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**Mapping heavy metal soil contamination and
investigating phytoremediation potential at an ex -
brownfield site in Kent, UK.**

By

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Canterbury Christ Church University

Thesis submitted for the degree

of

MSc by Research

2017

Contents

Abstract	5
Introduction	6
Heavy metal pollution and health	6
The Metals.....	7
Zinc	7
Nickel.....	8
Cadmium	9
Lead	10
Copper	11
Chromium.....	11
Iron	12
Site Description	13
Landfill known effects and problems	14
Brickworks known effects and problems	15
Policy and legislation.....	16
Remediation technologies	18
Remediation methods.....	19
Solidification.....	19
Soil Washing/flushing.....	19
Electrokinetic Remediation	20
Pyrometallurgical separation	21
Phytovolatilisation.....	21
Phytostabilisation.....	21
Phytodegradation.....	22
Phytoextraction	22
Cost of remediation methods	25
Hyperaccumulator Plants.....	27
Soil Properties	27
EC.....	28
Organic Matter	28
pH	29
Magnetic Susceptibility	30
Phytoremediation potential of species.....	31
<i>Trifolium repens</i>	32
<i>Trisetum flavescens</i>	32

<i>Lolium perenne</i>	33
<i>Plantago spp</i>	33
<i>Apiaceae spp</i>	33
<i>Agrostis stolonifera</i>	34
<i>Ranunculus spp</i>	34
<i>Rumex spp</i>	35
<i>Potentilla erecta</i>	35
<i>Vicia tetrasperma</i>	36
<i>Rubus fruticosus</i>	36
<i>Phleum spp</i>	37
<i>Pisum sativum</i>	37
<i>Salvia pratensis</i>	38
Study aims	38
Methods	39
Collection.....	40
Laboratory analysis	41
Soils	41
pH	41
EC.....	41
Organic matter	41
Magnetic susceptibility	42
Digestion.....	42
ICP – OES	43
Matrix matching	44
Statistical Analysis	44
GIS	44
Plants.....	45
Identification	45
Digestion.....	46
ICP – OES	46
Results	46
Discussion.....	53
Zinc	53
Nickel.....	53
Cadmium	54
Lead	54

Copper	55
Chromium.....	55
Iron	56
Soil Properties	56
Organic Matter	56
pH	57
Electrical Conductivity.....	57
Magnetic Susceptibility	58
Soil properties and Heavy metals.....	58
Landfills effect on the site	59
Phytoremediation potential of species.....	59
<i>Trifolium repens</i>	60
<i>Trisetum flavescens</i>	60
<i>Lolium perenne</i>	61
<i>Plantago spp</i>	61
<i>Apiaceae spp</i>	62
<i>Agrostis stolonifera</i>	62
<i>Ranunculus spp</i>	63
<i>Rumex spp</i>	63
<i>Potentilla erecta</i>	64
<i>Vicia tetrasperma</i>	64
<i>Rubus fruticosus</i>	64
<i>Phleum spp</i>	65
<i>Pisum sativum</i>	65
<i>Salvia pratensis</i>	66
Potential hyperaccumulators and remediation methods.....	66
Soil properties and the management method.....	68
Management methods for the site	68
Conclusion	71
Acknowledgements.....	72
References.....	72

Abstract

Milton Creek Country Park was identified as a contaminated public area with a need for remediation due to suspected high concentrations of heavy metal contamination. The extent of heavy metal contamination at Milton Creek Country Park, an ex-brownfield site, was quantified. Once known, the identification of accumulators and hyperaccumulator plants across the site was required to determine if plants that inhabit the site had any ability to remediate contamination: this would allow managers to utilize phytoextraction in order to provide cost-effective remediation of the site, whilst protecting the local ecosystem and providing a safe public green space.

Copper, zinc, nickel, iron, lead and chromium all showed above average concentrations within the soils of the site. Concentrations of lead (1896 ppm), chromium (114 ppm) and iron (44544 ppm) also provided cause for concern due to high peaking locations across the site, which are concentrations of concern to human health. 14 out of the 15 plants showed evidence of accumulation of heavy metal contaminants. *Trisetum flavescens* was the only species able to significantly uptake nickel, lead and chromium which is important in the remediation method. *Lolium perenne*, *Trisetum flavescens*, *Plantago spp* and *Agrostis stolonifera* were selected for the remediation of the site. These species cover a wide range of pH values as well as evidence they cover the remediation of all heavy metals required at the site. This will allow the mix of species to be able to be deployed at any area of the site which requires the most remediation. Other factors are discussed for the method, including the addition of the management of *Rubus fruticosus* by means of fruit removal to prevent the consumption of accumulated iron and the addition of *Trifolium repens* and fencing around areas of lead pollution. These additions are added to provide extra safety for the public, allowing the site to still be used but protecting the public's health from the pollution present.

Introduction

Heavy metal pollution and health

Soil pollution has gained significant traction from researchers and attention from the public over the last few decades (Garbisu and Alkorta, 2001) and even more so in the last few years (Paz-Alberto and Sigua, 2013) thanks to the rapid improvement in education and economic growth and development (Wang *et al.*, 2001). This increased attention towards soil pollution is due to the growth in contamination from military (warfare), industry (factory effluent), research and agricultural (fertilisers) activities (Paz-Alberto and Sigua, 2013). Included in these activities, an increasing amount of industry such as paper mills, land fill, chemical works (Freitas, Prasad and Pratas, 2004) and mining (Facchinelli, Sacchi and Mallen, 2001) contributing to anthropogenic pollution concentrations, have caused a global deterioration of the environmental quality (Garbisu *et al.*, 1997) within soils and the plants inhabiting them.

The term “heavy metals” is used to refer to any metals that in the past have densities that are greater than 4 g/cm³ (5 or more times, greater than concentrations in water) (Hawkes, 1997), or more recently greater than 5 g/cm³ (Jarup, 2003, Oves *et al.*, 2012, Chen *et al.*, 2015). Heavy metals themselves are such problematic contaminants due to their inability to degrade naturally and can remain unnoticed until high in the food chain (Wang *et al.*, 2001). Heavy metals are classified as both essential and non-essential to biological systems (Ali, Khan and Sajad, 2013). Some heavy metals are extremely important to life and are required by plants in relatively small amounts for them to survive, such as their need in the production of metalloproteins (Garbisu and Alkorta, 2001). In high concentrations, however, these metals that are required for health can be poisonous to plants and animals even at very low concentrations (Nagajyoti, Lee and Sreekanth, 2010). This can be due to the displacement of other key ions and minerals causing modifications to protein structures important to the internal mechanisms of the plants themselves (Beveridge and Doyle, 1989). In addition, with the biological half-life of heavy metal contaminants having the potential

to be several decades, they can pose a threat to the health of consumers for a long time after introduction to the food chain (Konotop *et al.*, 2012).

The Metals

Heavy metals, due to synergetic relationships formed between them, can greatly hinder the phytoremediative effects of a plant, if more than one is present (Lone *et al.*, 2008). Due to the nature of contaminations produced by anthropogenic processes such as landfill creation, several different heavy metal contaminants can be present at one time, and this can make phytoremediation of a site extremely difficult (Murakami, Ae and Ishikawa, 2007). Zinc is quite commonly associated with these interactions with other metals. Copper has been shown to have diminished removal efficiency by hyperaccumulators when in the presence of high concentrations of zinc, than if present alone in the soils (Ebbs and Kochian, 1997).

All guidelines of safe concentrations of contamination within the soils are taken from Nicholson and Chambers (2008) apart from iron which is taken from WebMD (2017)

Zinc

Zinc (Zn) as a micronutrient is important to plants in areas such as DNA transcription, nitrogen fixation and the processes related to plant growth and flowering (Sheoran, Sheoran and Poonia, 2016). The anthropogenic source that can produce zinc pollution is mostly related to the emission from smelting processes. Another significant source of zinc pollution is the burning of coal and domestic wastes (such as Milton Creek Country Park), metallurgical industry, municipal sewage, and in tyre wear from car traffic (Rauch and Pacyna, 2009). If these products are not burnt but discarded into landfill sites, they will break down and the zinc contained within will be released into the soils. Zinc is one heavy metal known to be able to be accumulated by many different plants (Kos, Greman and Lestan, 2003) due to their great tolerance of zinc (Dmuchowski *et al.*, 2014). On average, the concentrations of zinc found naturally in plant leaves that are not in the

direct influence of industrial emission can range between 10 and 70 mg/kg. If the content in the leaves reaches > 100 mg/kg, it can have adverse effects on sensitive species (Barker, 2015).

Some identified plants such as *Alyssum spp.* are able to take up above 1% of their dry biomass of zinc (Kos, Greman and Lestan, 2003) and *Thlaspi caerulescens* which has been shown to be able to accumulate concentrations of up to 4% within its dry root weight, without having any negative, poisonous effects on the plant itself (Brown *et al.*, 1995).

Nickel

Nickel (Ni) is a heavy metal pollutant that is highly abundant within the effluent from industries such as tanneries (Guimarães *et al.*, 2016) and waste in landfill (Méndez *et al.*, 2014). Nickel is regularly used in industry, to produce stainless steel, non-ferrous alloys and Ni-based super alloys (Coman, Robotin and Ilea, 2013). Consequently, nickel-containing wastes such as used batteries, wastewater and bleed-off electrolytes are generated, leading to environmental contamination (Coman, Robotin and Ilea, 2013, Denkhaus and Salnikow, 2002).

Soil properties have a major effect on the availability of nickel for uptake by plants. One of the main soil properties to affect the availability of the nickel is the soil pH (Wallace *et al.*, 1977, Sauerbeck, 1991) which goes some way to controlling the solubility and mobility of the nickel within the soils and into plants (Tye *et al.*, 2004). Nickel has had a large amount of research done on its phytoremediation potential of heavy metals, while also being largely focused around the effect that nickel has on wildlife such as mice (Guimarães *et al.*, 2016, Rabelo *et al.*, 2016) and on ground waters (Coman, Robotin and Ilea, 2013). Méndez *et al* (2014) talks in their research about the process of the removal of contaminated soils to land fill. Roccotiello *et al* (2015) also found that in plants such as *Alyssoides utriculata*, nickel uptake either resulted in poisonousness to the plant or stunting of the plant's growth. There are a number of papers such as that by Panwar *et al* (2002) and Kaviani *et al* (2017) that highlight a number of suitable remediating species that are well suited to Ni uptake. However, many are crop plants and not suitable for the remediation of a site such as Milton Creek Country Park.

Nickel is one example of a precious heavy metal that is a target for the use of phytoextraction as a method of phytomining of the metal due to its high value (Brooks *et al.*, 1998). This gives an added incentive for sites that have high contamination of nickel pollution, to use a phytoextraction method of removal in order to gain a monetary gain from its application.

Cadmium

Cadmium (Cd) pollution had drastically increased during the latter part of the 20th century and is still at high concentrations in areas today. One of the main reasons for this is cadmium in products such as stabiliser within PVC products, a colour pigment, within several alloys (Jarup, 2003) and most commonly in re-chargeable nickel–cadmium batteries which are rarely re-cycled, but often dumped along with household waste (Jarup, 2003). Cadmium metal is known for its ability to reside and stay within soils of a contaminated area without degrading for several decades (Christensen, Jensen and Christensen, 1996) such as Milton Creek Country Park with its many historical land uses. However, when living plants are present, it has an extremely high affinity for soil to plant transfer (Satarug *et al.*, 2011) and as such will collect in the fruit and stems of plants much more readily and quickly than most other heavy metal pollutants (Satarug *et al.*, 2011). However, according to Blum (1997), cadmium taken up by plants decreases in concentration as it moves up from roots to stem to leaves to fruit and lastly to seed. However, as many grasses are eaten by animals lower on the food chain, cadmium can still get into the human diet.

Trace amounts of cadmium have been seen to be beneficial to higher plants to achieve optimal growth (Verbruggen, Hermans and Schat, 2009). In most plants however, cadmium is non-essential and can have poisonous effects on many systems. As a result, large amounts of nutrient deficiency (Irfan *et al.*, 2013) can occur within plants exposed to high amounts of cadmium. Cadmium has also been said by Yang *et al.* (1996) to have an effect on the uptake of many other heavy metals such as iron, zinc and copper by lowering the amount that was accumulated.

According to Pinto *et al* (2004) and Prince *et al* (2002), Cadmium is also an important metal with regards to its ability to accumulate in high concentrations within plant tissues to concentrations that are poisonous to animals and humans, but without becoming phytotoxic to the plant itself. This high concentration of uptake is commonly identified within edible crop plants that are predominantly only grown to be eaten, such as lettuce (Crews and Davies, 1985), linseed (Marquard, Böhm and Friedt, 1990) and celery (Ni, Yang and Long, 2002). This means if used for remediation, they are then unable to be used for their prime use and as such the cost is increased if the product cannot be sold after phytoremediation and harvesting.

Lead

Lead (Pb) is found in soils through natural weathering processes of deposits via acid rain which in itself causes a base natural concentration of lead to be present in many soils nationally (Sharma and Dubey, 2005). The main sources of lead pollution are from emissions from manufacturing goods and vehicles, products sent to landfill such as batteries, industry that requires lead or discard lead by-products (Eick *et al.*, 1999). Other sources that can cause contamination to a lesser extent are mining and smelting of lead ores or compounds, metal plating, fertilizers and pesticides on agricultural land and additives in pigments found in paints (Eick *et al.*, 1999). A widespread environmental contaminant, lead is a highly poisonous heavy metal pollutant that due to its extensive uses, means it has become extremely widespread (Sharma and Dubey, 2005).

Lead is most abundantly found in the top most surface layers of soils in a polluted area and lead content of the soils are found to be significantly increased in cultivated soils near industrial sites (Abreu, Abreu and Andrade, 1998). The pH of the soil that lead resides in has a major effect on the solubility of the metal with solubility being low at neutral or alkaline concentrations (Huang and Cunningham, 1996). With the optimum pH of lead solubility being between 5 and 6.5 (Chlopecka *et al.*, 1996), this makes the application of plants for its phytoremediation a challenge with many plants preferring neutral 7 or 8.

Copper

Concentrations of copper (Cu) in topsoils within Europe vary within a wide range of between 0.8–256 mg/kg with the world average ranging between 14 -30 mg/kg (Karczewska *et al.*, 2015). This variance is strongly related to the regional and local geology of the areas with copper sources including the weathering of copper parent materials (Karczewska *et al.*, 2015). However, additional impacts from anthropogenic pollution such as emissions and copper metallurgy can also cause these concentrations to vary more and reach much higher concentrations (Svoboda, Zimmermannová and Kalač, 2000).

Copper is one nutrient that is essential to the many processes within plants including photosynthetic and respiratory electron transport chains, ethylene sensing, cell wall metabolism and in oxidative stress protection (Yruela, 2009). It is also essential for the microorganisms in the soils that form mutualistic relationships with the plants roots (Cornejo *et al.*, 2013). However, it is extremely poisonous if present in large quantities (Cuillel, 2009), especially to the roots of higher plants (De Vos *et al.*, 1991). The poisonousity of copper comes from its ability to form reactive oxygen species and the way it impairs proteins required for important key cellular processes, causing the inaction of enzymes and changing proteins structures (Yruela, 2009).

Chromium

Chromium (Cr) has been shown to be beneficial to the growth of some plant species in trace amounts (Samantaray, Rout and Das, 1998), however in higher amounts is highly poisonous to both plants and animals (Costa and Klein, 2006). Chromium occurs in several oxidation states in the environment ranging from Cr²⁺ to Cr⁶⁺ (Rodríguez *et al.*, 2007) and is the seventh most abundant element on the planet (Zhang, 2012). Chromium is widely used in industries such as metallurgy, electroplating, production of paints and pigments, wood preservation, chemical production and pulp and paper production (Ghani, 2011). All of these products can end up in land fill sites (Ghani, 2011) and come from industries, which discard their waste products and contaminate the area.

Chromium (VI) compounds, such as zinc chromates and lead chromates, are highly poisonous and carcinogenic in nature (Jaishankar *et al.*, 2014). Chromium (III) and Chromium (IV) can easily pass through the cell membranes of plants and animals (Jaishankar *et al.*, 2014). The presence of excess chromium that reaches beyond the limited concentrations can be extremely destructive to many of the plant's biological processes. Leaf biomass and number is seen to be reduced (Karunyal, Renuga and Kailash, 1994), plant growth and height has been seen to be reduced (Mei, Puryear and Newton, 2002) and a reduction in germination of seeds, root length and dry weight (Prasad, Greger and Landberg, 2001). Because it can so easily be taken up in the cell membrane of the plant's roots and shoots, it enters the food chain on consumption of these plant materials (Jaishankar *et al.*, 2014).

Iron

Iron (Fe) is one of the most critically crucial elements in the natural world for the survival and sustained growth of every living organism (Valko, Morris and Cronin, 2005). It is vital for enzymes such as catalase on enzymes required for the transport of oxygen (Vuori, 1995). Just like chromium, iron also readily converts between being ferrous and ferric ions (Phippen *et al.*, 2008), which helps facilitate its uptake into the various biological processes that go on within the plants. Iron, in its various forms, is the second most abundant metal that can be found both on the surface of the planet (EPA, 1993) and predicted to be abundant at deeper concentrations throughout the rest of the planet as well. The huge scale of iron processing, equaling several million tons in many places worldwide, determines and creates both local and broad scale contamination issues (Baldantoni *et al.*, 2014) and as this industry grows, so does the issue of iron contamination.

