	9.13	8.91	9.81	9.41	9.64	9.13	9.19	9.61
M2	9.35	9.15	8.89	10.51	9.17	9.35	9.61	9.33	9.75
M3	9.28	8.93	9.00	9.31	14.50	19.53	9.48	9.72	9.74
H1	9.77	9.73	8.89	9.42	8.19	8.80	9.88	9.39	10.16
H2	9.72	9.15	8.91	9.18	8.51	8.73	9.70	9.64	10.16
H3	9.06	9.04	8.82	8.23	8.45	8.78	9.62	10.05	10.43
Control 1	8.36	8.23	8.40	8.36	8.23	8.40	8.36	8.23	8.40
Control 2	8.62	8.10	8.18	8.62	8.10	8.18	8.62	8.10	8.18
Control 3	8.78	7.90	8.27	8.78	7.90	8.27	8.78	7.90	8.27
	60 days	120 days	200 days	60 days	120 days	200 days	60 days	120 days	200 days
	Mx	Mx	Mx	Lead	Lead	Lead	Zinc	Zinc	Zinc
L1	9.42	8.86	9.61	8.67	8.53	8.34	9.44	8.84	9.06
L2	8.30	8.93	9.83	8.36	8.16	8.16	9.61	9.02	5.50
L3	9.46	9.09	9.74	8.87	8.43	8.32	10.27	8.82	8.98
M1	9.61	9.24	9.88	9.06	8.43	8.80	9.88	9.73	9.84
M2	9.73	9.86	10.05	8.97	8.32	8.76	9.59	9.70	9.86
M3	9.66	9.86	10.21	8.71	8.58	8.71	10.07	9.86	9.92
H1	8.98	10.69	10.52	8.30	8.60	8.80	10.71	10.87	10.91
H2	10.01	10.23	10.41	9.02	8.51	8.73	10.98	10.60	11.09
H3	10.49	10.36	10.41	8.67	8.38	8.54	10.71	10.40	10.58
Control 1	8.36	8.23	8.40	8.36	8.23	8.40	8.36	8.23	8.40
Control 2	8.62	8.10	8.18	8.62	8.10	8.18	8.62	8.10	8.18
Control 3	8.78	7.90	8.27	8.78	7.90	8.27	8.78	7.90	8.27

Mx = Combination of arsenic, chromium, copper, lead, and zinc

Appendix 27: Showing plant biomass utilised after 200 days incubation

Treatment	BM added (g)	BM in 10 g soil at 60 days	BM left	% BM utilised
Arsenic	20.0	0.25	19.8	98.8
Chromuim	20.0	0.29	19.7	98.6
Copper	20.0	1.14	18.9	94.3
Mx	20.0	1.19	18.8	94.1
Lead	20.0	0.18	19.8	99.1
Zinc	20.0	0.74	19.3	96.3
Control	20.0	0.01	20.0	100.0
	BM added (g)	BM in 10 g soil at 120 days	BM left	% BM utilised
Arsenic	20.0	0.91	19.1	95.5
Chromuim	20.0	2.06	17.9	89.7
Copper	20.0	2.29	17.7	88.6
Mx	20.0	2.48	17.5	87.6
Lead	20.0	0.24	19.8	98.8
Zinc	20.0	1.68	18.3	91.6
Control	20.0	0.00	20.0	100.0
	BM added (g)	BM in 100 g soil at 200 days	BM left	% BM utilised
Arsenic	20.0	1.3	18.7	93.5
Chromuim	20.0	3.1	16.9	84.5
Copper	20.0	4.1	15.9	79.6
Mx	20.0	4.6	15.4	77.1
Lead	20.0	0.4	19.6	97.9
Zinc	20.0	2.9	17.1	85.4
Control	20.0	0.0	20.0	100.0

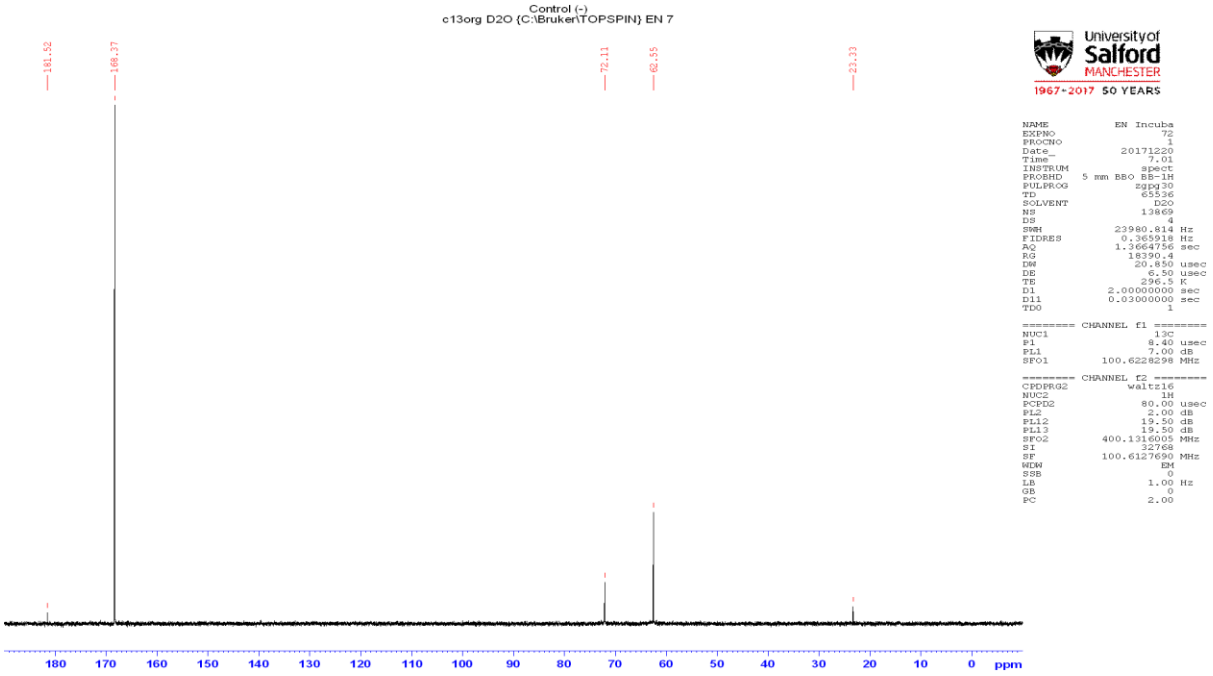
BM= Biomass, Mx = Combination of arsenic, chromium, copper, lead, and zinc

Appendix 28: Calculation steps to determine percentage heavy metal absorbed after 60 days incubation