Site Description

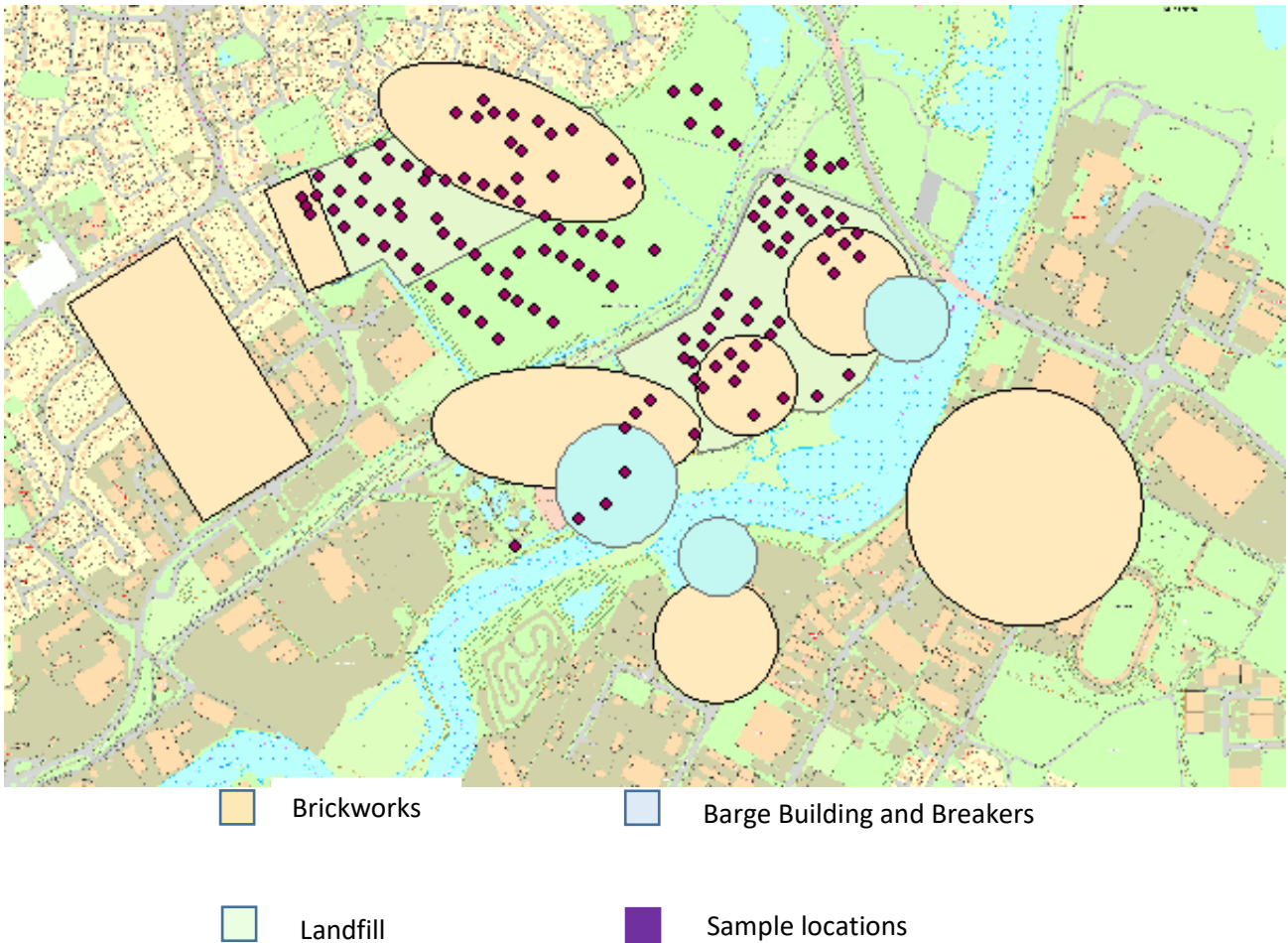


Figure 1 – Map of Milton Creek Country Park with historical industrial use

Milton Creek Country Park is a public green area in Kemsley, near Sittingbourne that is situated alongside the River Swale (51°21'17.3"N, 0°44'48.6"E). The ex-brownfield site started development into a public park in 2003. It has a wide range of past industrial uses: it is situated on old shipwright yards, brick works, a capped off land fill site and is downstream from a paper mill, as well as still being near industrial buildings and an active waste sorting site. During the 19th century, Milton Creek was central in Sittingbourne, supporting the town's paper mills and brickmaking as well as barge building industries. Early in the twentieth century an out port was developed, at the seaward end of the creek. In 1919 it was linked to Sittingbourne by a narrow gauge light railway, which became part of a dense network serving the brickfields and the docks

around Milton Creek. The twentieth century saw further changes to the creek; brick production stopped, Thames Barge building and commercial shipping ceased in the 1920s and, by 1969, the railway ceased to function. Many of the former quays became semi derelict and Milton Creek started to become inaccessible (Sheilsflynn, 2011). The area was then utilized for the landfill sites, being the site's latest industrial use in the late 1990s till 2003. As a result, it is the landfill which is thought to be the source of the high concentrations of pollution recorded during the repurposing of the site (see Figure 1).

There is no research around heavy metal contamination from barge building from the dates the industries were active. As such, theories can be suggested as to what heavy metals may be produced but more evidence is required. One that stands out is that iron is produced in large quantities from barge production. If barges were left to rust or broken down, high concentrations of iron might be left in the soils. Along with the rusting of old equipment from both the brick fields and barge building, concentrations of commonly used metals such as iron and copper as well as lead could be present in high quantities in areas where these industries resided. There are however, large amounts of research regarding heavy metals and landfill.

Milton Creek Country Park went through a major transformation during its conversion from an industrial brownfield site to a greenfield public park. During this transformation of the site, features such as the uneven landfill and the foundations of the old factory on the site had to be covered over. To do this, soil was brought in from different sources. However, these sources have not been recorded. As a result, the top most layer of the site's soils is made up of a mosaic of different potential soil types which could have effects on the soil properties that are affecting any contamination in the area.

Landfill known effects and problems

Landfill is a widely-accepted method of disposal for waste all over the world. This is largely because of its low operational costs as a whole (Xiaoli *et al.*, 2007). Municipal landfills can contain a mixture of household, commercial and industrial waste and can

often produce leachate with heavy metal pollutants with concentrations in to 1000's of parts per million (Baun and Christensen, 2004), however these concentrations can vary. Remon *et al* (2005) discuss that for an accurate risk assessment to be carried out on the risks a landfill can pose, it would have to take into account the risk of the heavy metal contamination transferring both down to the water table, and into vegetation and soil on top of the contaminated site via mobility of heavy metals in pooling water within the landfill over time. Due to this transfer, this study will quantify the concentration of contaminants in the topsoil and in plants in order to assess the risk posed. The limiting factor to this is that, as with this study, only the top 10 cm of soils could be collected, whereas if a core of up to 50 cm could be taken, an analysis of the change in concentration and thus the dispersal of the heavy metals up towards the top soils could also be carried out.

Remon *et al* (2005) go on to discuss that the amount of leaching that occurs from the landfill is closely dependent on the heavy metal concentration within the landfill from the wastes that had been permitted, and where within the landfill this resides. However, it will also depend greatly on the soil characteristics and on the plant species that are present (Welch, 1995; Ernst, 1996). Remon *et al* (2005) continue by saying that experiments should be aimed towards analysis of the plant communities that inhabit the site. This should be done by looking at species diversity, symptoms of poisonosity that they are expressing (yellowing or stunted growth etc.) and metal concentrations in above ground tissues, within different structures of the plants where the metals accumulate. As such, a method of assessing the landfill contamination via plant analysis has been implemented in the research within this paper, allowing not just for the correct analysis of the site via multiple methods of both plant and soil analysis, but also gaining an understanding of potential plants that are being utilised for their phytoavailability of the heavy metals.

Brickworks known effects and problems

The brickworks and fields at Milton Creek Country Park went through a number of stages and locations between 1835 and 1995 where they produced a mix of bricks and cement,

however the brickworks were moved off site during multiple selling of the company in the late 1800's and manufacturing was almost completely ceased as a result of the world wars (Miltoncreekmemories.co.uk, 2018). With the works producing concentrations of up to 50 million bricks a year, it could be estimated that there would be large amounts of particulate and metal pollution (Miltoncreekmemories.co.uk, 2018).

Contamination as a result of brick firing in kilns, as well as the drying process, is thought to be due to metals that both reside in the clays used in the making process and in the fuels used for heat (Passant et al., 2002). During the processes, heavy metals including lead, chromium, cadmium, and nickel have all been seen to be produced as particulates in the air (US EPA, 2000). These particles then settle in the surrounding areas meaning that pollution of this type can have an effect on areas a distance from the original source.

Policy and legislation

Different heavy metals have different natural concentrations that appear within soils. These natural concentrations will greatly depend on the parent geology of an area (Alloway, 2012). However, these concentrations change due to anthropogenic causes, either by increasing concentrations of metals above the background concentration or by adding heavy metals that should not naturally be there (lead and chromium for example) (Alloway, 2012). Under these circumstances, concentrations have to fall below concentrations set in EU legislation that determine if an area is safe to be in regarding the concentrations of different pollutants within soils (European Commission 2006), waters (European Parliament, 2000b) and the air (European Parliament, 2000a). In the EC Directive 86/278/EEC, only 6 heavy metals are given limit values, these being cadmium, copper, nickel, lead, zinc and mercury (European Communities, 1986). This only represents a small proportion of heavy metals that can pose a threat to health and plantlife.

Pax-Alberto and Sigua (2013) state that it is ignorance, lack of vision and carelessness of governments and district councils making the decisions regarding policies and implementation of remediation methods that cause issues around pollution of the sites. It is stated in the national planning and policy framework that for an area to be utilized

for a new means, the area must first undergo suitable treatment in order to make it suitable and safe for the area's new purpose (National Planning Policy Framework, 2012). The industries that resided on the site of Milton Creek Country Park were allowed to pollute the site with very little consequence. This is true for many areas around the world that had industrial processes being carried out upon them before reclamation to other means (Hanlon, 2015). This could be due to poor protection for the sites at the time of building, for example, with docklands, many pieces of scrap would be left to decay without the threat of repercussions (Hanlon, 2015). Similarly, past studies have shown that landfill sites which have been converted before more recent regulations were put in place might not have adequate protection to prevent leachate causing contamination of the surrounding soil (Bouzayani *et al.*, 2014) due to pooling water within contaminated areas. Milton Creek falls into this category: although remediation was first carried out on the site in order to turn it into a public park, appropriate policies and legislation were not in place to make the land fit for purpose, leading to the contamination of the site a decade in the future.

It has already been documented by the local government that Milton Creek Country Park, having been the site of many different industrial practices and processes, suffers from heavy metal contamination. However, an up-to-date and thorough investigation is required to quantify the contamination and then examine potential solutions, taking into account factors such as feasibility and cost. The health implications for people who are using the park for sports, recreation and particularly foraging on the site (e.g. for blackberries) raises great concern for the public's wellbeing (Carr *et al.*, 2007). With the capping of the landfill that resides underneath the park having been completed before a change in legislation, there is a potential issue of the leaching of the heavy metal pollutants out of the landfill and into the soils. This would be due to rainwater being able to reach the landfill waste and the pollutants getting into the water. This would then move the pollutants up into the soils, where the plants are then able to take them up, or into the River Swale that runs along the outer perimeter (Rattan *et al.*, 2005). As such these pollutants need to be removed in a safe, low cost and environmentally friendly way that will not cause more harm to the local area. Seeing as different metals

have different threshold values above which they are considered to pose a danger to the public, each metal has to be looked at on an individual basis.

Heavy metal contamination within the food chain deserves special attention in order to prevent this negative effect on not only humans but other animal life as well (Memon and Schröder, 2008). Attention should be focused on the plants found in contaminated areas that could be taking up the heavy metals, as by removing the contaminants from the primary producer concentration of the food chain will prevent the contaminants from entering or bioaccumulating at any other trophic concentration (Konotop *et al.*, 2012). A growing percentage of crops grown on contaminated soils are themselves becoming contaminated through the uptake into plant tissues and are subsequently causing more health issues through ingestion (Kos, Greman and Lestan, 2003). There is a large body of research on the health implications of the ingestion of various heavy metals on humans: copper, zinc, iron and cadmium all showed evidence of having negative or detrimental effects on human health (Jarup, 2003; Jaishankar *et al.*, 2014).

Remediation technologies

Phytoremediation is a natural and sustainably viable remediation technology of pollution management that has increasingly been implemented and is producing considerable research and economic interest all over the world (Pillai *et al.*, 2013).

Phytoremediation uses the application of plants and soil microbes that are associated with them to help reduce the concentrations or poisonous effects of contaminants in the environment (Greipsson, 2017). This process allows for the removal of contaminants such as heavy metals and organics that are residing in the soils and surrounding water bodies.

Phytoremediation of heavy metal pollutants in former brownfield sites such as landfill (Porębska and Ostrowska, 2006), mines (Fitamo and Leta, 2010) and mills (Mazumdar and Das, 2014) has been seen to be an effective way of removing such pollutants in a relatively short space of time. There are a number of other methods of remediation of heavy metals. These include the removal of the soils into areas where they can be

chemically treated, the use of electric charge to remove the metals directly from the soil and the solidification of the soils to stabilise the metals within (Mulligan, et al, 2001).

As phytoremediation methods are seen as a more environmentally ethical way of remediating a site due to its least disturbance to the surrounding wildlife and allows the area on the whole to still be utilised with minimum need for restriction of accesses, phytoremediation is the main focus of the research.

Remediation methods

Solidification

Solidification is the containment of a contaminated area with the physical encapsulation of the contaminants in a solid, which reduces the mobility of the heavy metals and prevents the take up of them by plants (Mulligan, Yong and Gibbs, 2001). Mulligan *et al.* (2001) goes on to say that this process has full scale applications for arsenic, lead and chromium, which are all metals known to be particularly hard to remediate due to their bonding and relationships with the surrounding soils. A more energy intensive version of solidification called vitrification can also be used that requires the addition of thermal energy. This involves electrodes that introduce thermal energy which help with the solidification process as they cool. However, with this process a number of other effects can occur that can cause problems and effect the efficiently. Glassy solids can form within the soils which can cause problems when it comes to extraction. Poisonous gases can also be given off which could then be released into the atmosphere and if done in situ, could pose a health threat to the local wildlife and human populations (Mulligan et al., 2001).

Soil Washing/flushing

Soil washing is the removal of heavy metals by the addition of chemicals such as hydrochloric acids or inorganic acids that lower the soils pH (Mulligan, et al. 2001). Soil flushing is the use of water to flush though the contaminated soils in order to leach the

contaminants from the soils. The water is then removed as it percolates through trenches and drains into the area (Mulligan, et al, 2001). In order for soil washing to take place, the soils must be removed from the site, costing large amounts of money for the process. Soil flushing doesn't require the soils to be removed from the site, however its success is highly dependent on a lot of factors. In order for this remediation technology to be successful, it is important that there is a high understanding of the hydrology of the site and the chemistry of the binding of the heavy metals within the soil. If this isn't known then the process cannot succeed due to a lack of hydrolyzed heavy metals (EPA, 1992).

Electrokinetic Remediation

Electrokinetic soil processing is the passing of a low-concentration current using electrodes across a cross section of the contaminated site. It can also be referred to as electrokinetic remediation, electroreclamation, and electrochemical decontamination (Kim, 2001). The success of electrokinetic remediation is highly dependent on the type and properties of the soils that the contamination is in, as well as the ionic state of the heavy metals present (Pamukcu and Wittle, 1992). Wang *et al* (2005) stated that they found that acidifying the soils below a pH 2.7 increased heavy metal removal. This concentration of pH is not a natural pH concentration for most plants to live in and so in an actively used park site, this type of remediation could be seen as impractical due to the requirement of plants for aesthetic reasons. Due to this dependence on the soil and ion conditions, studies show varying degrees of success with different metals. Kim (2001) publishes a success rate of 90% for the majority of metals. However, Pamukcu and Wittle (1992) show negligible concentrations of iron removal in all conditions they studied. In order for the metal to be physically removed from the soils after the attraction to the electrodes, other methods including the removal of the soil by pumping out have to be implemented. Although the cost will be lower due to the contamination being condensed into a smaller area, this process still costs a lot of money and so raises the cost of the remediation technology considerably (Kim 2001).

Pyrometallurgical separation

Pyrometallurgical separation uses high temperatures to volatilise heavy metals in contaminated soil. The contaminated area is removed from the site and placed into furnaces (Mulligan, et al, 2001). Temperatures of 200–700°C are used to evaporate and volatilise the contaminants. After volatilisation, metals are then recovered for re-use or immobilized. For some metals, such as lead, cadmium and chromium, fluxing or reducing agents might have to be added in order for the metals to reach melting point and to help with a uniform reaction (Mulligan, et al, 2001). Due to the high resource input for this process, to make its application cost effective, highly contaminated soils (5-20%) where the metals that are recovered can be profitable for the organisation (Mulligan, et al, 2001) are the only instances this can be used.

Phytovolatilisation

Phytovolatilization of heavy metals is the plant's ability to absorb through the roots and subsequently volatilize the contaminant into the atmosphere (Tangahu *et al.*, 2011). Some heavy metals can be biomethylated to form volatile molecules. These molecules can then be lost to the atmosphere through the leaves of the remediating plant (Raskin, Smith and Salt, 1997). The knowledge that plants are capable of phytovolatilisation (Raskin, et al, 1997) is relatively new, however, the role that microorganisms play and their importance in volatilization from soils has been known for much longer (Karlson and Frankenberger, 1989). Phytovolatilization is limited in its practical use due to the pollutant not being able to be collected and removed from the area completely. It is transferred from soil to atmosphere from where it can be redeposited in the soil again (Ali, et al, 2013).

Phytostabilisation

Phytostabilisation is the ability for the roots of a plant to take up, chemically reduce and then deposit or precipitate heavy metal pollutants in large quantities (Raskin *et al* 1994). It is also important that the roots of the plants use high root biomass and

although take up the metals in their roots, do not translocate this into the shoots (Alvarenga *et al.*, 2008). This process can be utilised in order to reduce the bioavailability within the soils and, as a result, prevent the heavy metal contaminants from being able to be introduced into the food chain or from washing into ground waters. Raskin *et al* (1994) goes on to say that remediation technologies such as this are greatly important in reducing the risks that heavy metal pollution can have to human health.

Phytodegradation

Phytodegradation is the breakdown of contaminants from the soils taken up by plants through many metabolic pathways within the plant itself. It can also take place as the breakdown of contaminants externally to the plant within the soils, through the effect of compounds produced by the plants extruded into the soils. (Tangahu *et al.*, 2011). Due to its nature, Phytodegradation is a limited method, able only to remove organic pollutants including synthetic herbicides and insecticides because heavy metals are non-biodegradable (Ali, Khan and Sajad, 2013).

Phytoextraction

Phytoextraction is the uptake of a contaminant from soils, or from water by the roots of plants and then being translocated to different tissues within the plant structure that are harvestable (Bhargava *et al.*, 2012). The overall aim is the removal of the pollutants in order to achieve long-term cleanup of the polluted sites (Sas-Nowosielska *et al.*, 2008). Utilising the removal of the harvestable parts of a plant to remove pollutants from the soils is a great step towards an environmentally friendly, green method of cleaning pollutions such as heavy metals (Luo, Shen and Li, 2005). With this ethos in mind, it is revolutionary within the world of pollution removal, with many recent studies around phytoextraction demonstrate the feasibility for its application as a commercially viable method of heavy metal removal (Escande *et al.*, 2014, Robinson, Anderson and Dickinson, 2015). If phytoextraction technologies are able to become economically viable, then they will be able to directly compete with other more

intensive methods of removal, such as the physical removal of the soils or the chemical treatment of the soils themselves *in situ* (Robinson, Anderson and Dickinson, 2015).

According to McGrath and Zhao (2003), phytoextraction is determined by two main factors, the biomass production and the metal bioconcentration factor. The bioconcentration factor is the measure of how well a plant can take up and subsequently transport heavy metals to the shoots, where it is easy for them to be collected for research and removal. This is done by the ratio of metal concentration in the plant shoots with the metal concentrations that are present in the soils. There are other uses and benefits that are gained by the use of phytoremediation technologies and not just pollution removal. Precious and expensive metals that have been lost to the ground such as gold (Maluckov, 2015), nickel (Anderson *et al.*, 1999) and platinum, can be collected (Kidd *et al.*, 2009) with the help of microorganisms to dissolve the target minerals (Maluckov, 2015), and recycled in a process known as phytomining, allowing for the exploitation of ore bodies which would have been deemed uneconomical to mine with the use of the more conventional methods (Brooks *et al.*, 1998).

Sheoran *et al* (2016) discussed in a review the main factors which have the biggest effect on phytoextraction. The first 4 factors - fast growth and high biomass, large root systems, a high tolerance for large concentrations of heavy metals within their plant tissues and a high translocation potential - all focus around the plant just being adapted to being good at removing metals in general, which would be expected of in a phytoremediator. The last two factors - adaptability to sites/environments and easy to manage in an agricultural setting - are the factors that allow this management method to be a viable, useful, economic choice for remediation of heavy metals (Kuppens *et al.*, 2015). Felix (1997) stated that the most limiting of these factors to the effectiveness of a accumulator is the availability of heavy metals to the plant roots in the soils. This means that a phytoextractor can be the most promising specimen in lab trials, but if heavy metals in the soils are not in a bioavailable form that is readily available to be taken up by the plants roots, it can no longer be an effective phytoextractor (Felix, 1997).

Heavy metal contamination is particularly hard to remediate. While other contaminants can be degraded into less poisonous compounds or volatilised as a gas, heavy metal contaminants cannot (Lasat, 2002). However, phytoextraction has been shown to work with the removal of heavy metal contaminants from soils. Phytoextraction allows for the heavy metals to be taken up and concentrated into the roots and shoots of the plants used. These plants can then be harvested and the heavy metals removed from the site of contamination completely (Garbisu and Alkorta, 2001). Depending on the concentrations of heavy metals within the plants, disposal processes can either be as a biofuel by the drying and ashing of the plants, or if concentrations are high and enough plant material is present, particularly sort after and expensive heavy metals can be collected from the plants in a recycling and reclamation scheme (Garbisu and Alkorta, 2001). For the metals that are suspected to be polluting the site, phytoextraction is seen to be the best suited for the remediation of the site. Phytoextraction, due to the pollutants that it takes up and the non-disruptive nature of the remediation is an ideal candidate for Milton Creek Country Park

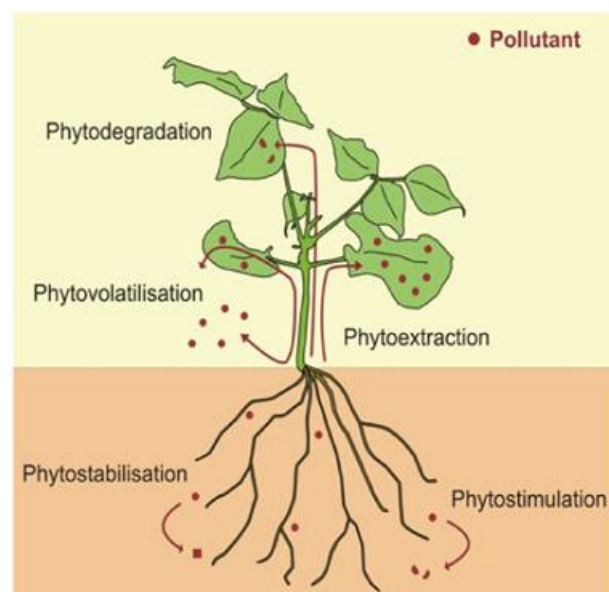


Figure 2 – Diagram showing the different processes heavy metals can go through in a plant (Pilon-Smits, 2005)

Cost of remediation methods

One of the biggest appeals of the use of phytoremediative methods for the removal of contaminants is the lower cost it carries as well as its intrinsic environmentally friendly application (Lasat, 2002, Tangahu *et al.*, 2011, Greipsson, 2017). Using bioremediation as a method for the cleaning of polluted sites can greatly reduce clean-up costs. This is because it treats the contamination *in situ*, uses the natural energy production of the sun and requires only the energy to set the plants out and reduces stresses on other areas of the environment by both the removal of the pollution from the site but also meaning there is little need for machinery (apart from tilling and harvesting) that can cause massive disruption to an ecosystem (Chapelle, 1997). Cunningham, Berti and Huang (1995) stated that the used of phytoremediation methods on average costs between \$0.2 – \$1 per m³ per year. This cost is substantially lower than the costs that can be involved with the use of other methods of removal of contaminants of the soil, which can range between \$10 and \$3000 per m³ per year, depending on how intensive the method of removal must be and what poisonous compounds are present in the soils. However, Glass (2000) with phytoremediation technology being a relatively new method and technology, costing can be hard. They went on to say that predictions can be made of phytoremediation being 50 to 80% less than alternative remediation methods, however again this will vary depending on the plants used and the rate at which the plants manage to remediate in that environment (Glass, 2000). A comparison of the different remediation costs is shown in table 2. Due to its significantly low cost, phytoremediation methods are the best to be implemented with Milton Creek Country Park as there is a lack of funding for large scale, more expensive methods for the cleaning of the site.

Technology	Description	Applicability	Costs (\$US/ton)
<i>Containment</i>			
Physical	Prevent movement by preventing fluid flow	Landfill covers and slurry walls	10–90
Solidification	Creation of an inert waste	Injection of solidifying chemicals	60–290
Vitrification	Application of electrical energy to vitrify contaminant	Shallow metal-contaminated soil, low volatility metals	400–870
<i>Ex situ treatment</i>			
Soil washing	Addition of surfactants and other additives to solubilize	For water soluble contaminants	25–300
Pyrometallurgical	Elevated temperature extraction and processing for metal removal	Highly-contaminated soils (5–20%)	200–1000
<i>In situ</i>			
Soil flushing	Water flushing to leach contaminants	For soluble contaminants	100–200
Electrokinetic	Application of electrical current	Applicable for saturated soils with low groundwater flow	Little info
Phytoremediation	Use of plants for metal extraction	Shallow soils and water	(50,000–200,000/acre)

Table 1 – Remediation technologies and their associated costs (Adapted from Mulligan, Yong and Gibbs, 2001)

Hyperaccumulator Plants

Hyperaccumulator plants are plants that are able to take up large amounts of heavy metal pollutants into their biomass, without a detrimental effect on plant growth or tissue yield (Johnston, Datta and Sarkar, 2005). Hyperaccumulator plants are commonly defined as being able to bioaccumulate a target heavy metal pollutant to concentrations greater than 2% within aerial structures of a plant once dried (Baker *et al.*, 1994). Clemens *et al* (2002) defines 3 key requirements that plants must have to be a hyperaccumulator: (i) enhanced root uptake and xylem loading of the heavy metals; (ii) mechanisms in place to allow excessive root to shoot translocation of the metals; and (iii) the ability to detoxify using methods such as chelation and sequestration from the leaves (Clemens, Palmgren and Krämer, 2002). As a result, hyperaccumulator plants are an extremely rare phenomenon. As of 2003, approximately 400 plant species had been identified as hyperaccumulators of heavy metals (McGrath and Zhao, 2003). In a recent review by Sheoran, Sheoran and Poonia (2016) the number had increased to 500 species, with predictions that the number of species identified will increase in the years to come.

One aspect of a phytoremediation management technique which is not controlled by the plants themselves is the bioavailability of the heavy metals that are within the soils. These unobtainable metals are present in different forms and are affected by these different soil properties.

Soil Properties

The bioavailability, availability and uptake of the heavy metals within soils are controlled on the most part by the absorption and desorption characteristics within the contaminated soils (Krishnamurti, Huang and Kozak, 1999). These characteristics are affected by many different soil properties such as metal content, pH (Antoniadis, Robinson and Alloway, 2008), electronic conductivity (EC), organic matter, and interaction between metals and other elements (Yang *et al.*, 2005) such as calcium in the rhizosphere. The physical and chemical properties of soil (pH, EC, organic matter content) can help determine the total accumulation and overall mobility of heavy metals

within the soils of the site, and as a result can represent some of the most important factors that effect and eventually lead to the spatial variation of the heavy metal contaminants in the soils (Doner, 1978, Micó *et al.*, 2006, Rodríguez Martín *et al.*, 2006). The differences that can occur within the physical and chemical properties of a soil are commonly quite small in the same type of soils that are from the same location, especially those from the same soil parent material. However, significant differences in the soil properties can exist greatly between different soil types that might have come from different original locations.

EC

Salinity (measured with electrical conductivity) is one of these properties. Salinity is known to be able to affect the mobility of copper, cadmium, lead and zinc (Acosta *et al.*, 2011). Each metal is affected by different salts causing the salinity.

Organic Matter

Organic matter relationships and its role on the availability of heavy metals has been extensively investigated over the past decade. Examples of this research in several experiments showed that heavy metal absorption into soil constituents decreases as there is an increase in the organic matter within that soil (Hettiarachchi *et al.*, 2003). As such it has been suggested that the overall availability of the heavy metal contaminants within the soils will increase over time as the organic matter content of the soils decreases as it decomposes (Antoniadis, Robinson and Alloway, 2008). Heavy metals chemically can bind to organic matter in the soils, precipitated as oxides, hydroxides, and carbonates, and embedded in structure of the silicate minerals (Sheoran, Sheoran and Poonia, 2016). As a result, these heavy metal pollutants are very difficult to be taken up by the roots of plants. As well as this, groups of metals that are bound to organic matter, carbonates and oxides are also removed totally from being phytoremediated (Li *et al.*, 2014).

The amount of bioavailable and unavailable heavy metals can greatly vary, with some metals such as zinc and cadmium being more readily bioavailable (Lasat, 2002) and metals such as lead being harder to obtain (Shakoor *et al.*, 2014) to raised amounts of organic content. However, with the addition of organic matter to a soil location, zinc becomes much less bioavailable to the plants and so phytoextraction was less effective (Romney *et al.*, 1977). Organic matter can act as a stabiliser by absorbing the heavy metals and keeping them in the ground (Antoniadis, Robinson and Alloway, 2008). However, with the organic matter being dissolved in soils, there is an increase in the mobility of the heavy metals within the soils and as a result this can cause an increase in the uptake of heavy metals into plant roots (Impellitteri *et al.*, 2002).

pH

Soil pH has been found to play one of the most important roles in determining metal speciation, solubility from mineral surfaces, movement, and eventual bioavailability of metals, (Zeng *et al.*, 2011) due to its strong effects on solubility and speciation of metals both in the soil as a whole and particularly in the soil solution (Mühlbachová, Šimon and Pechová, 2005). It is documented extensively that an increasing pH causes a decrease in the metal absorption into resident plants (Sukreeyapongse *et al.*, 2002) with the reverse increasing the bioavailability of the heavy metals within the soils and as a result, increasing their uptake (Wang *et al.*, 2006). The mobility and availability of the heavy metal pollutants have been seen to increase as the pH of the soil becomes more acidic (Brallier *et al.*, 1996). This enhances the uptake of heavy metals by plants, making phytoremediation methods much more useful and effective but can start posing a threat to human health in non-managed areas due to foraging of fruit from many areas that are left wild. It is documented extensively that an increasing pH causes a decrease in the metal absorption into resident plants (Sukreeyapongse *et al.*, 2002) with the reverse increasing the bioavailability of the heavy metals within the soils and as a result, increasing their uptake (Wang *et al.*, 2006). The pH is a critically essential property that has a major influence on the cation mobility within soils and regulates the solubility of heavy metal contaminants within soil environments (Kashem and Singh, 2001). Most heavy metal contaminants when in soils tend to become more readily available at more

acidic pH concentrations. The only exception to this is in the case of cadmium. The availability of cadmium greatly favors a much more alkaline pH as discussed by López-Arias and Rodríguez (2005) and tends to accumulate in high quantities in calcareous alkaline soil environments.

Magnetic Susceptibility

Magnetic susceptibility has been shown to be able to be used as a preliminary method of analysis to indicate the presence of many heavy metal contaminants including chromium, copper, cadmium, lead (Lu, Bai and Xue, 2007) and Nickel (Schmidt *et al.*, 2005). Magnetic susceptibility mapping of soils and sediments has become an important tool for estimating anthropogenic pollution around factories (Lu, Bai and Xue, 2007).

Research carried out by Wang and Qin (2005) showed that the strength of the correlation between the heavy metal and the magnetic susceptibility could greatly shift depending on which metal was being tested. Iron, lead, copper and zinc all showed a strong correlation with the magnetic susceptibility readings. Nickel, cadmium and chromium all showed a weaker correlation with the magnetic susceptibility readings that were taken. As such all heavy metals, apart from mercury showed some correlation between heavy metals presence and the magnetic susceptibility of the sample tested. This suggests, as did the research by Lu, Bai and Xue (2007) that magnetic susceptibility can be useful as a measuring tool for the presence of heavy metals. However, the usefulness of this tool is higher for those heavy metals that show a stronger correlation with the magnetic susceptibility than those which have a weaker one.

Differences between the results of the low magnetic susceptibility and the high magnetic susceptibility go some way as evidence that firstly, there are heavy metal pollutants present within the soil samples, but most importantly that the readings are not coming from ferrous metal nanoparticles and as such the metals that are within the soil can be said to have been produced recently through contamination from the

industry that was situated on the site before conversion into a country park, and have not been formed through natural processes and natural formations of the soil.

Phytoremediation potential of species

Roots, stems and leaves of plants all have different affinity for the storage of the bioaccumulated toxins and as such, different parts of a plant's structure store and accumulate different heavy metals (Sousa *et al.*, 2008). Stems and leaves to some extent can be used by a plant to store heavy metals (Shallari, 1998), however, a number of research papers do suggest that most of the heavy metal's contamination that is stored within a plants tissues are in the cell walls of the roots (Sousa *et al.*, 2008, Konotop *et al.*, 2012). These papers state that the roots retain most of the heavy metals and that any transfer of them up to the aerial structures of the plant is negligible (Mangabeira *et al.*, 2011, Sousa *et al.*, 2008). Mangabeira (2011) along with Harmens *et al* (1994) go on to say that this lack of presence in the aerial structures of the plants could be as a result of detoxification of the heavy metals through binding to organic acids and polypeptides and becoming accumulated within cytoplasm or vacuole.