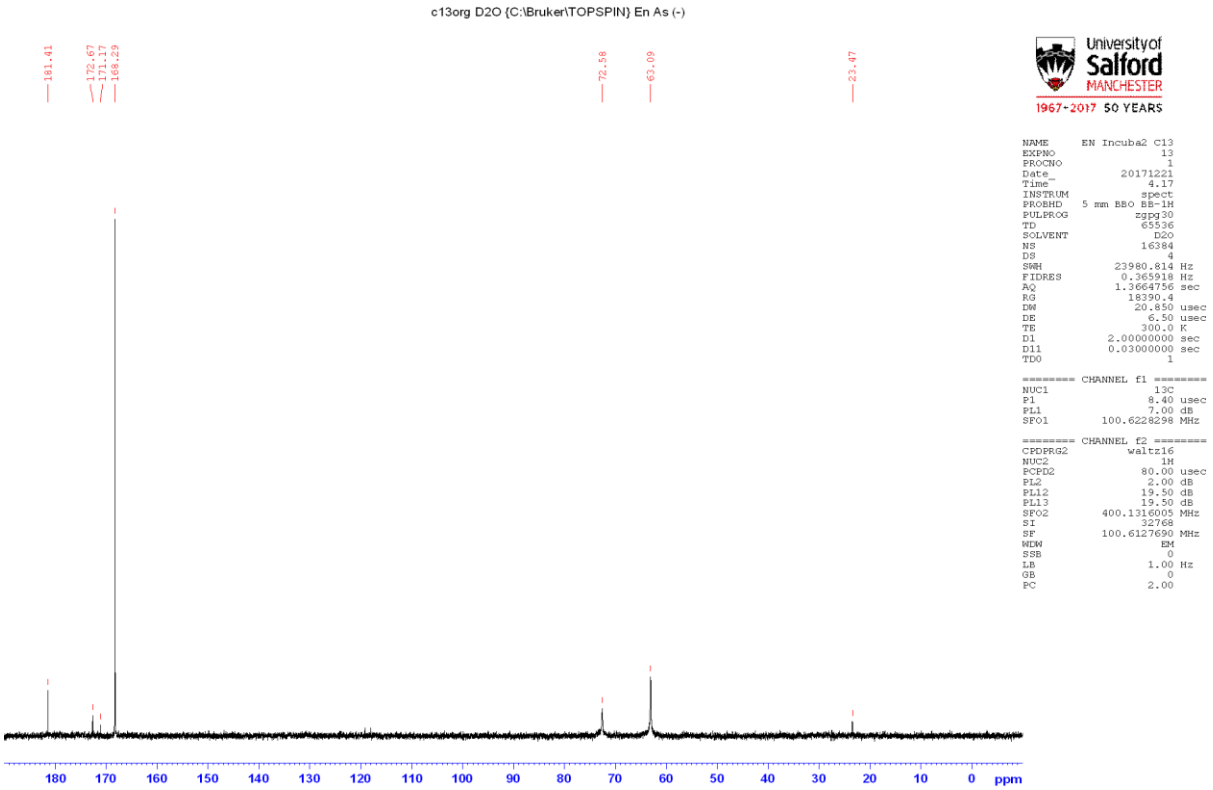
					Arsenic absorbed					Chromium absorbed
	Initial As	Final As	Initial-Final	Init-Fnal/init	in-fn/in*100	Initial Cr	Final Cr	Initial-Final	Init-Fnal/init	in-fn/in*100
L 1	64.77	34.25	30.52	0.47	47.12	159.15	94.72	322.13	0.40	40.48
L 2	64.87	33.56	31.30	0.48	48.26	182.49	93.24	446.23	0.49	48.91
L 3	64.69	35.32	29.37	0.45	45.40	149.77	94.41	276.78	0.37	36.96
M 1	114.71	60.97	53.74	0.47	46.85	257.19	187.02	350.86	0.27	27.28
M 2	115.10	57.36	57.74	0.50	50.16	338.34	186.48	759.28	0.45	44.88
M 3	114.75	40.90	73.85	0.64	64.36	269.91	186.48	417.13	0.31	30.91
H 1	213.43	84.48	128.95	0.60	60.42	531.51	372.96	792.74	0.30	29.83
H 2	213.67	81.03	132.64	0.62	62.08	573.30	372.96	1001.71	0.35	34.95
H 3	213.97	72.19	141.78	0.66	66.26	582.06	374.19	1039.32	0.36	35.71
					Copper absorbed					Lead absorbed
	Initial Cu	Final Cu	Initial-Final	Init-Fnal/init	in-fn/in*100	Initial Pb	Final Pb	Initial-Final	Init-Fnal/init	in-fn/in*100
L 1	143.66	126.30	17.36	0.12	12.08	186.21	46.23	139.99	0.75	75.18
L 2	155.84	123.81	32.03	0.21	20.55	186.10	24.58	161.51	0.87	86.79
L 3	182.39	123.07	59.32	0.33	32.52	186.73	72.59	114.15	0.61	61.13
M 1	257.33	241.50	15.83	0.06	6.15	369.48	51.02	318.46	0.86	86.19
M 2	239.40	239.06	0.33	0.00	0.14	368.93	58.16	310.78	0.84	84.24
M 3	249.49	239.06	10.43	0.04	4.18	372.26	72.69	299.58	0.80	80.47
H 1	602.56	479.91	122.65	0.20	20.36	735.97	105.16	630.82	0.86	85.71
H 2	613.38	489.13	124.25	0.20	20.26	737.30	133.58	603.72	0.82	81.88
H 3	722.16	479.41	242.75	0.34	33.61	738.45	137.48	600.97	0.81	81.38
					Zinc absorbed					Mx absorbed
	Initial Zn	Final Zn	Initial-Final	Init-Fnal/init	in-fn/in*100	initial Mx	Final Mx	Initial-Final	Init-Fnal/init	in-fn/in*100
L 1	372.74	357.52	15.21	0.04	4.08	926.52	659.02	267.51	0.29	28.87
L 2	356.13	350.10	6.03	0.02	1.69	945.41	625.30	320.12	0.34	33.86
L 3	361.31	353.52	7.79	0.02	2.16	944.88	678.91	265.97	0.28	28.15
M 1	609.04	483.65	125.40	0.21	20.59	1607.76	1024.16	583.60	0.36	36.30
M 2	519.15	484.57	34.58	0.07	6.66	1580.92	1033.06	547.85	0.35	34.65
M 3	494.94	483.82	11.11	0.02	2.25	1501.35	1022.95	478.40	0.32	31.86
H 1	907.34	742.94	164.40	0.18	18.12	2990.81	1785.44	1205.37	0.40	40.30
H 2	882.54	745.24	137.30	0.16	15.56	3020.19	1821.94	1198.25	0.40	39.67
H 3	803.26	745.05	58.21	0.07	7.25	3059.91	1808.33	1251.58	0.41	40.90

Mx = Combination of arsenic, chromium, copper, lead, and zinc

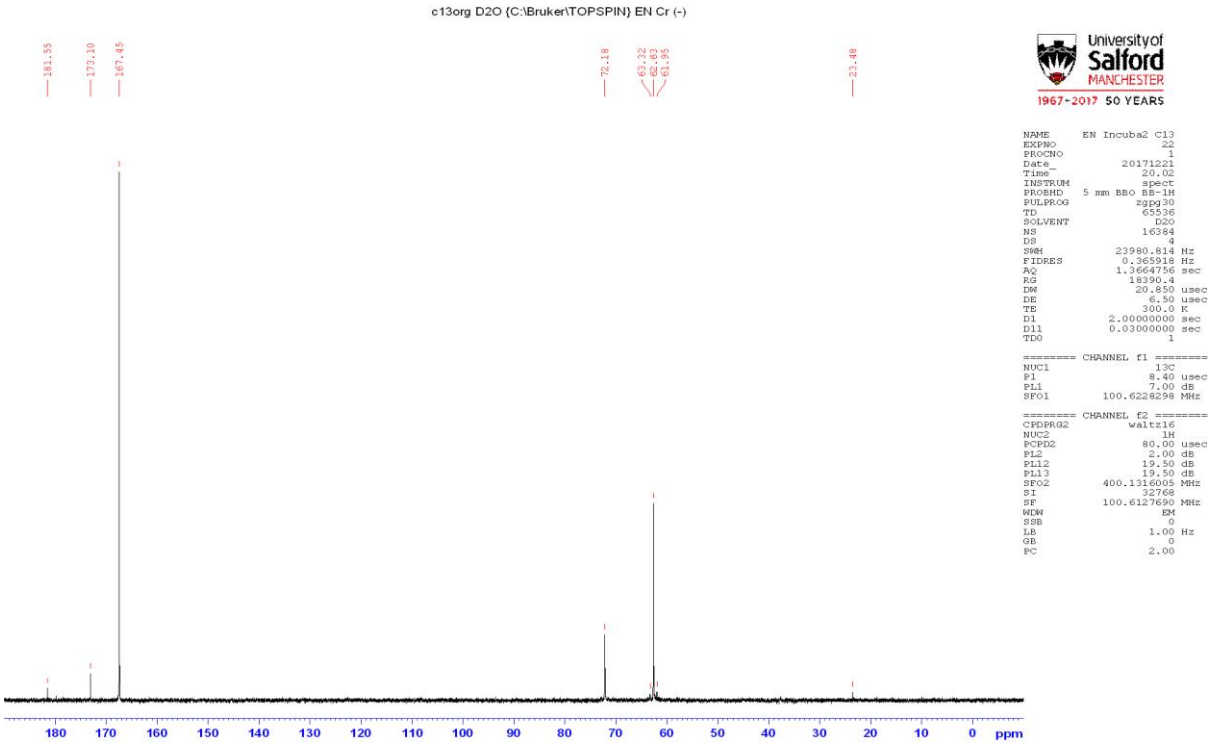
Appendix 29: Showing liquid state ^{13}C-NMR spectrum with different carbon species in humic acids fraction after 200 days control soil incubation



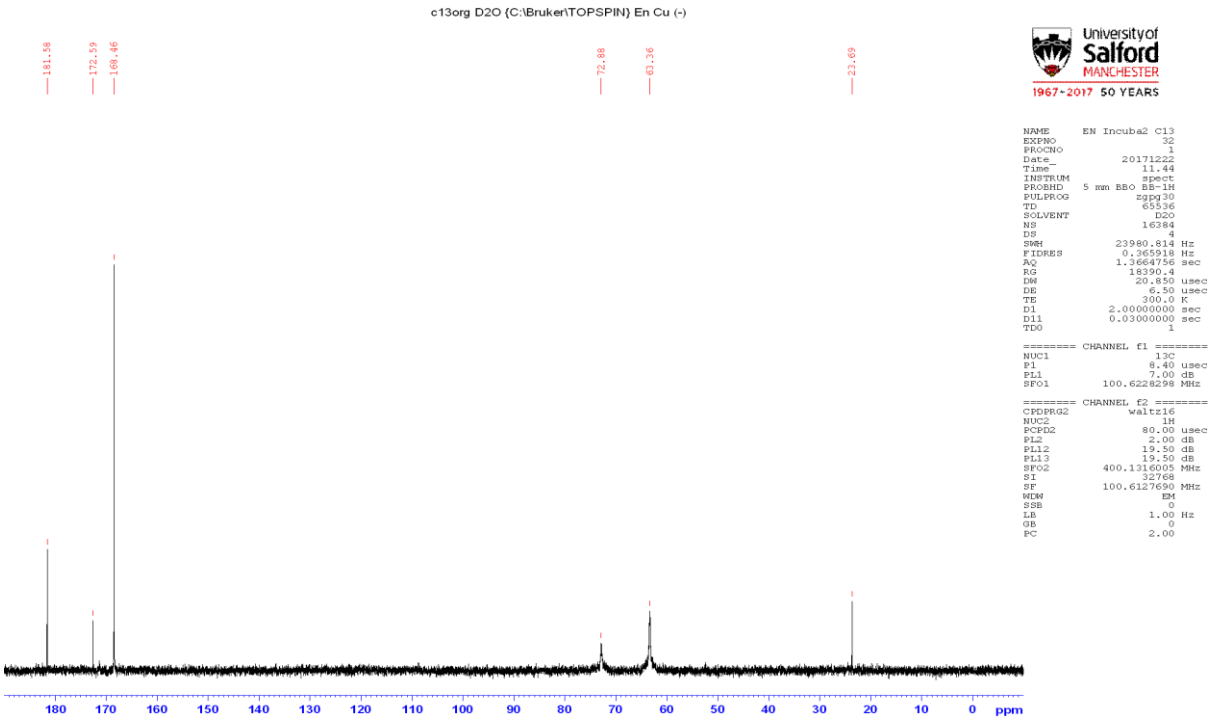
Appendix 30: Showing liquid state ^{13}C-NMR spectrum with different carbon species in humic acids fraction after 200 days arsenic soil incubation