There are three groupings of plants that are able to grow on metalliferous soils (Baker, 1981). Group one is the excluders; in these plants, metal concentrations within the shoots of the plants are sustained around a low concentration across many different soil contaminant concentrations and as such, prevent the uptake of the heavy metals and other poisonous compounds into their root cells (De Vos *et al.*, 1991). This allows for the plants to lock any contaminations within the soils in and prevent it from entering the food chain and from being eroded and entering the air (Lasat, 2002). Group 2 are the Accumulators; these plants do not prevent the take up of heavy metals or other toxins through the roots which allows them to take up, bioaccumulate and store these metals in plant structures above the ground, which can later be removed (Chehregani, Noori and Yazdi, 2009). This accumulator group is the main focus of this study. The last group are the indicators. This group exhibit the same concentration of toxins within their structures that are in the external concentrations in the ground, air or water. This

makes them good indicators as to what the concentrations of contamination are (McGrath, Zhao and Lombi, 2002) without disturbing the ground or water ecosystems.

As can be expected, the majority of the plant species that are to be found on the Milton Creek Country Park site are grasses. Grasses such as *Vetiveria zizanioides* seen in the research of Danh *et al* (2009) and Chen *et al* (2004) and of *Pennisetum glaucum* seen in the research of Xia (2004) and Zhang *et al* (2014) show that grasses can be used as bioaccumulates and as tools in phytoremediation projects in the past. A total of 15 different plant species were identified and analysed for their heavy metal content. Due to the season in which the samples were collected, a number of specimens could only be identified down to their genus.

Trifolium repens

Research on phytoremediation of heavy metals by *Trifolium repens* by Bidar *et al* (2008) showed results that indicated that *Trifolium repens* was able to grow extensively and form a plant cover on highly cadmium, lead and zinc soils. When up taking up these metals, the *Trifolium* stored much of the heavy metal pollutants within its roots and limited any spread into the aerial parts of the plant. These characteristics of *Trifolium* make it a suitable contender for a phytostabilization method of heavy metal pollution control on contaminated soils (Bidar *et al.*, 2008) but has limited benefit as a phytoremediator.

Trisetum flavescens

Trisetum flavescens are known to be a pioneer species on contaminated sites (Erdemir *et al.*, 2016). This indicates an affinity for the *Trisetum flavescens* to be able to withstand higher than average concentrations of contamination and still be able to grow and spread. Although, like many of the grasses that have been researched as part of this study, there is no data as to how effective *Trisetum flavescens* as a species is at accumulating heavy metals.

Lolium perenne

Lolium perenne has relatively little research focused around its relation to the phytoremediation of heavy metal contamination. Research around how *Lolium perenne* behaves in heavy metal contaminated conditions has been looked at in regards to Cd, Zn and Pb by Bidar *et al* (2007). Bidar suggests that *Lolium perenne* as well as *Trifolium repens* could both be considered suitable candidates for a biomanagement tool for heavy metal-polluted soils due to the concentrations of oxidative stress induced by the presence of the metals. Oxidative stress, as stated by Drzewiecka *et al* (2012), has a close link to the phytoremediation potential of the plant in question.

Plantago spp

Plantago major, one species within the genus *Plantago* is well known for its abilities to accumulate many different heavy metals as well as for the phytostabilisation of metals not able to be accumulated. The higher translocation factors of cadmium, iron and lead within the shoots of *Plantago major* makes it suitable for phytoextraction of these heavy metal contaminants from the soil, while the lower translocation factors of nickel, chromium, zinc, and copper make *Plantago major* suitable for phytostabilization (Galal and Shehata, 2015). In a study carried out by Yoon *et al.*, (2006) *Plantago major* was seen to be accumulating lead up to concentrations of 249 ppm within its roots and 67 ppm in its shoots, as well as copper up to 150 ppm. *Plantago orbignyana* has been shown to be a hyperaccumulator of both lead and zinc showing concentrations of between 6070 to 8240 ppm of lead within its tissues and concentrations between 8290 to 11,560 ppm of zinc. It has been theorised that the hyperaccumulation trait of the *Plantago* genus must have a phylogenetic origin as nine out of 15 *Plantago* species presented hyperaccumulation traits for copper, zinc, lead and iron (Serrano *et al.*, 2016),

Apiaceae spp

Apiaceae spp in the form of celery, has been shown to have an affinity to accumulate cadmium from soils however not to hyperaccumulation concentrations (Mendez and Maier, 2007). The same has been found for zinc (Parmar *et al.*, 2013) where although

concentrations are accumulated there in no hyperaccumulation present. Although *Apiaceae spp* has also been found to accumulate other heavy metals that can be in soils such as arsenic (Tremlová *et al.*, 2016), the metals that are of interest do not appear to be accumulated to any concentrations that might be considered hyperaccumulated. Due to its presence at the site however, analysis will be done to confirm or deny this theory.

Agrostis stolonifera

Agrostis stolonifera that has been grown for a long period of time on areas that are contaminated with nickel pollution has been shown to produce a tolerance to high nickel concentrations as a result of morphological changes within the species (Reeves, Brooks and Macfarlane, 1981). As well as these changes, the *Agrostis stolonifera* was able to accumulate concentrations of nickel between 300 – 1,100 ppm within their plant tissues (Reeves, Brooks and Macfarlane, 1981). This same morphological changes, resistance and subsequent accumulation of metals has also been seen with copper (Wu, Bradshaw and Thurman, 1975). *Agrostis stolonifera* is also known to be a common find over metal contaminated soils and due to rooting into the stratum directly, provides surface stability of the contaminated soils, preventing movement by wind or water (Bradshaw and Baker, 2003).

Ranunculus spp

Ranunculus spp has been seen to have the rare ability to accumulate chromium heavy metal, although not hyperaccumulating, concentrations of 14 ppm (Porębska and Ostrowska, 1999). *Ranunculus spp* has also demonstrated a tolerance for growing on contaminated soils containing lead, copper and iron in high concentrations while also accumulating small concentrations of each of the metals into its tissues (Maric, Antonijevic and Alagic, 2012). However, evidence of *Ranunculus spp* being used or found to be a hyperaccumulator is not to be found. Analysis will be carried out to determine if the chromium uptake as well as the uptakes of copper, lead and iron from

research by others can be found at the site and if the conditions of the site allow for hyperaccumulation of these metals to take place.

Rumex spp

One species within the *Rumex* genus, *Rumex acetosella*, has been discussed in research by Wenzel *et al.*, (2003), who say that both species are heavy metals excluders, preventing the uptake of many heavy metals into their systems. This can make their use as hyperaccumulators null and void, for if they don't take up heavy metals, what use can they be as accumulators? *Rumex nepalensis* has shown concentrations of lead uptake of 33 ppm (Nazir *et al.*, 2011) which is not a very common ability within plants due to its poisonosity. Concentrations of lead, cadmium and zinc have also been found to be accumulated by *Rumex crispus*. As such, there is evidence that suggests that *Rumex spp* can be both excluders and accumulators of heavy metals and although there is little evidence to suggest hyperaccumulation, this will be clarified within the research carried out in this study.

Veronica chamaedrys

The research around any heavy metal accumulation into *Veronica chamaedrys* is extremely sparse with no papers directly stating any uptake of metals into the species at all. However, being common in Kentish grasslands as well as other areas of England, it is not surprising that it can be found at Milton Creek Country Park. With preliminary results from the site showing high concentrations of contamination of many different heavy metals across the site, it is of interest to see if the *Veronica chamaedrys* that is found at the site is an excluder, accumulator or even hyperaccumulator of any of the heavy metals that are present.

Potentilla erecta

Potentilla erecta has been used in phytoremediation studies as an indicator of how acidic the soils of the location are (Jones, O'Reilly and Morgan, 2007). Oxbrow and

Moffat (1979) state that *Potentilla erecta* is able to grow without hindrance above areas with high concentrations of lead pollution. However, very little research apart from Oxbrow and Moffat talks about *Potentilla erecta* in relation to heavy metals and no research has been found to discuss any phytoremediation potential of this species. Being common to the area of Kent, and known to grow at Milton Creek Country Park, if found in sampling, analysis for accumulation of all metals including lead would be of interest.

Vicia tetrasperma

Vicia tetrasperma has very little research surrounding it in regards to accumulations of heavy metal contamination. Its close relative *Vicia villosa* however has numerous papers documenting its accumulation efficiency. Showing high accumulation rates for copper, zinc lead and most importantly chromium with 4500 ppm within its tissues (Wang *et al.*, 2002). If the ability to hyperaccumulate heavy metal contamination is a genetic trait within the *Vicia* genus, it is possible that *Vicia tetrasperma* might have some of the accumulation ability that is found within the *Vicia villosa* species. Tests of samples that are found at the site will be carried out to determine if this theory holds any truth.

Rubus fruticosus

Rubus fruticosus or blackberry provides a large opportunity for phytoextraction. Being fast growing and able to be cut back and regrow its vast biomass, it provides an opportunity for extraction and removal while not requiring replanting each harvest (Erturk, Yerlikaya and Sivritepe, 2007). Erturk *et al* (2007) state that blackberry shows an affinity for remediation of both zinc and copper, however copper showed to decrease the biomass of the bushel as concentration increased. *Rubus spp* showed in research Yoon *et al.*, (2006) to accumulate copper up to concentrations of 265ppm, 8 times more than was in the soils at the location. Due to the local population foraging from blackberry bushes that inhabit the site, as well as birds and small mammals, it is important that *Rubus fruticosus* is analysed to deem if the area is safe for public use or if some extra management method will have to be in place to protect them.

EU regulations take into account the safe concentrations of each heavy metal contaminant that can be consumed by a person via the consumption of edible crops and animals or the inhalation of these metals from particulates in the air. However, there is little in the current legislation regarding the concentrations found directly in the soil. This makes gauging whether the concentrations found in soils at Milton Creek Country Park pose a risk to human health more difficult to determine. However, using the guidelines for consumption, it is possible to consider whether concentrations in the soil are dangerous to the health of the population through the accidental ingestion of or exposure to soils, or by comparing data on concentrations in plants to threshold values for the consumption of foragable plants or crops.

Phleum spp

Species within the *Phleum* genus have demonstrated accumulation of heavy metals in previous research. *Phleum pratense* has shown high remediation and relocation of nickel contamination of near 90ppm within its leaves (Vara Prasad and De Oliveira Freitas, 1999). Accumulation of zinc and lead has also been found in *Phleum pratense*, however, with high concentrations of these contaminants, *Phleum pratense* becomes highly sensitive (Atabayeva, 2016). Other species within the genus such as *Phleum phleoides* have been shown to also take up very small concentrations of heavy metals such as nickel and copper, with negligible take up of cadmium and chromium (Salihaj, Bani and Echevarria, 2016).

Pisum sativum

There are many research papers documenting *Pisum sativum* as a well-known hyperaccumulating species. Tariq and Ashraf (2016) demonstrate the ability of *Pisum sativum* to accumulate copper and lead (with highest removal efficiency of 96.23%) from concentrated contamination in pot experiments, showing the species resistance to high concentrations of contamination. Cadmium contamination in the soils where *Pisum sativum* is present, has shown the effect of a lower uptake of nutrients into the plant. This would hinder the growth of the plant and as such, reduce the species' ability to

accumulate the heavy metals from the soils (Hernández, Gárate and Carpena-Ruiz, 1997). However, Hattab *et al.*, (2009) showed that although cadmium can cause a reduction in growth and photosynthesis, at certain concentrations *Prisum sativum* is able to accumulate cadmium within its leaves. When tested with copper, once concentrations of 10 ppm were reach, accumulation stopped (Hattab *et al.*, 2009).

Salvia pratensis

No specific research into *Salvia pratensis*'s ability to accumulate has been carried out. Studies looking at grasslands including *Salvia pratensis* have shown high accumulation results for copper and lead (576 ppm and 421 ppm) (Manu *et al.*, 2017). This suggests that some species within the plants of the grasslands have the ability to hyperaccumulate the heavy metals, however, whether this is as a result of *Salvia pratensis* is yet to be seen. Multiple examples of research (Štrba, Turisová and Aschenbrenner, 2014, Manu *et al.*, 2017) demonstrate that *Salvia pratensis* is able to tolerate and grow in contaminated soils containing copper suggesting a resistance. Related species *Salvia sclarea* has shown the ability to hyperaccumulate lead and to accumulate cadmium and zinc (Angelova *et al.*, 2016). As tests, have not been seen to be carried out on *Salvia pratensis*, the potential for accumulation or even hyperaccumulation is there in evidence from broad studies and genetically close species.

Study aims

This paper focuses on firstly quantifying and mapping the concentrations of various heavy metals in the soil at Milton Creek Country Park. It will then examine the risk posed by pollutant accumulation within plants, that can then be consumed or otherwise introduce the heavy metals into the surrounding wildlife and local population (Wang *et al.*, 2005) such as inhalation or breaks in skin. Finally, the study will examine whether phytoremediation is a viable method for removing these pollutants from the area using natural means. If potential phytoremediators can be identified, this could help prevent problems both now and with future use. In conclusion there will be a plan of how the

resulting accumulating plants can be used as a management tool for the soil contaminants present.

The hypothesis for this research is that historical industry within the site area have affected levels of heavy metal contamination and plants that are found within the site will have a natural affinity for the accumulation of heavy metals. This will be proved with the answering of the following research questions;

- To what extent do plants found on the site bioaccumulate different heavy metals from contaminated soil in above-ground plant material.
- Identify plant species from the site which are tolerant to certain heavy metals.
- Investigate the effect of historical land use on heavy metal pollution at Milton Creek Country Park.
- Which plants would be best used as a local wild bioremediation tool for Milton Creek Country Park?

Methods

Analysis of the different plants at the site and the amounts of pollutants present in each will give an insight into each one's ability to take up each heavy metal. However, they are only accumulators of a small number of the metals and not all heavy metals are being looked at within his study.

Results were also split into species by an in-depth separation of plant samples taken from each sampling site. Where it was not possible for the species of a plant to be found, genus was used. If genus could not be found, if plant material was different from other identified plants then it was kept separately as an unidentified species known to be different. All other plant material was classified as unknown for each site. This allowed for a complete bio diversity and species variation to be looked at for future interest. Results showing which species are removing the most of each heavy metal pollutant from the soils in their sample locations was as a result collected. It then allowed for

which species located at Milton Creek Country Park are best used for phytoremediation across the heaviest polluted areas of the site (Porębska and Ostrowska, 2006) to be theorized from the result.

Samples of both the soils and plants at 148 locations across the site were collected from 2-x2-cm quadrat from transects across the site (figure 1), to a sward height of 5cm, all taken in early August 2015. Extraction of heavy metals from the root matter, plants tissues and soils were carried out with the use of a microwave digester. ICP-OES was then used to measure for concentrations of cadmium, copper (Pahlsson, 1989), nickel (Ahmad and Ashraf, 2011) lead, zinc, iron and chromium. The concentrations of heavy metals found within the plant material at each site were then cross referenced with results of heavy metal concentrations taken from the soil samples. GIS analysis was used to determine pollution mapping of the site.

Collection

At each sampling point, GPS co-ordinates were recorded in order to allow for spatial analysis. For soil sampling, a 10cm x 10cm x 10cm soil turf was extracted using a spade, placed in a sealed sample bag and transported to the laboratory. Soil was stored in a fridge at 4°C for up to 24 hours before being placed in aluminium foil and oven-dried at 90°C for approximately 96 hours. Once dry, soils were stored in sealed sample bags until required.

At each location, plant material was taken from a 20x20 quadrat to a sward height of 5cm was taken to cut close to ground concentration to ensure as much of the plant material would be collected as possible. These samples were then stored in labelled sample bags. Once returned to the laboratories the plant samples were placed at -20°C prior to analysis.

A number of species and genera were identified during the analysis phase of the research. The species and genera in question have all been analysed due to different characteristics that are of interest, including hyperaccumulation affinity, potential issues with consumption of contaminated fruit, or the fact that they are not abundant but

present at the site. The species and genus found and analysed were *Lolium perenne*, *Trifolium repens*, *Rubus fruticosus*, *Potentilla erecta*, *Trisetum flavescens*, *Agrostis stolonifera*, *Plantago spp*, *Apiaceae spp*, *Ranunculus spp*, *Rumex spp*, *Veronica chamaedrys*, *Vicia tetrasperma*, *Phleum spp*, *Prisum sativum* and *Salvia pratensis*.

Laboratory analysis

Soils

pH

The pH of the soil samples was measured using a Palintest Micro 500 pH probe. Samples were prepared by placing 3g of oven dried soils sample into a 25ml falcon tube. The falcon tubes were then filled with distilled water up to the 15ml mark to allow for room for the probe to take measurements within the water element of the test and to produce a 1:5 dilution (Department of Sustainable Natural Resources, n.d.). Samples were then shaken in a Excella E25 heated orbital shaker for 1 hour at 25°C at 120RPM. Samples were left to settle for 10 minutes, to allow partial separation of the water and soil.

EC

Electrical conductivity of the soil samples was carried out with the use of a WPA CMD 200 electrical conductivity meter. This was done using the same samples as the pH readings and was carried out simultaneously to ensure consistency within the samples was the same.

Organic matter

To gain the measurement for organic matter a method put forward by Robertson (2011) was followed and modified to fit with the equipment available. Oven dried soils samples (1g) was placed inside a previously weighed ceramic crucible and then stored in a desiccator. The samples were then placed inside a Muffle Furnace at 550°C for 6 hours

and left to cool until the next day, at which point each was reweighed. Loss of weight on ignition was calculated as a proxy for organic matter, taking into account the crucible weight.