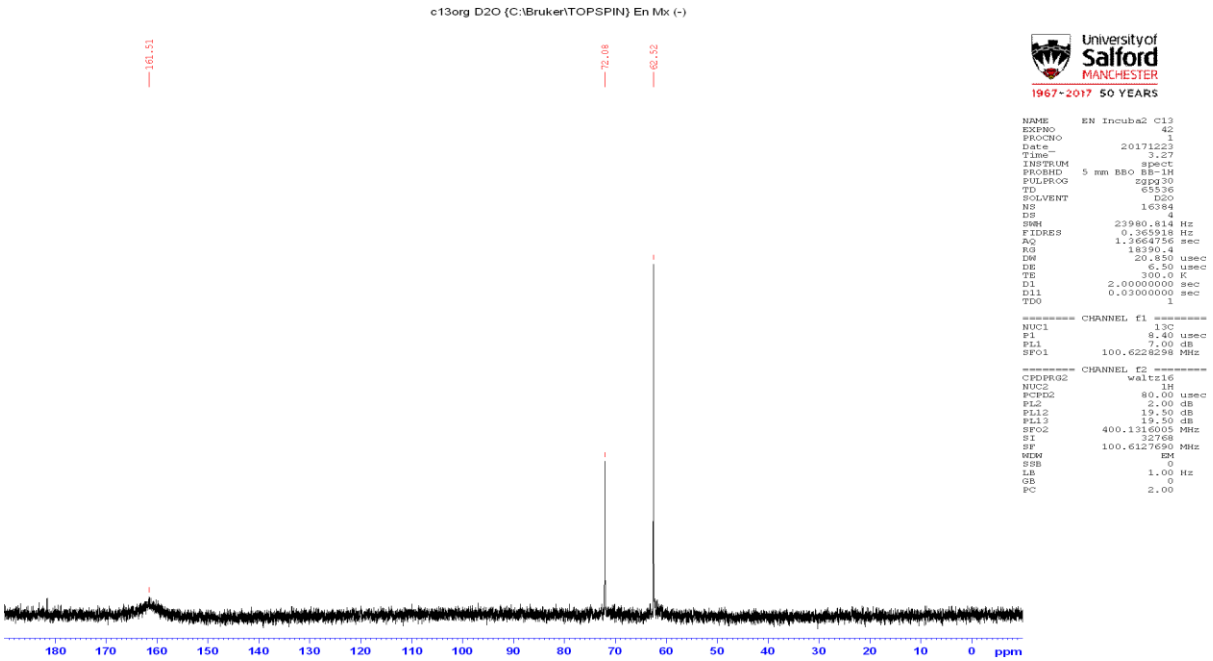
Appendix 31: Showing liquid state ^{13}C-NMR spectrum with different carbon species in humic acids fraction after 200 days chromium soil incubation



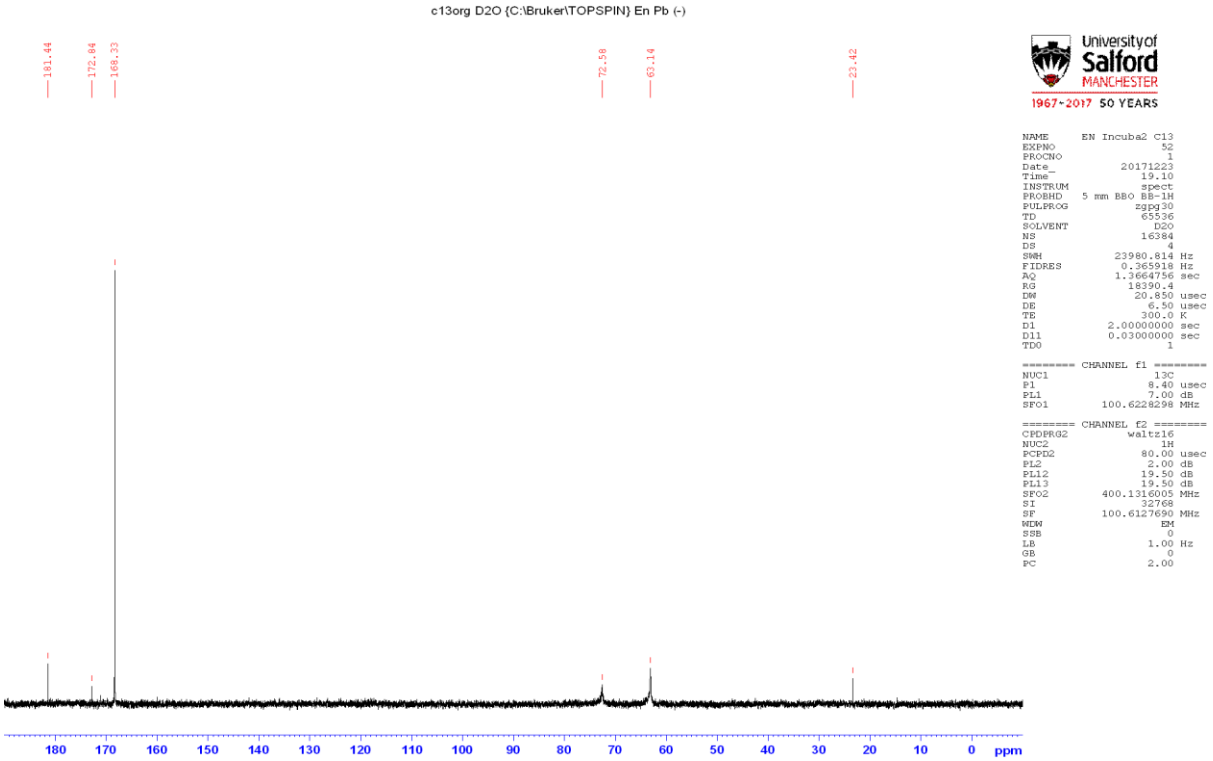
Appendix 32: Showing liquid state ^{13}C-NMR spectrum with different carbon species in humic acids fraction after 200 days copper soil incubation



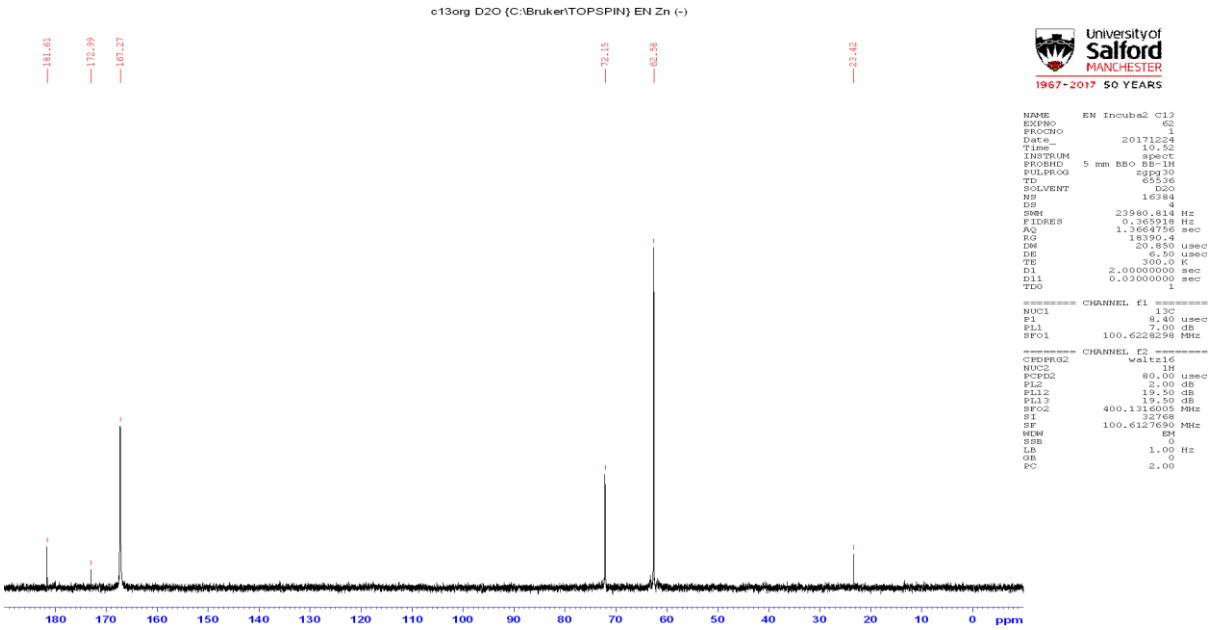
Appendix 33: Showing liquid state ^{13}C-NMR spectrum with different carbon species in humic acids fraction after 200 days Mx soil incubation



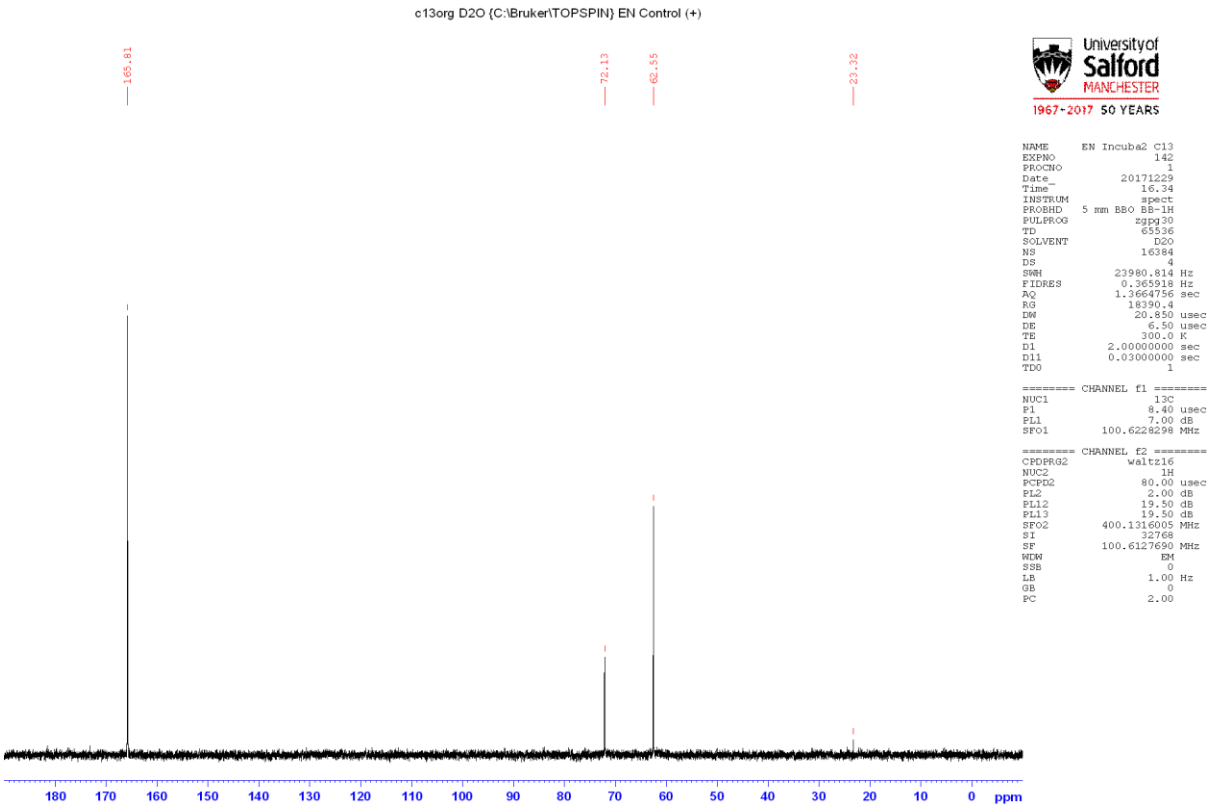
Appendix 34: Showing liquid state ^{13}C-NMR spectrum with different carbon species in humic acids fraction after 200 days lead soil incubation



Appendix 35: Showing liquid state ^{13}C-NMR spectrum with different carbon species in humic acids fraction after 200 days zinc soil incubation



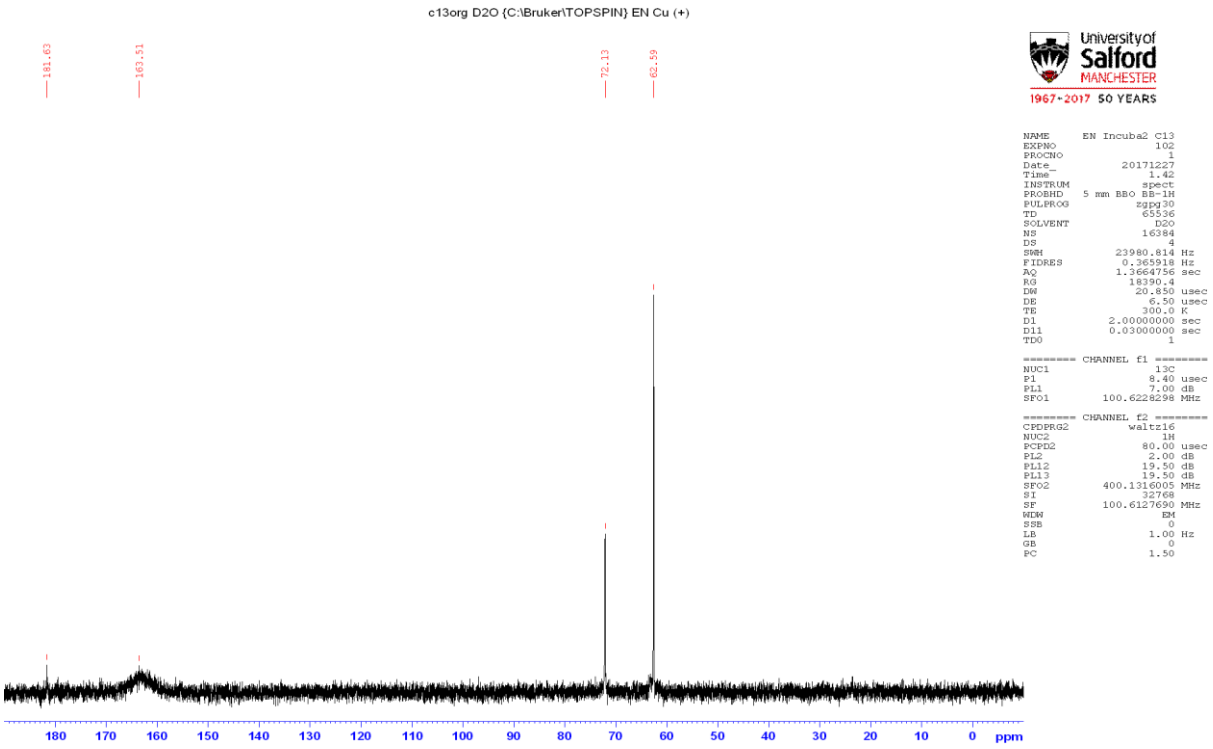
Appendix 36: Showing liquid state ^{13}C-NMR spectrum with different carbon species in fulvic acids fraction after 200 days Control soil incubation



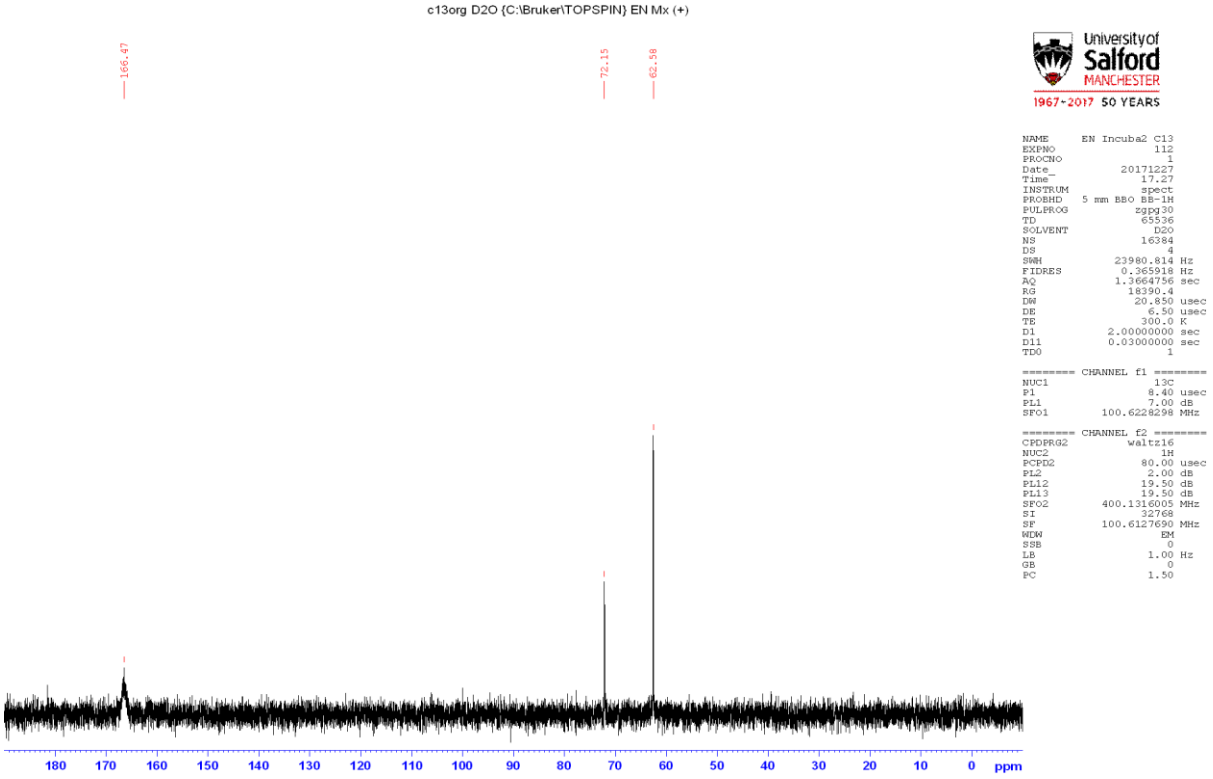
Appendix 37: Showing liquid state ^{13}C-NMR spectrum with different carbon species in fulvic acids fraction after 200 days arsenic soil incubation