Magnetic susceptibility

Magnetic susceptibility was measured using a Barrington MS2 magnetic susceptibility meter. Each dried soil sample was ground down to a fine powder and large stones removed, in order for no interference with the apparatus. The ground samples were then placed into 1cm³ clear plastic containers and compressed down to remove air gaps from the prepared samples and to produce sample of the approximate same mass and volume. Each container was topped with cling film to prevent movement that could affect the result from the magnetic susceptibility meter. Samples were first run on the low frequency setting, then were removed to allow for calibration before the next sample was inserted. Time between samples and calibrations was kept to a consistent 3 seconds to prevent duration from being a distinguishing factor. All samples were run afterwards on the high frequency setting, following the same principles, and both readings were recorded.

A large difference in the low and high frequency readings can indicate the presence of microparticles of metallic and ferrous metals. This would indicate that the metals within the soils are produced from natural sources and ground down over years of weathering. This is because the magnetic field that is oscillated next to the soil samples is increased in oscillation speed which only smaller particles can keep up with, showing their presence (Sassa, 2017). However, a small difference between the low and high frequency readings indicate that metals within the soil samples have not been created and distributed by natural sources and come from anthropogenic causes.

Digestion

Digestions of the soils was carried out in order to allow for their heavy metal analysis with the use of the ICP – OES using a LAT Microwave Digester. 1g samples were weighed

out and oven dried. Prior to analysis, microwave digester vessels were run on a digestion cleaning cycle using HNO₃ (68% / 16.23 M) and HCL (37% / 10.15 M) to assure that all residues of metals and organics were removed from the vessels. Soil samples were ground down and excess stones removed if present. This is to ensure complete digestion of the soil samples while in the microwave digester. 1g of each soil sample was weighed and then placed into a digestion vessel. To these (2.35ml of 65% HNO₃ and 7ml of 37% HCL) were added, ensuring that the whole sample within the vessel was covered. Each vessel was then shaken gently to coat the sample in the acid and then placed within the microwave digester on a 2-hour cycle. Once digested, each sample was left to cool overnight. The vessels, once cooled, were decanted into 100ml glass volumetric flasks. The digestion vessels were rinsed using distilled water to collect any residual digested sample that remained and this was added to the volumetric flasks. The samples were then diluted up to the 100ml mark using distilled water, creating a 1:100 dilution factor. These were then stored for further analysis at a later date.

ICP – OES

An ICP–OES Optima 8000 was used for the analysis of the following metals: copper, nickel, zinc, iron, cadmium, chromium and lead using the soil samples previously digested in the Microwave Digester. In order for the heavy metal standards obtained to be comparable with the microwave digested samples for both the soils and the plant digestions, matrix matching was carried out. Each sample was loaded into a 15ml falcon tube, calibration samples of 5ppm, 10ppm, 20ppm, 30ppm, 40ppm, 50ppm and 100ppm as well as a blank were run.

Reference standards were placed periodically between samples as a method of QC to ensure continued accuracy on low concentration reading to ensure the results for each sample were accurate. Each sample was tested in sequence for each heavy metal, and 3 repetitions of each carried out.

Matrix matching

Matrix matching of all reagents and samples that are used during the process of the analysis of the samples using an ICP-OES is very important and can have significant effects on the resulting data. Matrix matching is the use of the same solutions or solvents in all the components for your analysis such as blanks, standards and the samples themselves making the acid composition, total dissolved solids and acid concentrations match making all components to be run through the ICP-OES compositionally the same (Murry, Miller and Kryc, 2000). This was carried out to match standards with the samples produced from digesting in order to eliminate potential data discrepancies.

Statistical Analysis

All data collected for the analysis of the soils was tested for normality using Minitab's normality test. Due to non-normal distribution of the data, the non-parametric test, Spearman's correlation was used to determine relationships between the soil properties of the site and the contamination concentrations of heavy metals.

GIS

Geographical information systems are a method of depicting data which has been measured along with geographical references to create a visual interpretation of data analysis. GIS has a large diversity of application and allows for the depiction of data from areas of science, economics and mathematics in a geographical representation (Maguire, Goodchild and Rhind, 1991).

Inductively coupled plasma optical emission spectrometry is a technique used for the detection of trace elements, such as heavy metals, within a sample. These samples can be from both the soils (Bettinelli *et al.*, 2000) that are being analysed and the plant samples that are being tested for their phytoextraction efficiency. All samples must first

be digested in a microwave digester to extract the heavy metals from the samples. The methods for this are different for both plants and soils.

Inverse distance weighted interpolation is a form of spatial interpolation geometry. This method is based on the first rule of geography, which states that “everything is related to everything else, but near things are more related than distant things” (Tobler, 1970). Effectively, this means that the closer two objects are, the more similar their values will be, and vice versa. This measurement uses the distance between the interpolation spot and the sample spot, with the spot near the interpolation being weighted more heavily than those at a greater distance (Zhou *et al.*, 2016).

Producing a visual image analysis of an area, allows for the easy representation of data such as contamination of a site, making the data more flexible to the audiences that might be interested in its worth. Visually depicting contamination with red for high and green for low, quickly gets the point across of the concentrations and exactly where they are. If this is done at a site where the owners have a limited scientific background, then being able to get scientific data across in this manner is a major benefit.

Plants

Identification

Identification of the species found at the site was carried out to obtain likely hyperaccumulating species. All plant samples that had been collected at the site were separated out and identified to species if possible, and if not then genus. Samples were first removed from the -20°C freezer and left to thaw in the fridge overnight. Once thawed, the samples were separated out by observation, into categories dependent on key physiological features and traits including, ligule size, presence of auricles, presence of hair, height and the width shape and roughness of the blades. The most identifying feature of the grasses and other plants, the flowering heads and bodies, could not be used within this identification due to the time of season that the samples had to be taken from the site; this made the process more difficult in regards to identifying to the

species concentration. This process was carried out over several weeks using The Wild Flower Key (Rose *et al.*, 2006).

Once completed, the bags on sample were weighed, dried in an oven at 70°C and then weighed again. Once oven-dried, plant identification was re-checked, and a number of plants were selected for further analysis using the ICP-OES, based on confidence with identification and whether they had been identified to the species concentration.

Digestion

For each sample to be run, 0.4g of plant material was weighed out. These samples were ground down to ensure complete digestion of the plant samples while in the LAT microwave digester as due to some samples being thick and woody, if not treated in this manner, would protrude from the acid and as a result be less likely to be digested. Samples were then placed into washed digestion vessels, as per the method for soil analysis. To these (5ml of 68% HNO₃ and 3ml of 35% H₂O₂) were added, ensuring that the whole sample within the vessel was covered. Each vessel was then shaken gently to coat the sample in the acid and then placed within the microwave digester. Once digested, each sample was left to cool overnight. The vessels, once cooled, were decanted into 100ml glass volumetric flask. The digestion vessels were rinsed using distilled water to collect any residual digested sample that remained, and this was added to the volumetric flask. This was then filled up to the 100ml mark using distilled water, creating a 1:250 dilution factor. These were then catalogued and then stored to be analysed at a later date.

ICP – OES

The samples were then tested using the ICP–OES using the same method as previously implemented for soil analysis

Results

Concentrations of each heavy metal contamination were measured, and basic statistics carried out to show the variation between the concentrations of each metal across the

site (table 2). Spearman’s correlations were then used to indicate relationships between the soil properties across the site and each heavy metal (table 3). Magnetic susceptibility had the strongest correlations between metals and its readings while each other property had effects on only one of the metals.

Heavy Metal	Median (ppm)	Mean (ppm)	Standard deviation (ppm)	Max ppm	min ppm
Cu	1196.92	1214.37	53.67	1484.22	1139.12
Fe	14663.10	15074.46	7728.21	44544.40	0.00
Ni	16.10	17.85	7.54	53.57	0.00
Cd	0.00	0.52	2.57	17.90	0.00
Pb	75.43	122.70	192.77	1895.94	0.00
Zn	334.70	488.70	374.10	2124.57	10.31
Cr	22.12	24.15	12.22	114.52	0.00

Table 2 – Basic statistics of heavy metals present in the soils

P-value	Copper	Iron	Nickel	Cadmium	Lead	Zinc	Chromium
Organic Matter	0.125	0.380	0.688	0.010	0.110	0.136	0.811
Electrical conductivity	0.777	0.714	0.048	0.518	0.486	0.090	0.230
pH	0.391	0.352	0.012	0.110	0.173	0.054	0.088
Magnetic Susceptibility	0.000	0.747	0.150	0.566	0.000	0.001	0.951

Table 3 – P-Values of relationships between soil properties and heavy metals using a Spearman’s correlation.

Mann – Whitney tests were then conducted on each metal result from areas which reside on either landfill and no landfill use to determine a causality between concentrations of the heavy metals and the lands historical industry (table 4). Cu, Fe, Ni and Cr all reported significance back indicating a relationship between there capped landfill areas and peaks in contamination by these heavy metals. Figure 3 displays Boxplots of these comparisons in order to visualize the significant differences.

Landfill	P-value	No Ex Land Use	P-value	Landfill and Brickworks	P-value	Landfill
Cu	0.0003	Cu	0.3752	Cu	0.0006	Cu
Fe	0.0038	Fe	0.1773	Fe	0.1480	Fe
Ni	0.0004	Ni	0.0476	Ni	0.1136	Ni
Cd	0.3742	Cd	0.9545	Cd	0.3454	Cd
Pb	0.1205	Pb	0.2220	Pb	0.0112	Pb
Zn	0.1308	Zn	0.1442	Zn	0.0128	Zn
Cr	0.0000	Cr	0.0006	Cr	0.6811	Cr

Table 4 – P-values from Mann – Whitney showing comparisons between Landfill, areas with no landfill land use and landfill that historically had brickworks.

In order to visually display the findings to greater understand how each soil property and contamination is affecting the site, inverse distance weighting was carried out and plotted on top of the sample area (figures 4 and 5). These maps show changes in the concentrations across the site, helping lead to a greater understanding of each historical land use (figure 1) and how they have affected concentrations of each variable across the site.

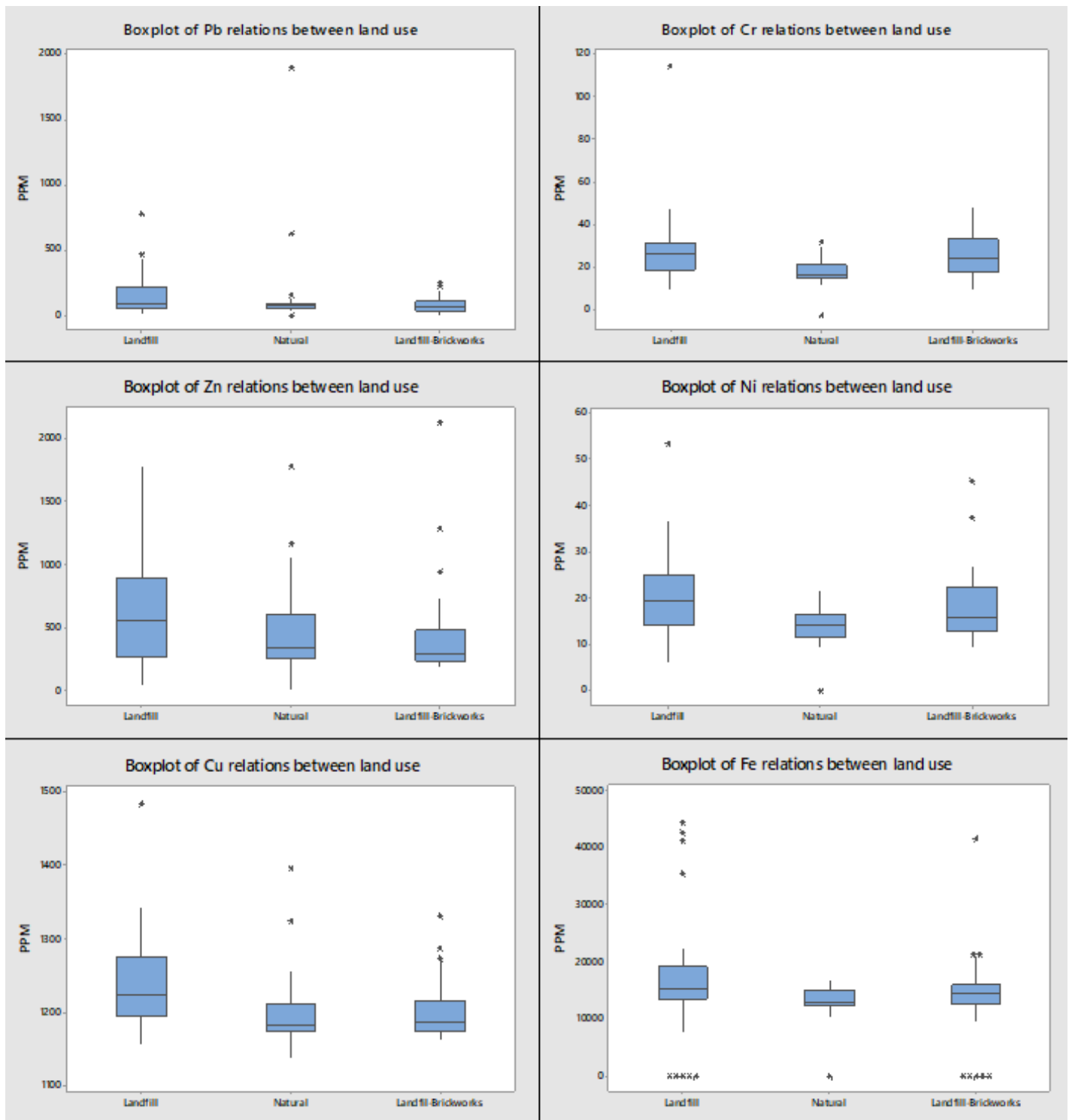


Figure 3 – Boxplots as visual representation of differences between landfill, landfill-brickworks and natural (non-landfill) areas.

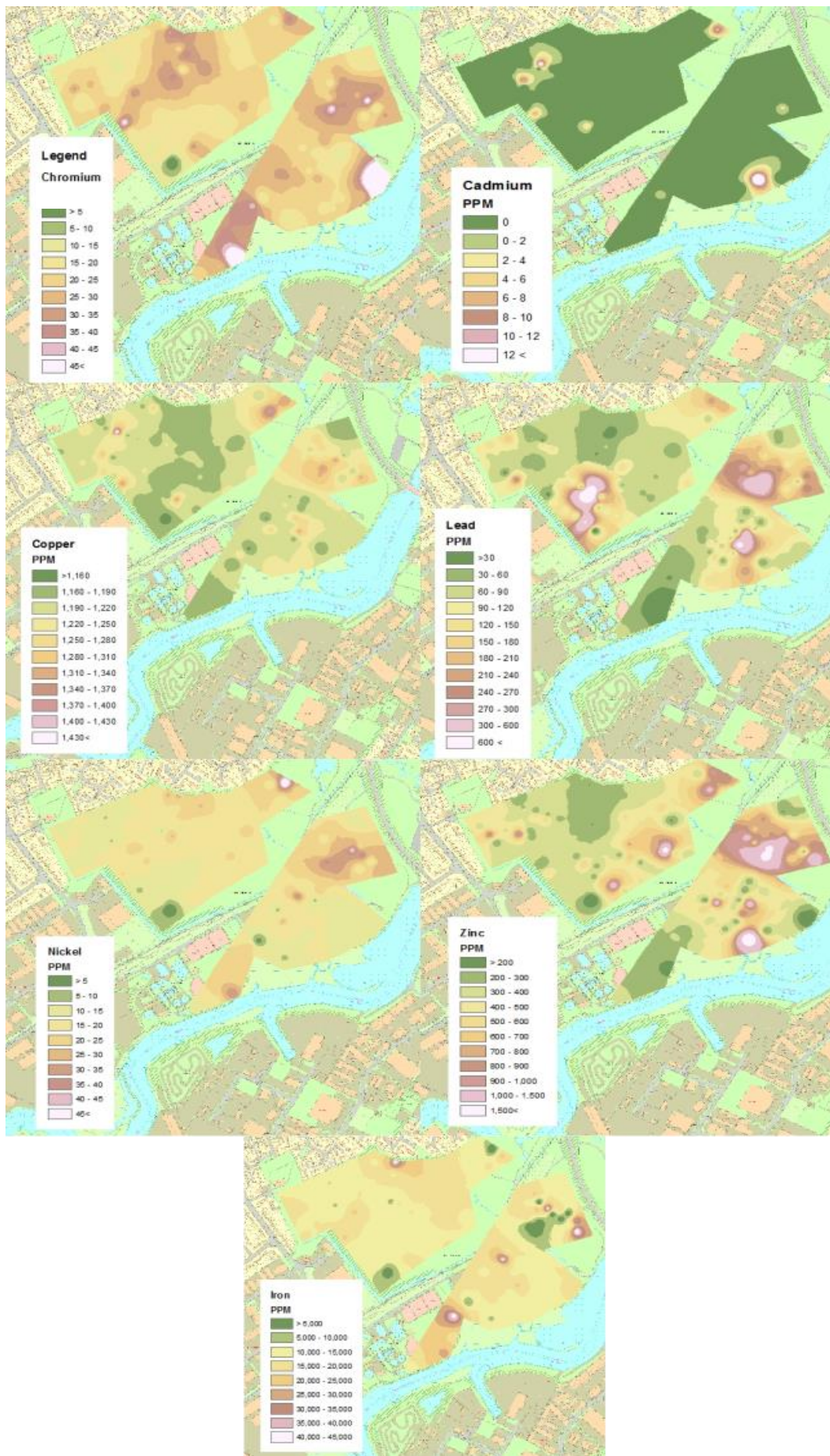


Figure 4 – Inverse distance weighting of heavy metal contaminants

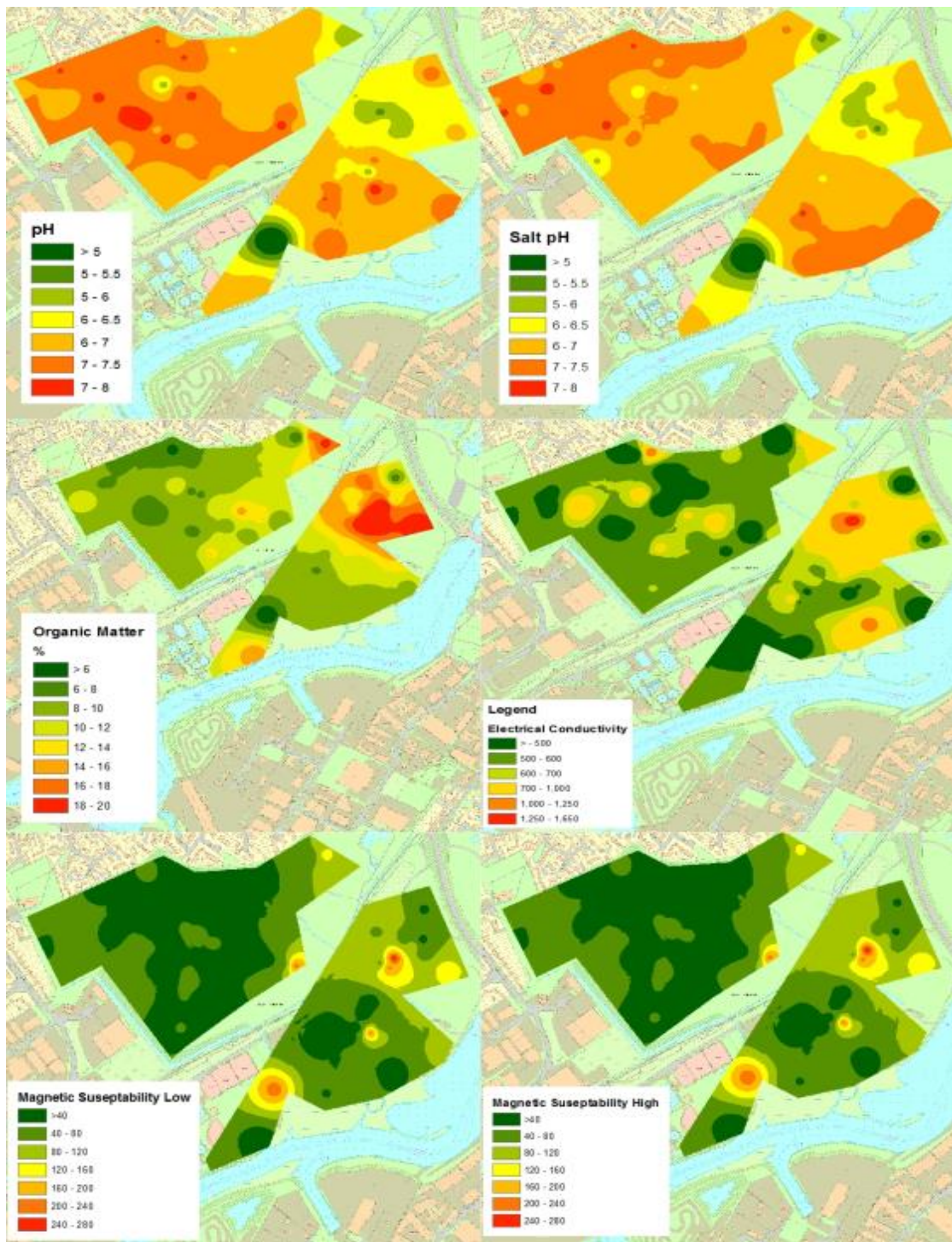
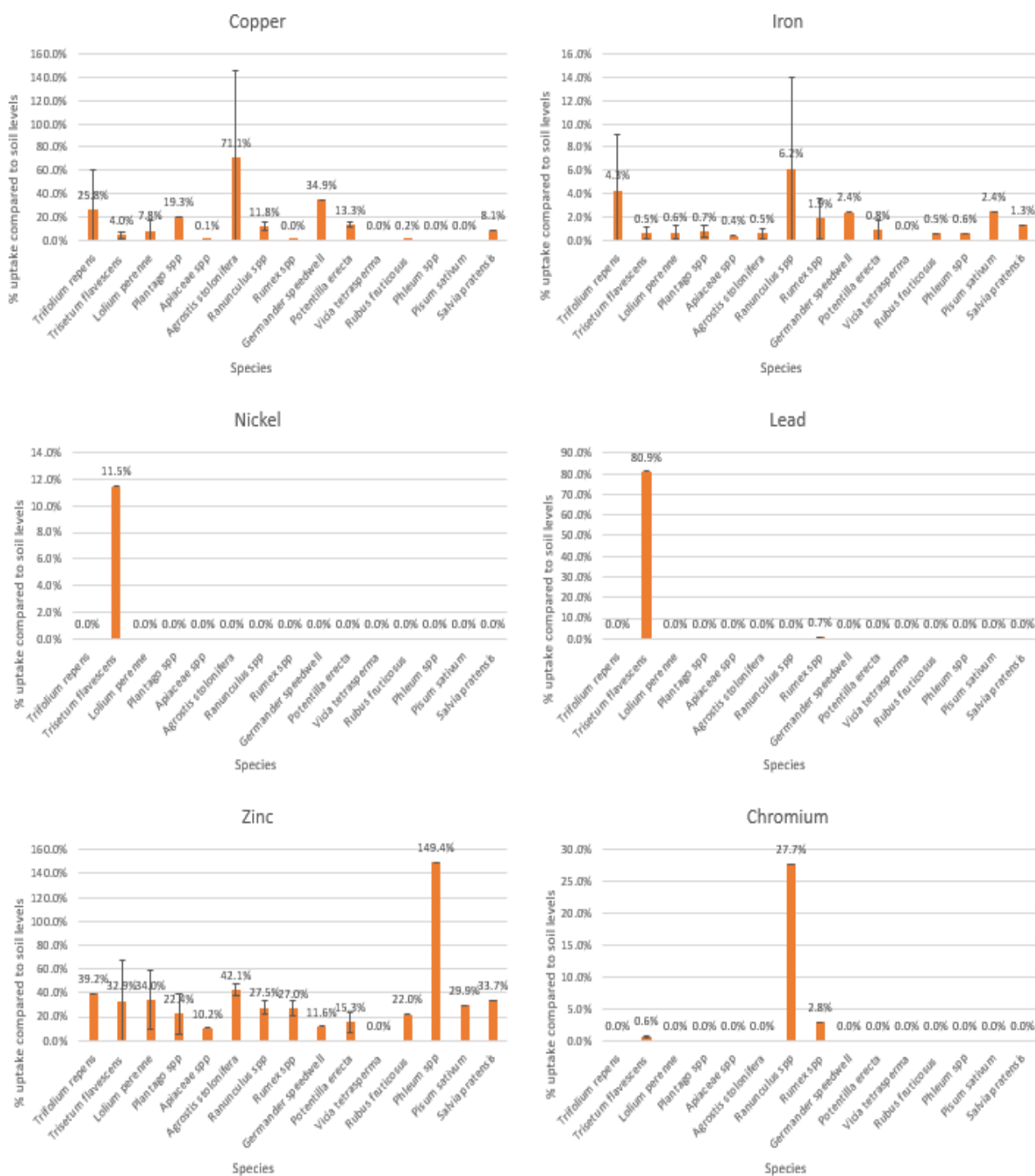


Figure 5 – Inverse distance weighting of soil properties at Milton Creek Country Park

Figure 6 – Graphs showing % accumulation of heavy metals within plant compared to soils



Discussion

Zinc

Zinc concentrations at the site were highly variable, with a standard deviation of 374 ppm. Concentrations were consistently above concentrations that would be safe for consumption by animals, with concentrations safe for ingestion ranging between 150 – 200 ppm (shown in table 2) (however this goes up to 300 ppm if the pH is over 7, reducing mobility) and the mean concentrations achieved at the site being 488 ppm. Although the site is not used for livestock, multiple foraging plants are on site meaning this shows a risk for high concentrations of zinc within plants on the site for foraging animals and people. As can be seen in the GIS analysis (figure 4) for heavy metals, much of the higher concentrations of zinc contamination are to the east of the site, with its highest peaking area showing concentrations of 2125 ppm (table 2). The location of these higher concentrations can be seen in figure 1 and can be seen to be located on the site of the landfill, however table 4 shows no significant correlation between concentrations of zinc on landfill areas and concentrations on areas with no historic land use (p-value 0.1308). If the elevated concentrations within the soils are not being caused by the landfill areas, a combination of landfill pollution, other industries across the site and the movement of new soil to the site might all play a part in these readings.

Nickel

Concentrations of nickel across the site remained relatively constant around 17.8 ppm (table 2). Even with a peak of 53 ppm to the east of the site, nickel concentrations within the soils don't surpass guidelines for safe concentrations within soils, with 50 ppm being acceptable at pH 5 and then increasing up to 110 ppm at above a pH of 7 (Tóth et al., 2016). The concentrations that are present at the site, being spread out, do not seem to come from any one source of industry and as shown in table 4, there is a high significance between landfill (0.0004) and the rest of the site showing in this case that nickel is not caused by leaching from the capped landfill areas and as such from some

other source. The p – value for a relationship between landfill and brickwork sites compared to no previous land use is much weaker than that of landfill. This could indicate a difference in the utilisation of landfill areas that previously were used for brickfields.

Cadmium

Few results for cadmium concentration were recorded across the majority of the site, not breaking any concentrations for health. However, at seven points across the site, cadmium concentrations peaked at above 12 ppm and reaching over 17.9 ppm again in the east of the site (table 2). Safe concentrations for cadmium, regardless of pH concentration, are 3 ppm. As such these areas, once again seeming to be situated on landfill locations, however due to the limited number of samples displaying these high concentrations there is no significant correlation that can be drawn to show that landfill is the cause of this cadmium contamination (table 4).

Lead

Lead concentrations vary across the site with a standard deviation of 192 ppm (table1). Acceptable concentrations of lead within soils is deemed to be 400 ppm in all pH conditions. The majority of the site fell below this category with an average of 122 ppm (table 2). There were however peaking areas in the centre of the site (figure 4) and to the east of the site that go above this 400 ppm guideline. 2 of these 3 major peaks correlated with the location of the brickwork areas, the 3rd peak however doesn't correspond with any industrial area but no significant correlation showing causality can be drawn (table 4) so more research into how this area has become contaminated to this concentration must be conducted.

Lead concentrations across the entire site could pose a large threat to human health. Concentrations of lead reach up to 1896 ppm. The EPA's standard for lead in play areas is 400 ppm and being a park of public access, Milton Creek Country Park falls into the category. The higher concentrations of lead that pose the threat to children's health at

the site are seen in the west of the site, as well as central where the soils of the site are more exposed, increasing the risk of ingestion or entry into the blood through cuts and scrapes. The Centers for Disease Control in the USA has set the upper limit for blood lead for adults at 10 µg/dl and for children at 5 µg/dl. With areas at above recommended concentrations of 400 ppm, the area is already causing risk to children's health. In areas between 1000 and 2000 ppm children should not be allowed onto the affected area. All this will have to be taken into account for the management method of the site.

Copper

Copper concentrations across the site are consistently above concentrations that are deemed safe. With the highest safe concentration being 200 ppm in pH 7 soils, copper is consistently between 1100 ppm and 1500 ppm across the whole site (table 2). Once again, these concentrations don't correlate with any one industry from the sites history due to its consistence across the whole site. With copper being consistently above this concentration across the site, this rules out the idea of targeted remediation of the certain areas of the site, as the whole area will need to be targeted. However, with there being a significance between the landfill areas and the concentrations of copper contamination (p-value 0.0003), these areas should be the target for the remediation of copper first.

Chromium

Concentrations of Chromium allowed to be within the soil can reach to 400 ppm. No concentrations at the site reach up to this concentrations with peaks of 114 ppm (table 2) situated to the east on top of the ex-landfill – brickworks, with a p – value of 0.000 and 0.0006 showing that the landfill areas are directly linked to the chromium peaks. Although 400 ppm is deemed to be safe within soils, chromium at these concentrations can be poisonous depending on the state and mobility of the metal, as such this area still needs to be monitored to ensure these peaks do not pose a threat in the future to the health of locals.

Iron

Concentrations of ingestion of iron of over 20ppm have been known to cause iron poisoning and concentrations above 50ppm can cause high poisonosity (WebMD, 2017). As such, in order to gauge worrying concentrations of iron within the soils, health risks will be used as a measure due to a lack of legislation for iron. Concentrations within the soils at Milton Creek Country park averaged 15000 ppm. This is a extremely high concentration of iron with the maximum peak reaching 44500 ppm. Even though not all of this will be available for the uptake of plants and so ingestion into humans, these concentrations cause sever concern and as such need to be heavily investigated in regard to forgeable plants. Interestingly, one of the largest peaks for iron can be linked (as seen in figures 1 and 3) to the barge building that was associated with the site.

Soil Properties

Organic Matter

Organic matter was consistently low (below 10%) across the majority of the site. Concentrations got higher towards the north east of the site, reaching concentrations of up to 20%, as shown in figure 5. Higher concentrations of organic matter within soils can form strong complexes with heavy metals and prevent them from being mobile helping prevent their uptake by plants as evident with copper in research by Temminghoff, Van der Zee and de Haan (1997) where at 3.9 pH only 33% of copper bound, but at 6.6 pH 99% was bound. With low concentrations across the site, it could be assumed that little binding would be taking place and so virtually no effect on the mobility. However, even at higher concentrations organic matter is dependent on the pH of the soils when it comes to its own mobility (Temminghoff, Van der Zee and de Haan, 1997) and so organic matter on its own has less of an impact.

pH

The pH of the site sat between the ranges of 5 and 7. This range is the average range for this area with between 5 pH and 7 pH being the optimal range for the majority of the plant species that would be expected to be found at the site. There are a number of sites towards the north west of the site which do go above this average, reaching up to 8 pH. pH values can have a very strong relationship with the mobility of heavy metals within the soils. Cadmium and zinc have been shown to increase in mobility as the soils become more acidic (Calmano, Hong and Förstner, 1993). With much of Milton Creek Country Park being around 5 pH, this could mean relatively high mobility of these metals. Within the ranges. Chromium has been shown to become more mobile at higher pH (Zayed and Terry, 2003). However, with the concentrations at the site staying below 8, the mobility will not be effected to a great extent. Many heavy metals around the concentration of Milton Creek are less mobile as at 7 pH, retention of them in the soils increases dramatically (Harter, 1983) however, even with the slightest rise or dip, this can change however.

With the pH of the site being varied, the management method that is to be implemented, would need to take this variation into account. This would mean further study into the effect of the pH on the selected remediation species to determine how best to implement a plan that will remediate all areas of the site, no matter the pH level.

Electrical Conductivity

The electrical conductivity was measured to determine if the salinity of the area would change across it. This is due to it in the past being underwater and within a floodplain of the River Swale now. As was expected, EC concentrations were consistently low across the majority of the site. However, as sampling approached the river, concentrations rose indicating a rise in salinity. Salinity can cause an increase in the mobility of heavy metals within the soils (Acosta et al., 2011) and allow them to be taken up more easily within plants. Each metal is affected by different salts causing the salinity. As such, although salinity can be said to promote mobility at higher concentrations, such

as those to the east of Milton Creek Country Park, it cannot be predicted how mobility will be affected without more research being carried out.

Magnetic Susceptibility

Magnetic susceptibility was consistently low with the exception of 4 peaks across the site (as visible in figure 4). These peaks coincide with both the landfill to the east of the site, and the barge building to the south of the site (as depicted in figure 1). In table 3, magnetic susceptibility is shown to have a significant relationship with copper (p-value 0.000), lead (p-value 0.000) and zinc (p-value 0.001). This indicates that the relationships between these metals and magnetic susceptibility is so strong that the concentrations of magnetic susceptibility should be able to be used as a rough determinate of concentrations of these heavy metals. However, as all these metals show a relationship, it would be impossible to know what of these metals was being indicated by the changes in the readings of magnetic susceptibility and so this as a method of testing for metals individually is largely unreliable.