Appendix 38: Showing liquid state ^{13}C-NMR spectrum with different carbon species in fulvic acids fraction after 200 days chromium soil incubation

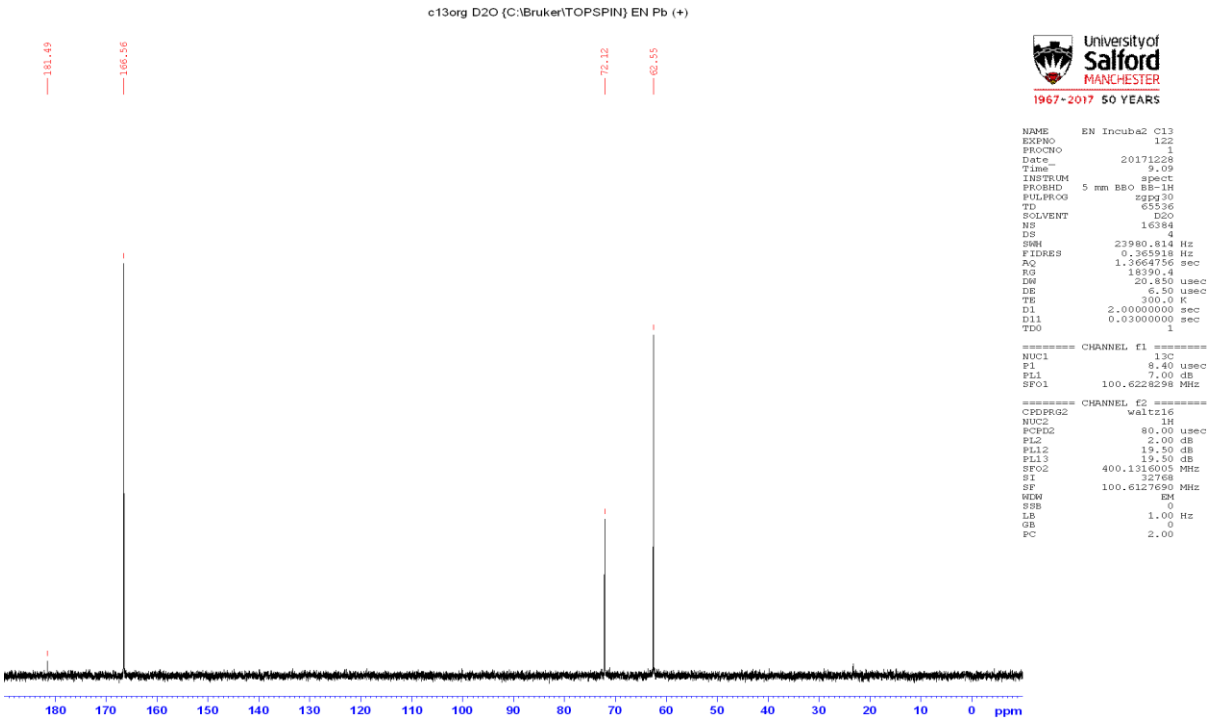
Appendix 39: Showing liquid state ^{13}C-NMR spectrum with different carbon species in fulvic acids fraction after 200 days copper soil incubation



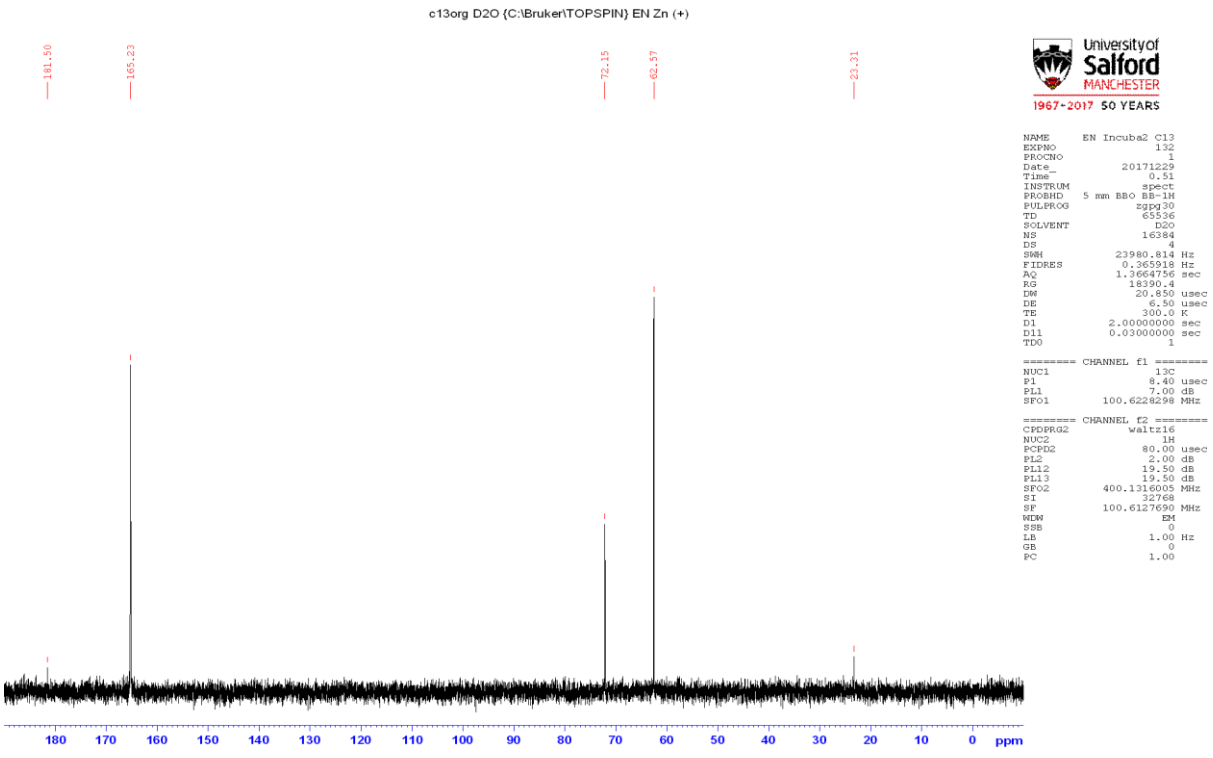
Appendix 40: Showing liquid state ^{13}C-NMR spectrum with different carbon species in fulvic acids fraction after 200 days Mx soil incubation



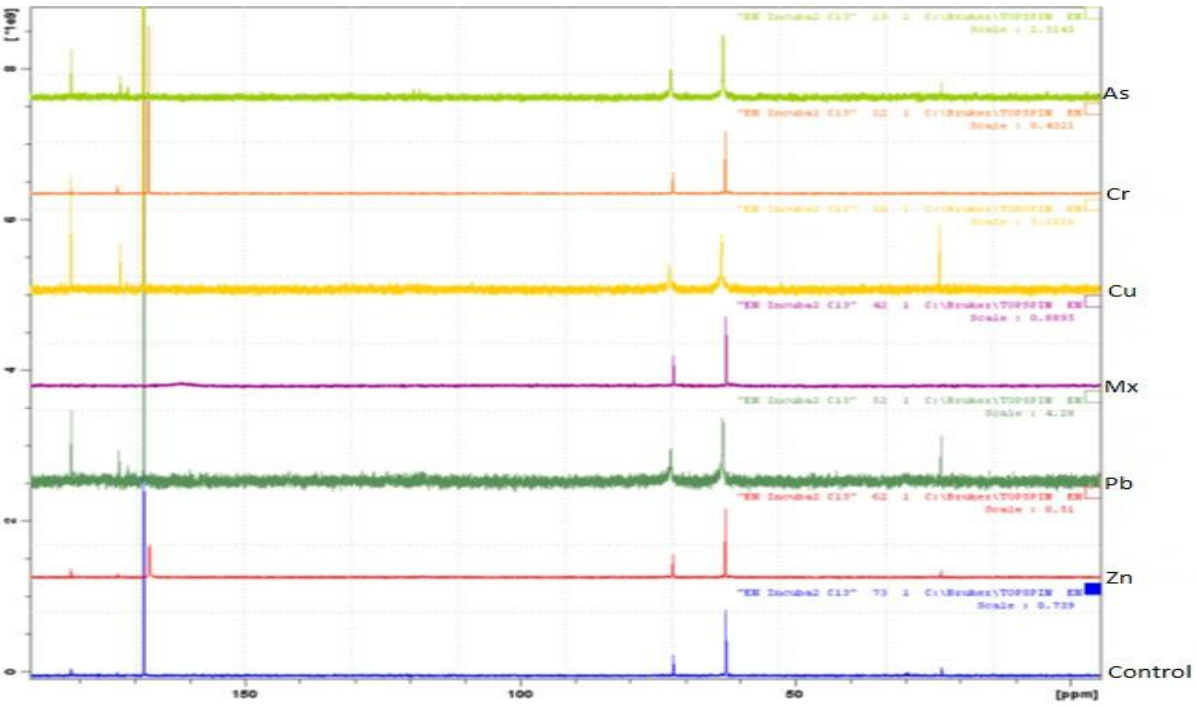
Appendix 41: Showing liquid state ^{13}C-NMR spectrum with different carbon species in fulvic acids fraction after 200 days lead soil incubation



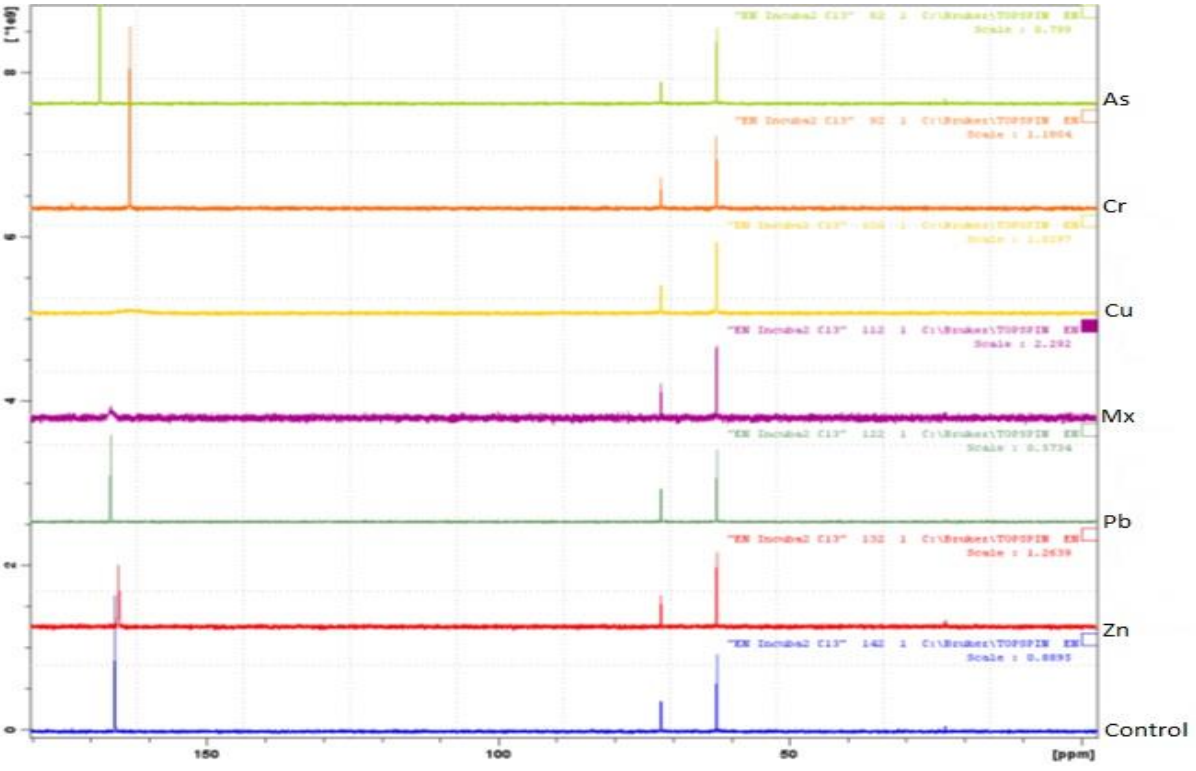
Appendix 42: Showing liquid state ^{13}C-NMR spectrum with different carbon species in fulvic acids fraction after 200 days zinc soil incubation



Appendix 43 Liquid state ^{13}C-NMR spectrum with different carbon species in humic acids (HA) fraction after 200 days heavy metal soil incubation



Appendix 44 Liquid state ^{13}C-NMR spectrum with different carbon species in fulvic acids (FA) fraction after 200 days heavy metal soil incubation



Appendix 45: Diversity indexes

Sample ID	Total read bases (bp)	Total reads	GC (%)	AT (%)	Q20 (%)	Q30 (%)
RB 0-15 cm	81,595,080	271,080	56.728	43.27	83.884	75.372
RB 0-15 cm	60,938,052	202,452	56.704	43.30	83.771	75.194
RB 15-30 cm	77,100,548	256,148	56.907	43.09	84.371	75.978
RB 15-30 cm	66,131,506	219,706	56.989	43.01	82.438	73.556
GSM 0-15 cm	79,848,076	265,276	55.680	44.32	85.336	77.088
GSM 0-15 cm	87,299,632	290,032	55.687	44.31	84.821	76.444
GSM 15-30 cm	75,414,346	250,546	55.946	44.05	85.066	76.799
GSM 15-30 cm	87,712,604	291,404	55.910	44.09	84.322	75.921
GSM-N 0-15 cm	80,193,624	266,424	55.334	44.67	84.192	75.831
GSM-N 0-15 cm	78,706,082	261,482	55.399	44.6	84.456	76.041
GSM-N15-30 cm	84,684,544	281,344	55.549	44.45	84.697	76.436
GSM-N15-30 cm	81,415,684	270,484	55.501	44.5	83.796	75.297
Total	941,039,778	3,126,378	672	528	1,011	910
Mean	78,419,981.5	260,531.5	56.0	44.0	84.3	75.8

The ratio of reads that have a Phred quality score of over 20= (Q20)

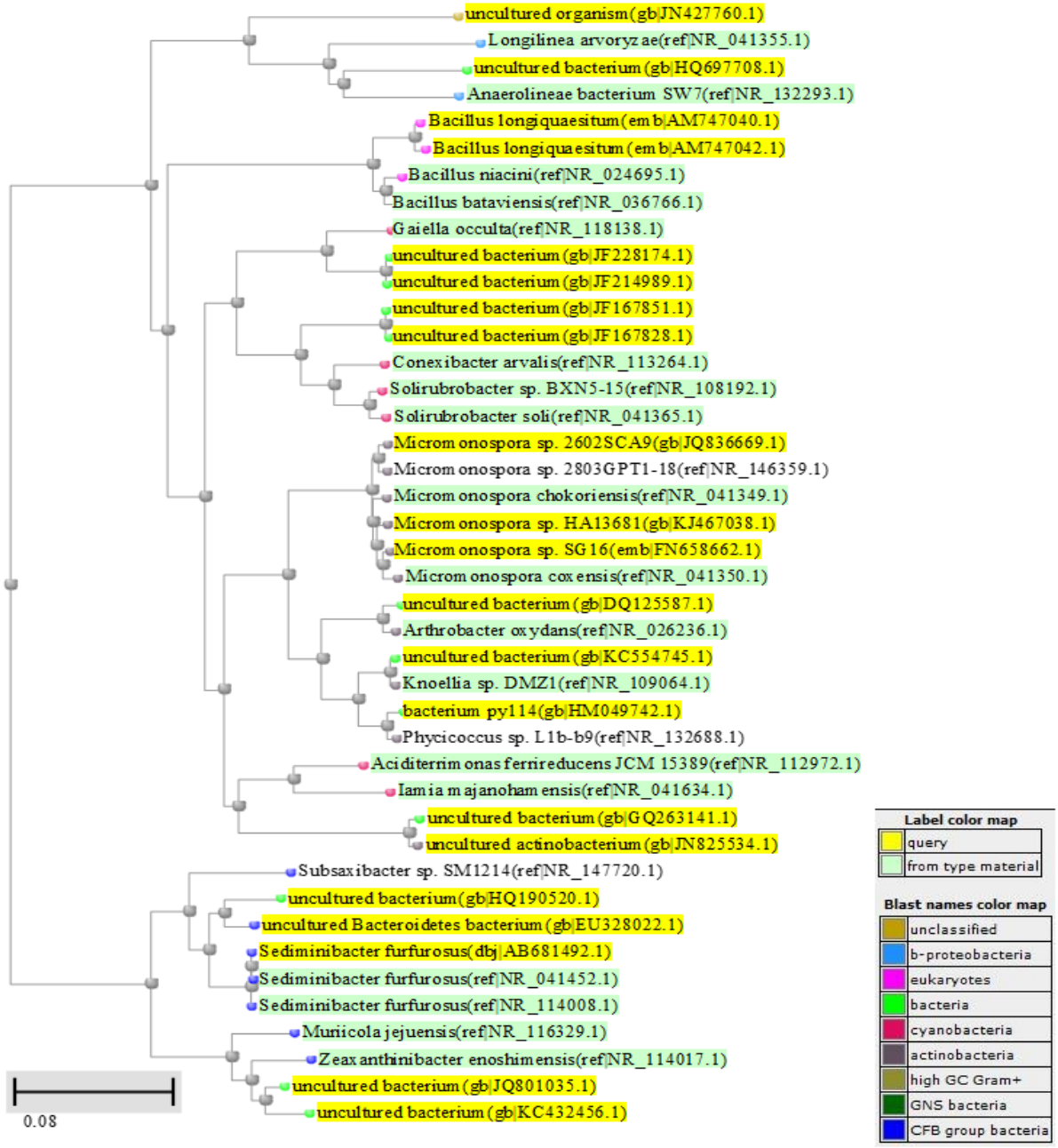
The ratio of reads that have a Phred quality score of over 30= (Q30)

Guanine-Cytosine content= GC

Adenine-Thymine content= AT

%= percentage

Appendix 46: Showing Bacterial community clustering using phylogenetic tree



Appendix 47: Showing percentage relative abundance in taxa Phyla under different land uses/land covers