Soil properties and Heavy metals

After carrying out a Spearman's correlation, a number of relationships between soil properties and heavy metals at the site have been observed (as seen in Table 3). Cadmium showed significance with the organic matter at the site (p = 0.010). This relationship however could be due to the lack of cadmium pollution across the majority of the site appearing to match up with the consistent organic matter concentrations. Nickel showed relationships with both pH (p = 0.012) and electrical conductivity (p = 0.048). This relationship could mean a direct link between the amount of nickel within the soils and the pH and EC at which the soils are. As such, a site containing nickel would have variances from the normal pH and EC of the local area meaning the pollution would have even more of an impact on the local ecosystem, but also be more visible during analysis. Lastly, copper (p = 0.000), lead (p = 0.000) and zinc (p = 0.001) all showed correlations with the magnetic susceptibility of the site. With all 3 of these metals

seemingly linked to the landfill to the east of the site, it could be suggested that this link could be used as an indication of heavy metal pollution from remediated landfill sites.

Landfills effect on the site

With landfill the most recent industrial land use for the site of Milton Creek Country Park, the idea that raised concentrations could be directly linked to these capped off areas is a viable suggestion. Due to a large proportion of the landfill areas of the site also historically being areas associated with brickworks and brickfields, these land uses had to be separated when testing against areas of no past landfill use. Cu (p-value 0.0003), Fe (p-value 0.0038) Ni (p-value 0.004) and Cr (p-value 0.0000) all tested to be significantly different under Mann – Whitney tests (shown in table 4 and visualised in figure 3. This significance when scrutinised with boxplots (figure 3) and inverse distance weighting (figure 4) can be drawn to show that landfill areas are having a significant effect on the concentrations of these four heavy metal pollutants by causing the concentrations to increase considerably.

All significant readings for sites consisting of both landfill and brickworks that were found against no land use areas all corresponded with significant values with landfill areas. This can draw the conclusion that no additional concentrations of contamination can be seen from the brickwork areas. It can be seen however that there are three significant differences between landfill and landfill-brickworks areas (Table 4). Reasons for this significance is unclear, as these areas were all used and eventually capped landfill areas. If the areas had been utilised in slightly different ways during the landfill, due to residual structures or foundations of the brickworks, this could suggest a reason for these differences.

Phytoremediation potential of species

A total of 15 different plant species were identified and analysed for their heavy metal content. Due to the season in which the samples were collected, a number of specimens could only be identified down to a genus concentration. A number of the identified

species also have a low number of samples, due to the species or genus being less abundant at the site. These plants were still run and analysed to determine for what reason their abundance at the site is diminished. Also error bars were added in order to show the large variation the uptake of some heavy metal had within certain species (figure 6).

Trifolium repens

Trifolium repens showed an increased ability to be able to take up three of the tested heavy metals (copper, iron, and zinc) with concentrations of copper and zinc showing 25% that of the soils. The amounts there were taken up by the *Trifolium repens* samples were massively varied, demonstrated by the extent of the standard deviation error bars on graph 1. As well as the large deviation, *Trifolium repens* is an unlikely candidate for remediation purposes. Having a small overall biomass per plant means that in order to have phytoremediation on the scale required to clean a site such as Milton Creek Country Park, there would need to be many individual plants, which would create an imbalance in the local plant ecosystem.

Trisetum flavescens

In figure 6, it is evident that *Trisetum flavescens* shows the best affinity for phytoremediation regarding the variety of heavy metals that it can take up. Six of the seven heavy metals (not cadmium) were extracted by the *Trisetum flavescens* samples tested. With the most abundant of samples at the site with 15 samples from the sampled areas, the species has already shown the ability to grow to a large extent on the soil types that are present. With the soil types being so varied, this is another key characteristic that increases *Trisetum flavescens* phytoremediation potential. However, the concentrations at which the samples of *Trisetum flavescens* took up the heavy metals in the soil were low compared to the concentrations at which other species could extract, being the third lowest for copper (4%), average for the uptake on zinc (32.9%) and iron and chromium all at concentrations below 1%. Although lead and nickel are shown to have high percentage concentrations of total soil concentration, only one of

the 15 samples showed the ability to take lead and nickel up, and so these high results have to be scrutinised and looked at again in further research.

Lolium perenne

Lolium perenne presented a varied ability between the different samples for accumulation of the heavy metals. Copper (7.8%), iron (0.6%), zinc (34%) all showed evidence of remediation within the majority of the *Lolium perenne* samples to a varying degree of success, however for all four of these metals, accumulation concentrations varied considerably as evident by the high standard deviation error bars. Like *Trisetum flavescens*, there were a larger number of samples found compared to most of the other species. *Lolium perenne* however, showed more accumulation than the *Trisetum flavescens*, even though its only remediated three rather than six of the metals. This makes *Lolium perenne* a better candidate than *Trisetum flavescens*, regarding the quantity of available contaminations accumulated from the soil source.

Plantago spp

Due to the sampling of the plant species having been carried out outside the flowering season, some of the samples were hard to identify down to species concentration. *Plantago spp* was one species where many samples were available to be analysed, however these could only be identified down to genus concentration confidently. *Plantago spp* was found to only accumulate the three commonly remediated metals in this experiment (copper, iron and zinc). Although displaying a relative accumulation to the soils for copper of 19.3%, for iron and zinc, relatively low concentrations compared to the other species were found (0.7% and 34%). Although lower in accumulation than some, the rate of accumulation across the samples collected showed a consistency with the rate of uptake. This is an important aspect of a hyperaccumulating plant as this means that different soil conditions across the site such as the pH were not affecting the

Plantago spp ability to accumulate. This however does have to be compared to its rate of accumulation in order to consider it a viable phytoextracting method for the area.

Apiaceae spp

For some genus and species, not many samples were discovered in the sampled areas. A number of these were analysed to see if these less abundant species showed any affinity for accumulation, and if not, this could be the reason for low abundance. If they did show high accumulation concentrations, then other reasons such as outcompeting by other species would have to be looked into. *Apiaceae spp* is one of the samples where only one could be found from the selected sample areas. With this limited evidence, a very low accumulation rate was seen in copper, iron and zinc, having the lowest remediation concentration for all of these out of every genus and species that was analysed. This means this would not be seen as a good target for remediation as it is not demonstrating the hyperaccumulation that other species have across multiple samples.

Agrostis stolonifera

Agrostis stolonifera is another species that provided only a limited number of samples. However, *Agrostis stolonifera* showed the highest percentage accumulation relative to its sample soils of all sampled plants for copper at 71.1%. *Agrostis stolonifera* also showed the second highest concentration for zinc (42.2%) and small amounts of iron (0.5%). For copper, however, there was a high variance between the samples. This could come down to the pH or other soil properties of the location affecting the concentrations of accumulation. For zinc, the variance is relatively low for the concentration of accumulation that is taking place, showing that the *Agrostis stolonifera* has some concentration of hyperaccumulating ability associated with zinc. For the purposes of Milton Creek Country Park however, *Agrostis stolonifera* would be low on the list of potential hyperaccumulators to use.

Ranunculus spp

Ranunculus spp is the most promising genus for its accumulation of iron and chromium. Although a small number of samples of *Ranunculus spp* were collected from the site, the samples that were found showed the highest iron (6.2%) of all the genus and species that were tested. Concentrations of copper (11.8%) and zinc (27.5%) accumulation were also recorded. These concentrations are not the highest amongst species but with the ability to remediate other heavy metals in high concentrations mean *Ranunculus spp* is a good candidate for further research. *Ranunculus spp* also showed the highest accumulation of chromium (27.7%) which is of great importance as there are not many species of plant that have the ability to hyperaccumulate chromium. This high reading of chromium was not shared by all samples of *Ranunculus spp* tested; however, this does make the *Ranunculus spp* a genus for further research in the future.

Rumex spp

Rumex spp showed no affinity for the accumulation of copper unlike the majority of other species that were tested. *Rumex spp* did present accumulations of the other two common remediated metals in this study in iron (1.9%) and zinc (27%). *Rumex spp* is notable however for its apparent ability to accumulate lead (0.7%), which is one of two genus or species in this study. It also showed accumulation of chromium at 2.8%. With both lead and chromium being notoriously difficult to remediate due to the relationship with the organic matter content of the soils and the pH, *Rumex spp* shows promise of a future accumulating genus. The need for a greater number of samples in order to assess *Rumex spp* full potential as a remediator of lead and chromium is important. This is due to only one of the samples showing results for the two metals.

Veronica chamaedrys

Veronica chamaedrys was an uncommon species at the site, only providing one sample. This means that the result can only give a suggestion as to how affective *Veronica chamaedrys* would be at hyperaccumulating heavy metals on the whole. The *Veronica chamaedrys* exhibited accumulation of the four widely accumulated heavy metals on the site, being copper, iron, nickel and zinc. Above average concentrations of

accumulation for the site were found in the *Veronica chamaedrys* for iron (2.4%). High concentrations of copper accumulation were also found (34.9%). However, concentrations of zinc accumulation were low compared to the other species analysed.

Potentilla erecta

The results gained for *Potentilla erecta* showed that the accumulation rate within the samples was variable across the sample locations. However, although variable most samples were within 7% of each other for the higher accumulating metals (copper at 13.3% and zinc at 15.3%). *Potentilla erecta* also showed accumulation of iron at 0.8%, which is at a low concentration of accumulation. Although there is a consistency with the samples collected, with the concentrations being as low as they are for the four heavy metals accumulated, *Potentilla erecta* does not look like a potential candidate for hyperaccumulation methods of remediation.

Vicia tetrasperma

Vicia tetrasperma showed no accumulation ability of any of the heavy metals. This is surprising as all plants require concentrations of key heavy metals such as iron, zinc and copper to survive. *Vicia tetrasperma* was scarce at the site and was only found in one sample location. This could show that the heavy metals were having an adverse effect on the *Vicia tetrasperma* causing no uptake of any heavy metals into the plant and causing its growth to be impaired. It might also be due to being out-competed by species that are better adapted to grow in harsher soil conditions and contamination concentrations.

Rubus fruticosus

The *Rubus fruticosus* sample is of specific interest in this research due to its potential impact on human health in the area due to foraging being common. Very low concentrations of uptake of copper were recorded of 0.2%, representing 2.5 ppm, which is within recommended concentrations for infants of a maximum of 3 ppm ingestion (Stern, 2010). This is below health limits and would be safe for consumption. Iron

concentrations within the *Rubus fruticosus* sample reached 84 ppm. Although this was only 0.5% of the soil concentration, according to Sipahi *et al* (2002), concentrations of ingestion of over 20ppm can cause cases of iron poisoning and concentrations above 50ppm causing severe poisonosity (WebMD, 2017). With concentrations of over four times the 20ppm and over the 50 ppm threshold, this could pose a serious threat to the population if it were to also accumulate within the berry of the *Rubus fruticosus*. Further research is required to determine if this occurs. There were no recorded accumulation concentrations of nickel, cadmium, lead or chromium. This is promising due to these four metals all being poisonous to human health at low doses. Zinc concentrations that were accumulated within the *Rubus fruticosus* of 65.3% were not above concentrations that would cause risk to human health which start to be seen at intakes of 150–450 mg of Zn per day (Hamilton, Gilmore and Strain, 2000).

Phleum spp

Phleum spp is intriguing in its results for zinc accumulation. Its uptake of 149.4% compared to the soil contamination concentrations, which equates to 524ppm is a large uptake of zinc that would normally cause poisonosity. This could be the reason behind the lack of any copper uptake within the plant sample as high concentrations of zinc have been shown to cause deficiency of copper within both plants and humans. With only displaying minimal iron uptake of below 0.1%, it is the amount of zinc uptake that makes this genus intriguing. The lack of abundance however makes the effectiveness of *Phleum spp* as a hyperaccumulator for the site questionable. The zinc can be taken up in such high concentrations that it is having an adverse effect on growth due to copper deficiency. If the plants only take up zinc, in doing so it causes the plant to be unsuccessful in growth, this does not make it a prime candidate for remediation of the site.

Pisum sativum

Due to the finding of just one sample of *Pisum sativum*, and it known for being grown as a domestic crop in gardens, it can be deemed that this specimen could be a loan sample

of the species as it is not commonly found growing in the area if not cultivated. No uptake of copper was seen in the analysis of any concentration. Iron was accumulated at 2.4% of soil concentration concentrations which is third highest out of species tested, however still low for a hyperaccumulating species. *Pisum sativum* also took up zinc at 29.9% of the concentration of the soil at 78.3 ppm which is a natural concentration for plants to be able to withstand and not exceptional amongst other species tested.

Salvia pratensis

Salvia pratensis exhibited accumulation compared to the locations soils of 8.2 % for copper contamination which is 95.4ppm within the plant sample. It also exhibited 1.3% iron accumulation and 33.7% of zinc. These concentrations are average compared to other accumulation rates from the site for other species.

Potential hyperaccumulators and remediation methods

A number of species and genus that were collected and analysed during this research have shown promise as phytoremediators. In order for the method chosen for the site to be effective, all six heavy metals need to be taken into account. For this to occur, multiple species and genus will have to be implemented together to get the most benefit from the application. That being said, cadmium was only found in a small number of locations on the site, as shown in the inverse distance weighting in image 2. Also, with the highly-varied concentrations of all the contaminants across the site, slightly different management methods must be implemented across the site depending on the combination of contaminants.

Lolium perenne and *Trisetum flavescens* are the first species of interest for the remediation method. Although only demonstrating average concentrations of accumulation of copper, iron and zinc, the abundance of the two species at the site mean that if it can grow across the entire of Milton Creek Country Park, then there are more individuals remediating as a multitude of individuals taking up average amounts is

better than two or three taking up large amounts of heavy metal contamination. Both these species can be found in the same location. This means that the species do not out-compete each other but can live together. This aspect makes them a good basis for a remediation method. *Trisetum flavescens* has also demonstrated the ability to be able to take up concentrations of lead, nickel and chromium which are not commonly accumulated heavy metals and is a rare attribute. For nickel, *Trisetum flavescens* was the only species tested from the site that showed any concentration of accumulation. This makes *Trisetum flavescens* more of an ideal candidate for the remediation of Milton Creek Country Park. However, more species are required if all metals are to be remediated to the full extent.

To be able to remediate the heavy metal contaminants that have very high concentrations of contamination, other species will need to be introduced into the remediation methods mix. For some metals, such as lead, nickel and chromium, this cannot be done as *Trisetum flavescens* was the only species that demonstrated the ability to hyperaccumulate these metals. For zinc, copper and iron, which are all in high concentrations across the entirety of the site, this can be done. *Agrostis stolonifera* and *Plantago spp* are ideal for the mix. Both *Agrostis stolonifera* and *Plantago spp* accumulate higher concentrations of copper than either *Lolium perenne* and *Trisetum flavescens*; they also remediate average concentrations of zinc. By adding these two species into the mix a higher quantity of the most abundant heavy metals will be removed, speeding up the remediation process, allowing for the site to be utilised in other ways that are not allowed while contamination is present.

Implementing a mix of *Lolium perenne*, *Trisetum flavescens*, *Plantago spp* and *Agrostis stolonifera* has the added benefit of keeping as much biodiversity present at the site as possible. Taking away too much biodiversity could impact the site in a negative way for the other wildlife that inhabit the site. By keeping the species richness as high as possible while also using a large number of the targeted plants limits the negative effects that can occur, such as deterring bees from pollinating. A lower biodiversity could also cause rare species that inhabit the site to not be able to sustain themselves; this would be a largely negative outcome of the remediation method and in many cases where

species might be protected by law, illegal. As such, every effort must be made to keep these species in the area and comfortable with the surrounding ecosystem.

Soil properties and the management method

The mix of species is very important for the management method due to its ranges of pH tolerances as well as ranges of organic matter. With the pH of the site varying between just below 5 pH and just above 8 pH, a spread of species that are able to cope in the differing soil property is essential. The combination of four species *Lolium perenne*, *Trisetum flavescens*, *Plantago spp* and *Agrostis stolonifera* means that each area of the site has the potential to have one of the hyperaccumulating species growing and remediating the heavy metal contamination. *Trisetum flavescens* survives between the pH values of 5.5 and 7.5. therefore, this will allow the species to grow in the majority of areas which show peaks in lead, nickel and chromium, where *Trisetum flavescens* either is the only species found to remediate or the best. *Plantago spp* is able to survive at pH concentrations up to 9.0 pH. This means that the heavy metals that are at the higher concentrations such as iron and zinc as some of the higher concentrations of these heavy metals are situated in areas that are at 8.0 pH values or above.

As a number of the species that were tested had only a small sample size, seeing if the soil properties of the site in question had any effect on either their abundance or growth would be difficult. In order to do so, another test would need to be carried out where these identified species would be collected from across the site, wherever found and then compared with soil property results in order to get a clear understanding on any link between the two.

Management methods for the site

Along with the need to remediate the site, another focus of this study was to gauge any health risks that could be as a result of the heavy metal contamination at the site. As such, one issue that must be considered in the management plan for the site is the heavy metal contamination that is evident within the *Rubus fruticosus*. Being a prevalent area

for foraging, the slightly elevated concentrations of copper and zinc within the species, although not life threatening on their own, could cause problems in people with already increased concentrations in their systems. The major concern with the results for *Rubus fruticosus* is the concentrations of iron within the sample being above 50 ppm, which has been known for causing severe poisonosity in humans. As such, a decision will need to be made as to the best course of action to prevent the foraging of fruit from the bush. This can be the putting up of signage, warning the public of the dangers. Although it could be effective, this method would not prevent the foraging of the fruit by birds and small mammals, which these concentrations of contaminants could also be harmful. The removal of the bushes entirely would be a heavily work intensive process as well as expensive. Low costs are one of the main reasons for utilising a phytoremediation method of clearing a site and so this method again would not be massively suitable. The removal of the fruit from the bushes would again require a high concentration of work to manage. This method does however keep the plant within the ecosystem, which is important as a habitat for local wildlife. The flowering of *Rubus fruticosus* is also important for the attraction of pollinating insects. As such, this method, although highly work intensive for managers of the park, could be seen as the best balance of removing the threat of ingestion of the heavy metal contaminants whilst also maintaining a good balance for the local ecosystem.