Kingdom	Phylum	RB 0–15 cm (%)	RB 0–15 cm (%)	RB 15–30 cm (%)	RB 15–30 cmv	GSM 0–15 cm (%)	GSM 0–15 cm (%)	GSM 15–30 cm (%)	GSM 15–30 cm (%)	GSM-N 0–15 cm (%)	GSM-N 0–15 cm (%)	GSM-N15–30 cm (%)	GSM-N15–30 cm (%)
Archaea	__"Thaumarchaeota"	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Bacteria	Other	0.20	0.10	0.23	0.14	0.09	0.04	0.04	0.07	0.02	0.02	0.03	0.02
Bacteria	__	9.08	9.35	10.03	10.36	7.30	7.53	7.30	7.53	6.66	6.37	5.73	6.04
Bacteria	__"Acidobacteria"	9.52	8.85	6.26	6.80	5.18	5.36	4.59	4.60	4.62	4.29	4.26	4.12
Bacteria	__"Actinobacteria"	34.91	35.21	35.97	33.91	24.44	24.02	21.67	21.13	17.01	17.39	19.75	18.94
Bacteria	__"Armatimonadetes"	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00
Bacteria	__"Bacteroidetes"	6.45	6.22	5.35	5.28	12.28	12.04	12.75	13.02	17.05	16.86	14.34	14.85
Bacteria	__"Chloroflexi"	7.71	6.58	6.91	5.77	10.61	12.78	17.87	19.29	16.17	15.69	16.22	15.96
Bacteria	__"Deferribacteres"	0.00	0.00	0.00	0.00	0.03	0.03	0.10	0.07	0.01	0.01	0.01	0.03
Bacteria	__"Deinococcus-Thermus"	0.00	0.00	0.01	0.00	0.00	0.01	0.03	0.02	0.02	0.01	0.09	0.13
Bacteria	__"Fusobacteria"	0.00	0.00	0.00	0.00	0.06	0.08	0.03	0.01	0.10	0.03	0.06	0.02
Bacteria	__"Gemmatimonadetes"	1.37	1.58	2.45	2.36	1.05	1.19	1.03	1.02	1.07	1.25	1.51	1.51
Bacteria	__"Planctomycetes"	1.42	1.86	1.24	1.38	1.19	1.06	0.94	0.90	1.03	1.11	0.96	0.89
Bacteria	__"Proteobacteria"	24.10	26.36	26.35	28.31	30.36	28.96	30.07	28.91	32.23	33.73	33.53	34.12

Bacteria	__"Spirochaetes"	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.00	0.00	0.00
Bacteria	__"Tenericutes"	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00
Bacteria	__"Verrucomicrobia"	1.76	1.82	1.40	1.91	0.74	0.77	0.48	0.49	1.07	0.78	0.59	0.70
Bacteria	__Candidatus Saccharibacteria	0.06	0.08	0.05	0.02	0.29	0.28	0.15	0.28	0.28	0.22	0.21	0.24
Bacteria	__Cyanobacteria/Chloroplast	1.81	0.58	0.17	0.57	2.54	2.15	0.05	0.07	0.63	0.45	0.23	0.19
Bacteria	__Firmicutes	0.54	0.36	3.09	2.53	3.32	3.25	2.44	2.20	1.46	1.31	2.00	1.93
Bacteria	__Hydrogenedentes	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Bacteria	__Ignavibacteriae	0.18	0.11	0.06	0.05	0.20	0.16	0.27	0.24	0.22	0.17	0.13	0.15
Bacteria	__Latescibacteria	0.11	0.17	0.03	0.06	0.07	0.08	0.02	0.03	0.17	0.13	0.14	0.08
Bacteria	__Microgenomates	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Bacteria	__Nitrospirae	0.73	0.69	0.38	0.48	0.09	0.14	0.07	0.04	0.13	0.14	0.13	0.05
Bacteria	__Parcubacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.01	0.01	0.01	0.02
Bacteria	__candidate division WPS-1	0.02	0.02	0.01	0.02	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00
Bacteria	__candidate division WPS-2	0.01	0.03	0.00	0.01	0.04	0.03	0.01	0.03	0.00	0.00	0.00	0.00
Unassigned	Other	0.02	0.01	0.03	0.05	0.03	0.01	0.03	0.01	0.01	0.02	0.04	0.03

Appendix 48: Showing percentage relative abundance in taxa Order under different land uses/land covers

kingdom	**Order**	RB 0–15 cm **(%)**	RB 0–15 cm **(%)**	RB 15–30 cm **(%)**	RB 15–30 cm **(%)**	GSM 0–15 cm **(%)**	GSM 0–15 cm **(%)**	GSM 15–30 cm **(%)**	GSM 15–30 cm **(%)**	GSM-N 0–15 cm **(%)**	GSM-N 0–15 cm **(%)**	GSM-N15–30 cm **(%)**	GSM-N15–30 cm **(%)**
Archaea	__Nitrososphaerales	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Bacteria	Other	0.20	0.10	0.23	0.14	0.09	0.04	0.04	0.07	0.02	0.02	0.03	0.02
Bacteria	__	9.08	9.35	10.03	10.36	7.30	7.53	7.30	7.53	6.66	6.37	5.73	6.04
Bacteria	__	0.04	0.00	0.05	0.09	0.07	0.17	0.09	0.09	0.00	0.00	0.00	0.00
Bacteria	__	1.18	1.01	0.85	0.68	0.43	0.45	0.87	0.60	0.59	0.60	0.56	0.55
Bacteria	__	0.01	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	__	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	__	2.65	2.30	1.34	1.39	1.36	1.39	0.85	0.90	0.98	0.90	0.97	1.11
Bacteria	__	0.48	0.51	0.34	0.50	0.15	0.12	0.10	0.09	0.18	0.20	0.17	0.14
Bacteria	__	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00
Bacteria	__	0.00	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Bacteria	__	0.00	0.00	0.00	0.00	0.02	0.01	0.05	0.05	0.01	0.01	0.02	0.02
Bacteria	__	0.14	0.20	0.03	0.09	0.03	0.04	0.06	0.03	0.07	0.07	0.06	0.09
Bacteria	__	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00

Bacteria	__	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.02	0.01	0.00	0.00	0.00
Bacteria	__	0.90	1.01	1.04	1.07	1.27	1.02	1.15	1.24	1.51	1.31	1.49	1.33
Bacteria	__	1.22	0.88	0.84	0.95	1.02	1.54	0.60	1.01	0.52	0.47	0.40	0.32
Bacteria	__	0.10	0.13	0.06	0.08	0.01	0.04	0.03	0.02	0.04	0.09	0.03	0.05
Bacteria	__	2.66	2.65	1.41	1.73	0.65	0.45	0.36	0.33	0.61	0.61	0.53	0.48
Bacteria	__	0.14	0.13	0.17	0.18	0.11	0.12	0.17	0.12	0.03	0.00	0.00	0.01
Bacteria	__	0.00	0.00	0.10	0.03	0.04	0.02	0.19	0.07	0.07	0.02	0.03	0.02
Bacteria	__	0.42	0.42	0.64	0.52	0.55	0.56	1.14	1.15	1.14	0.95	1.71	1.88
Bacteria	Other	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.01
Bacteria	__	4.75	5.25	3.63	3.71	1.95	1.99	1.83	1.62	2.66	3.21	2.82	2.71
Bacteria	__Acidimicrobiales	10.4	10.62	11.23	11.35	4.87	4.96	4.38	4.59	4.93	5.02	5.05	4.89
Bacteria	__Actinomycetales	8.48	8.51	11.50	10.25	11.55	10.85	8.74	7.03	5.20	5.31	5.34	5.17
Bacteria	__Coriobacteriales	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01
Bacteria	__Gaiellales	8.33	8.49	6.95	6.04	2.65	2.62	1.36	1.45	0.20	0.20	0.21	0.36
Bacteria	__Nitriliruptorales	0.00	0.00	0.01	0.00	0.01	0.03	0.09	0.07	0.03	0.04	0.00	0.03
Bacteria	__Rubrobacterales	0.03	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	__Solirubrobacterales	2.49	1.89	2.01	2.03	2.84	3.00	4.14	5.21	2.85	2.65	4.61	3.89
Bacteria	__	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00

Bacteria	__Armatimonadales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00
Bacteria	Other	0.00	0.00	0.00	0.00	0.05	0.01	0.02	0.02	0.01	0.02	0.05	0.01
Bacteria	__	0.39	0.21	0.17	0.13	1.72	2.41	2.09	2.08	1.72	1.71	1.74	1.62
Bacteria	__	3.19	3.30	1.24	2.25	0.85	0.85	0.45	0.49	0.71	0.62	0.63	0.56
Bacteria	__"Bacteroidales"	0.00	0.00	0.00	0.00	0.19	0.35	0.26	0.24	0.19	0.10	0.10	0.12
Bacteria	__Cytophagales	0.03	0.02	0.03	0.05	0.36	0.30	0.69	0.73	0.52	0.61	0.46	0.52
Bacteria	__"Flavobacteriales"	1.91	1.70	3.47	2.31	7.68	6.52	7.99	8.17	11.72	11.71	9.50	9.94
Bacteria	__"Sphingobacteriales"	0.93	0.99	0.43	0.53	1.43	1.61	1.25	1.29	2.18	2.10	1.86	2.08
Bacteria	Other	0.87	0.79	0.59	0.46	0.48	0.46	0.39	0.45	0.42	0.43	0.39	0.33
Bacteria	__	3.85	3.30	4.07	3.61	3.73	4.69	6.19	7.21	6.71	6.76	8.22	7.93
Bacteria	__	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00
Bacteria	__Anaerolineales	0.81	0.62	0.86	0.68	3.83	4.64	8.37	8.64	6.35	6.14	5.05	5.20
Bacteria	__Caldilineales	0.99	0.85	0.43	0.39	1.05	1.50	1.48	1.50	1.44	1.35	1.23	1.30
Bacteria	__"Chloroflexales"	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Other	0.02	0.02	0.05	0.02	0.27	0.26	0.29	0.27	0.30	0.27	0.17	0.19
Bacteria	__	0.13	0.22	0.23	0.06	0.06	0.06	0.08	0.12	0.00	0.02	0.13	0.17
Bacteria	__Sphaerobacterales	1.02	0.75	0.68	0.55	1.19	1.16	1.06	1.12	0.96	0.73	1.02	0.85
Bacteria	__Deferribacterales	0.00	0.00	0.00	0.00	0.03	0.03	0.10	0.07	0.01	0.01	0.01	0.03

Bacteria	__Deinococcales	0.00	0.00	0.01	0.00	0.00	0.01	0.03	0.02	0.02	0.01	0.09	0.13
Bacteria	__"Fusobacteriales"	0.00	0.00	0.00	0.00	0.06	0.08	0.03	0.01	0.10	0.03	0.06	0.02
Bacteria	__Gemmatimonadales	1.37	1.58	2.45	2.36	1.05	1.19	1.03	1.02	1.07	1.25	1.51	1.51
Bacteria	__	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	__Phycisphaerales	0.01	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00
Bacteria	Other	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.00	0.01	0.02	0.00	0.01
Bacteria	__	0.04	0.09	0.00	0.02	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.02
Bacteria	__Planctomycetales	1.37	1.74	1.22	1.37	1.16	1.05	0.94	0.88	1.01	1.06	0.93	0.86
Bacteria	Other	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.01
Bacteria	__	0.00	0.00	0.00	0.00	0.05	0.04	0.03	0.04	0.06	0.14	0.06	0.06
Bacteria	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.01	0.01	0.01	0.01
Bacteria	__	1.60	1.50	0.79	0.98	1.09	1.28	0.67	0.81	0.61	0.61	0.62	0.66
Bacteria	__Alphaproteobacteria_incertae_sedis	0.04	0.00	0.02	0.01	0.10	0.10	0.09	0.06	0.07	0.07	0.03	0.04
Bacteria	__Caulobacterales	0.24	0.17	0.14	0.20	0.32	0.37	0.15	0.24	0.08	0.06	0.08	0.06
Bacteria	__Rhizobiales	10.01	11.44	6.04	8.77	4.98	4.02	5.14	4.60	4.38	4.45	4.38	4.48
Bacteria	__Rhodobacterales	0.63	0.72	0.20	0.16	1.38	1.13	0.79	0.83	1.58	1.66	1.19	1.23
Bacteria	__Rhodospirillales	1.50	1.76	1.65	2.02	1.88	1.90	1.86	1.60	1.41	1.44	1.33	1.26

Bacteria	__Sneathiellales	0.02	0.00	0.00	0.00	0.02	0.05	0.09	0.03	0.11	0.08	0.03	0.01
Bacteria	__Sphingomonadales	1.64	1.50	2.18	1.62	2.05	3.00	1.30	1.75	0.97	0.87	0.77	0.93
Bacteria	Other	0.00	0.03	0.02	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00
Bacteria	__	0.64	0.68	0.44	0.44	0.32	0.30	0.38	0.41	0.39	0.27	0.36	0.40
Bacteria	__Burkholderiales	0.40	0.74	0.59	0.65	0.95	0.61	0.87	0.61	0.77	0.76	0.71	0.89
Bacteria	__Gallionellales	0.01	0.00	0.00	0.00	0.02	0.03	0.22	0.17	0.01	0.01	0.02	0.01
Bacteria	__Hydrogenophilales	0.00	0.00	0.01	0.01	1.20	1.20	2.44	2.46	1.52	1.45	1.62	1.65
Bacteria	__Methylophilales	0.00	0.00	0.00	0.00	0.15	0.19	0.09	0.12	0.18	0.12	0.16	0.25
Bacteria	__Nitrosomonadales	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.05	0.05	0.03	0.06
Bacteria	__Rhodocyclales	0.08	0.02	0.00	0.03	0.16	0.16	0.06	0.07	0.31	0.35	0.19	0.18
Bacteria	__	0.27	0.34	0.25	0.33	0.34	0.33	1.52	1.20	0.84	1.01	0.88	1.00
Bacteria	__Bdellovibrionales	0.08	0.06	0.22	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	__Desulfobacterales	0.00	0.00	0.01	0.00	0.36	0.29	0.69	0.49	0.35	0.41	0.26	0.26
Bacteria	__Desulfovibrionales	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00
Bacteria	__Desulfuromonadales	0.46	0.45	0.56	0.57	1.12	1.07	1.43	1.48	1.44	1.50	1.33	1.42
Bacteria	__Myxococcales	0.46	0.66	0.38	0.35	0.39	0.45	0.89	0.92	0.54	0.60	0.51	0.54
Bacteria	__	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Bacteria	__Campylobacterales	0.00	0.02	0.06	0.13	2.75	2.83	2.35	2.56	1.55	1.16	0.77	0.96

Bacteria	Other	0.01	0.02	0.00	0.02	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.01
Bacteria	__	2.38	2.82	1.31	1.95	2.47	2.38	3.71	3.44	4.46	4.52	4.15	3.63
Bacteria	__"Enterobacteriales"	0.07	0.11	0.55	0.65	0.14	0.02	0.00	0.06	0.09	0.08	0.03	0.03
Bacteria	__"Vibrionales"	0.00	0.00	0.00	0.00	0.14	0.06	0.08	0.08	0.01	0.00	0.01	0.00
Bacteria	__Aeromonadales	0.57	0.40	1.38	2.51	1.20	1.03	0.08	0.02	0.55	1.17	1.17	1.89
Bacteria	__Alteromonadales	0.08	0.03	0.60	0.54	1.30	0.63	1.21	1.76	4.53	4.97	8.17	6.30
Bacteria	__Chromatiales	0.07	0.04	0.00	0.02	1.03	0.45	0.34	0.24	0.16	0.13	0.13	0.13
Bacteria	__Gammaproteobacteria_incertae_sedis	0.00	0.00	0.00	0.00	0.74	0.78	1.64	1.25	1.11	1.10	0.66	0.80
Bacteria	__Legionellales	1.04	1.14	1.13	1.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	__Oceanospirillales	0.00	0.00	0.00	0.00	0.01	0.04	0.25	0.22	0.02	0.01	0.00	0.01
Bacteria	__Pseudomonadales	1.43	1.30	7.32	4.76	2.87	3.62	1.16	0.97	3.18	3.74	3.21	4.31
Bacteria	__Thiotrichales	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.06	0.06	0.05	0.05
Bacteria	__Xanthomonadales	0.39	0.41	0.50	0.44	0.82	0.58	0.50	0.36	0.82	0.87	0.57	0.63
Bacteria	__Spirochaetales	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.00	0.00	0.00
Bacteria	__Acholeplasmatales	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00
Bacteria	__	0.06	0.01	0.01	0.03	0.02	0.01	0.02	0.03	0.05	0.02	0.02	0.03
Bacteria	__	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00

Bacteria	__Opitutales	0.02	0.02	0.02	0.01	0.03	0.03	0.02	0.01	0.03	0.05	0.02	0.04
Bacteria	__Puniceicoccales	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.02
Bacteria	__	1.02	1.17	1.11	1.58	0.30	0.26	0.11	0.16	0.02	0.01	0.00	0.00
Bacteria	__	0.64	0.60	0.26	0.30	0.18	0.23	0.17	0.14	0.35	0.24	0.17	0.22
Bacteria	__Verrucomicrobiales	0.02	0.02	0.01	0.00	0.19	0.22	0.16	0.14	0.61	0.47	0.39	0.41
Bacteria	__	0.06	0.08	0.05	0.02	0.29	0.28	0.15	0.28	0.28	0.22	0.21	0.24
Bacteria	__	1.81	0.58	0.17	0.57	2.54	2.14	0.05	0.07	0.61	0.42	0.22	0.16
Bacteria	__	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.03	0.01	0.03
Bacteria	__Bacillales	0.54	0.36	2.99	2.48	2.54	2.52	1.96	1.76	0.78	0.81	1.65	1.62
Bacteria	__Lactobacillales	0.00	0.00	0.01	0.03	0.02	0.02	0.01	0.01	0.11	0.08	0.05	0.04
Bacteria	__Clostridiales	0.00	0.00	0.08	0.03	0.76	0.71	0.47	0.43	0.56	0.42	0.30	0.27
Bacteria	__	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Bacteria	__Ignavibacteriales	0.18	0.11	0.06	0.05	0.20	0.16	0.27	0.24	0.22	0.17	0.13	0.15
Bacteria	__	0.11	0.17	0.03	0.06	0.07	0.08	0.02	0.03	0.17	0.13	0.14	0.08
Bacteria	__	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Bacteria	__"Nitrospirales"	0.73	0.69	0.38	0.48	0.09	0.14	0.07	0.04	0.13	0.14	0.13	0.05
Bacteria	__	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.01	0.01	0.01	0.02
Bacteria	__	0.02	0.02	0.01	0.02	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00

Bacteria	__	0.01	0.03	0.00	0.01	0.04	0.03	0.01	0.03	0.00	0.00	0.00	0.00
Unassigned	Other	0.02	0.01	0.03	0.05	0.03	0.01	0.03	0.01	0.01	0.02	0.04	0.03