It has become apparent that the soil properties on the site are quite varied in there levels. Each species of plant to be implemented would have a preferred level of each soil property. In order for the management method to work at its full potential, a study on how the soil properties (pH , organic matter and EC particularly) interact with the species would need to be carried out. A comparison cannot be drawn up from the result collected as the sampling size of each species was not big enough to draw any definitive conclusions as to any link. Pot experiments with each variable tested would be the best way in ensuring the remediation plants chosen are the best for the management required.

Lead concentration concentrations across the entire site could pose a large threat to human health as discussed before. With the issues of the site crossing away from plant contamination to the health of the public, other management techniques will need to

be implemented regarding public safety. One of the major issues brought up by the results is the high concentration of lead contamination on and around the area where the clear ground and children's play park are situated. To secure the areas of high contamination, there are three options; remove, cover or cordon off. With the main objective to remediate the contamination using phytoremediation methods, it would be obvious that the removal of the contaminants is the primary source of action. However, with this taking time and other methods being destructive and high in cost, cover and cordoning will be the next course of action.

Covering the area in foliage is said to reduce the risk of the lead contamination from entering the blood from cuts and prevents the soil from forming dust and being inhaled. The recommended species for phytoremediation (*Lolium perenne*, *Trisetum flavescens*, *Plantago spp* and *Agrostis stolonifera*) would provide some of this cover, with *Agrostis stolonifera* providing surface stability with its root structure (Bradshaw and Baker, 2003). However, in order to produce a protective cover, a plant such as *Trifolium repens* would be ideal to introduce into the mix. With *Trifolium repens* having a large leaf base and many samples in one area, this would form a protective layer while also contributing to the remediation of the contaminants in the soil.

Cordoning of the area from the public in the areas that present with concentrations between 1000 and 2000 ppm will have to be considered. With these concentrations being highly dangerous and with recommendations that children are not allowed in areas with this concentration, fencing the ground off and producing appropriate signage explaining the issues to the public would be the best course of action. Therefore, covering the area in the phytoremediation plants and introducing *Trifolium repens*, whilst also fencing the areas off, the risk of lead poisoning to children and adults on the site of Milton Creek Country Park would be reduced to the minimum which could be achieved.

Conclusion

Concentrations of heavy metal contamination including copper, iron, nickel, lead, zinc and chromium all showed as high across Milton Creek Country Park, with some areas reaching dangerous concentrations for lead, iron and chromium. A number of the species that had been taken from sample areas at the site showed high accumulation rates with some displaying hyperaccumulating concentrations for certain metals.

Lolium perenne, *Trisetum flavescens*, *Plantago spp* and *Agrostis stolonifera* have all been specified as practical choices for a phytoremediation method for Milton Creek Country Park due to their high range of survivable pH and coverage of accumulation of all heavy metals found in high concentrations at the site. This includes *Trisetum flavescens* which was the only species that was tested at the site which showed an ability to be able to accumulate concentrations of nickel, lead and chromium, which were the most important metals to remove. Extra aspects to the remediation method that will be put in place have also been suggested including the management of *Rubus fruticosus* at the site due to risk from high iron concentrations to people's health. This will be done by the removal of the fruit from the plant and signage indicating the concern and action being undertaken. Issues regarding lead pollution concentrations were also taken into account with the suggestion of the covering of open areas with phytoremediation foliage with the addition of *Trifolium repens*, while also cordoning of the area with fences and signs to minimise the risk to the high numbers of children that use the site each day.

More research will need to be carried out regarding how the suggested species interact with each other as well as how the differing soil properties of the site might affect their use as hyper-accumulators.

Also, more research into the amount of chromium pollution of the site and its situation next to the River Swale will need to be conducted to determine if the contamination coming from high points of contamination might be polluting the water source and if so to what extent this might be and if this might cause issues with human health and the health of the surrounding ecosystem.

Acknowledgements

Firstly, I would like to acknowledge Canterbury Christchurch University for facilitating me and enabling me to carry out my Masters research. I would like to thank Dr Naomi Rintoul for all her hard work supporting and guiding me through my research. I would like to thank the lab technicians especially to Tom Briggs, without whom I would not have collected data on time. I would like to thank my family, whose support has been unwavering and ever-present. Lastly, I would like to thank my partner Alan Clarke, who has helped me get through my Masters Degree (one very hard and troubling time) while also helping me deal with one of the hardest years.

References

Abreu, C., Abreu, M. and Andrade, J. (1998). Distribution of lead in the soil profile evaluated by DTPA and Mehlich-3 solutions. *Bragantia*, 57(1), pp.185-192.

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

Ali, H., Khan, E. and Sajad, M. (2013). Phytoremediation of heavy metals—Concepts and applications. *Chemosphere*, 91(7), pp.869-881.

Alloway, B. (2012). Sources of Heavy Metals and Metalloids in Soils. *Heavy Metals in Soils. Environmental Pollution*, (22), pp.11-50. Springer, Dordrecht.

Alvarenga, P., Gonçalves, A., Fernandes, R., de Varennes, A., Vallini, G., Duarte, E. and Cunha-Queda, A. (2008). Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass. *Science of The Total Environment*, 406(1-2), pp.43-56.

- Anderson, C., Brooks, R., Chiarucci, A., LaCoste, C., Leblanc, M., Robinson, B., Simcock, R. and Stewart, R. (1999). Phytomining for nickel, thallium and gold. *Journal of Geochemical Exploration*, 67(1-3), pp.407-415.
- Angelova, V., Ivanova, R., Todorov, G. and Ivanov, K. (2016). Potential of *Salvia sclarea* L. for Phytoremediation of Soils Contaminated with Heavy Metals. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, 10(12), pp.776-786.
- Antoniadis, V., Robinson, J. and Alloway, B. (2008). Effects of short-term pH fluctuations on cadmium, nickel, lead, and zinc availability to ryegrass in a sewage sludge-amended field. *Chemosphere*, 71(4), pp.759-764.
- Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B. and Mittal, N. (2008). Heavy metal accumulation in vegetables irrigated with water from different sources. *Food Chemistry*, 111(4), pp.811-815.
- Atabayeva, S. (2016). Heavy Metals Accumulation Ability of Wild Grass Species from Industrial Areas of Kazakhstan. *Phytoremediation*, pp.157-208.
- Baker, A. (1981). Accumulators and excluders -strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, 3(1-4), pp.643-654.
- Baker, A., McGrath, S., Sidoli, C. and Reeves, R. (1994). The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. *Resources, Conservation and Recycling*, 11(1-4), pp.41-49.
- Baldantoni, D., Cikatelli, A., Bellino, A. and Castiglione, S. (2014). Different behaviours in phytoremediation capacity of two heavy metal tolerant poplar clones in relation to iron and other trace elements. *Journal of Environmental Management*, 146, pp.94-99.
- Barker, A. (2015). *Handbook of plant nutrition*. Boca Raton, Fla. [u.a.]: CRC Press.
- Baun, D. and Christensen, T. (2004). Speciation of Heavy Metals in Landfill Leachate: A Review. *Waste Management & Research*, 22(1), pp.3-23.

- Bettinelli, M., Beone, G., Spezia, S. and Baffi, C. (2000). Determination of heavy metals in soils and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis. *Analytica Chimica Acta*, 424(2), pp.289-296.
- Beveridge, T. and Doyle, R. (1989). *Metal ions and bacteria*. 1st ed. New York [etc.]: Wiley, pp.31-90.
- Bhargava, A., Carmona, F., Bhargava, M. and Srivastava, S. (2012). Approaches for enhanced phytoextraction of heavy metals. *Journal of Environmental Management*, 105, pp.103-120.
- Bidar, G., Garçon, G., Pruvot, C., Dewaele, D., Cazier, F., Douay, F. and Shirali, P. (2007). Behavior of *Trifolium repens* and *Lolium perenne* growing in a heavy metal contaminated field: Plant metal concentration and phytotoxicity. *Environmental Pollution*, 147(3), pp.546-553.
- Bidar, G., Pruvot, C., Garçon, G., Verdin, A., Shirali, P. and Douay, F. (2008). Seasonal and annual variations of metal uptake, bioaccumulation, and toxicity in *Trifolium repens* and *Lolium perenne* growing in a heavy metal-contaminated field. *Environmental Science and Pollution Research*, 16(1), pp.42-53.
- Blum, W. (1997). Cadmium uptake by higher plants. In: *fourth international conference on the biogeochemistry of trace elements*. Berkeley, USA, pp.109-110.
- Bradshaw, A. and Baker, A. (2003). *The Restoration and Management of Derelict Land*. World Scientific, p.Chapter 5.
- Brallier, S., Harrison, R., Henry, C. and Dongsen, X. (1996). Liming effects on availability of Cd, Cu, Ni and Zn in a soil amended with sewage sludge 16 years previously. *Water, Air, and Soil Pollution*, 86(1-4), pp.195-206.
- Brooks, R., Chambers, M., Nicks, L. and Robinson, B. (1998). Phytomining. *Trends in Plant Science*, 3(9), pp.359-362.
- Brown, S., Angle, J., Chaney, R. and Baker, A. (1995). Zinc and Cadmium Uptake by Hyperaccumulator *Thlaspi caerulescens* Grown in Nutrient Solution. *Soil Science Society of America Journal*, 59(1), p.125.

Calmano, W., Hong, J. and Förstner, U. (1993). Binding and Mobilization of Heavy Metals in Contaminated Sediments Affected by pH and Redox Potential. *Water Science and Technology*, 28(8-9), pp.223-235.

Carr, R., Zhang, C., Moles, N. and Harder, M. (2007). Identification and mapping of heavy metal pollution in soils of a sports ground in Galway City, Ireland, using a portable XRF analyser and GIS. *Environmental Geochemistry and Health*, 30(1), pp.45-52.

Chapelle, F. (1997). *Bioremediation: nature's way to a cleaner environment*. Fact Sheet FS-054-95. U. S. Department of Interior: U.S. Geological Survey, pp.1-4.

Chehregani, A., Noori, M. and Yazdi, H. (2009). Phytoremediation of heavy-metal-polluted soils: Screening for new accumulator plants in Angouran mine (Iran) and evaluation of removal ability. *Ecotoxicology and Environmental Safety*, 72(5), pp.1349-1353.

Chen, H., Teng, Y., Lu, S., Wang, Y. and Wang, J. (2015). Contamination features and health risk of soil heavy metals in China. *Science of The Total Environment*, 512-513, pp.143-153.

Cheng, S. (2003). Heavy metals in plants and phytoremediation. *Environmental Science and Pollution Research*, 10(5), pp.335–340.

Chlopecka, A., Bacon, J., Wilson, M. and Kay, J. (1996). Forms of Cadmium, Lead, and Zinc in Contaminated soils from Southwest Poland. *Journal of Environment Quality*, 25(1), p.69.

Christensen, J., Jensen, D. and Christensen, T. (1996). Effect of dissolved organic carbon on the mobility of cadmium, nickel and zinc in leachate polluted groundwater. *Water Research*, 30(12), pp.3037-3049.

Clemens, S., Palmgren, M. and Krämer, U. (2002). A long way ahead: understanding and engineering plant metal accumulation. *Trends in Plant Science*, 7(7), pp.309-315.

Coman, V., Robotin, B. and Ilea, P. (2013). Nickel recovery/removal from industrial wastes: A review. *Resources, Conservation and Recycling*, 73, pp.229-238.

Cornejo, P., Pérez-Tienda, J., Meier, S., Valderas, A., Borie, F., Azcón-Aguilar, C. and Ferrol, N. (2013). Copper compartmentalization in spores as a survival strategy of arbuscular mycorrhizal fungi in Cu-polluted environments. *Soil Biology and Biochemistry*, 57, pp.925-928.

Costa, M. and Klein, C. (2006). Poisonousity and Carcinogenicity of Chromium Compounds in Humans. *Critical Reviews in Poisonousology*, 36(2), pp.155-163.

Crews, H. and Davies, B. (1985). Heavy metal uptake from contaminated soils by six varieties of lettuce (*Lactuca sativa* L.). *The Journal of Agricultural Science*, 105(03), p.591.

Cuilliel, M. (2009). The dual personality of ionic copper in biology. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 65(1-2), pp.165-170.

Cunningham, S., Berti, W. and Huang, J. (1995). Phytoremediation of contaminated soils. *Trends in Biotechnology*, 13(9), pp.393-397.

De Vos, C., Schat, H., De Waal, M., Vooijs, R. and Ernst, W. (1991). Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. *Physiologia Plantarum*, 82(4), pp.523-528.

Denkhaus, E. and Salnikow, K. (2002). Nickel essentiality, poisonousity, and carcinogenicity. *Critical Reviews in Oncology/Hematology*, 42(1), pp.35-56.

Department of Sustainable Natural Resources (n.d.). Soil survey standard test method pH: 1:5 soil : water suspension. Department of Sustainable Natural Resources.

Dmuchowski, W., Gozdowski, D., Brągoszewska, P., Baczewska, A. and Suwara, I. (2014). Phytoremediation of zinc contaminated soils using silver birch (*Betula pendula* Roth). *Ecological Engineering*, 71, pp.32-35.

Doner, H. (1978). Chloride as a Factor in Mobilities of Ni(II), Cu(II), and Cd(II) in Soil1. *Soil Science Society of America Journal*, 42(6), p.882.

Drzewiecka, K., Mleczek, M., Waśkiewicz, A. and Goliński, P. (2012). *Abiotic Stress Responses in Plants*. 1st ed. New York, NY: Springer Science+Business Media, LLC.

Ebbs, S. and Kochian, L. (1997). Poisonousity of Zinc and Copper to Brassica Species: Implications for Phytoremediation. *Journal of Environment Quality*, 26(3), p.776.

Eick, M., Peak, J., Brady, P. and Pesek, J. (1999). Kinetics of lead adsorption/desorption on goethite: Residence time effect. *Soil Science*, 164(1), pp.28-39.

EPA (1992). *Chemical Dehalogenation Treatability Studies Under CERCLA An Overview*. Office of Solid waste and Emergency Response, pp.1-8.

EPA (1993). *Standard Methods for the Examination of Water and Wastewater*. US: American Public Health Assoc.

Erdemir, Ü., Arslan, H., Güteryüz, G. and Güçer, Ş. (2016). Elemental Composition of Plant Species from an Abandoned Tungsten Mining Area: Are They Useful for Biogeochemical Exploration and/or Phytoremediation Purposes?. *Bulletin of Environmental Contamination and Poisonousology*, 98(3), pp.299-303.

Ernst, W. (1996). Bioavailability of heavy metals and decontamination of soils by plants. *Applied Geochemistry*, 11(1-2), pp.163-167.

Erturk, U., Yerlikaya, C. and Sivritepe, N. (2007). In vitro Phytoextraction Capacity of Blackberry for Copper and Zinc. *Asian Journal of Chemistry*, 19(3), pp.2161-2168.

US EPA, (2000) Compilation Of Air Pollutant Emission Factors, Volume I, Fifth Edition.

Escande, V., Garoux, L., Grison, C., Thillier, Y., Debart, F., Vasseur, J., Boulanger, C. and Grison, C. (2014). Ecological catalysis and phytoextraction: Symbiosis for future. *Applied Catalysis B: Environmental*, 146, pp.279-288.

European Commission (2006). The Soil Thematic Strategy. European Commission.

European Communities (1986). EC 86/278/EEC. *Official Journal of the European Communities*, 181(10), p.5.

European Parliament (2000a). Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe. European Parliament.

European Parliament (2000b). Water Framework Directive. European Parliament.

Facchinelli, A., Sacchi, E. and Mallen, L. (2001). Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. *Environmental Pollution*, 114(3), pp.313-324.

Felix, H. (1997). Field Trials for in Situ Decontamination of Heavy Metal Polluted Soils using Crops of Metal-Accumulating Plants. *Zeitschrift für Pflanzenernährung und Bodenkunde*, 160(4), pp.525-529.

Freitas, H., Prasad, M. and Pratas, J. (2004). Plant community tolerant to trace elements growing on the degraded soils of São Domingos mine in the south east of Portugal: environmental implications. *Environment International*, 30(1), pp.65-72.

Galal, T. and Shehata, H. (2015). Bioaccumulation and translocation of heavy metals by *Plantago major* L. grown in contaminated soils under the effect of traffic pollution. *Ecological Indicators*, 48, pp.244-251.

Garbisu, C. and Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology*, 77(3), pp.229-236.

Garbisu, C., Alkorta, I., Carlson, D., Leighton, T. and Buchanan, B. (1997). Selenite bioremediation potential of indigenous microorganisms from industrial activated sludge. *Microbiologia*, 13(4), pp.437-444.

Ghani, A. (2011). Effect of chromium poisonosity on growth, chlorophyll and some mineral nutrients of *Brassica juncea* L. *Egyptian Acad J Biol Sci*, 2(1), pp.9-15.

Glass, D. (2000). Economic Potential of Phytoremediation. In: I. Raskin and B. Ensley, ed., *Phytoremediation of Poisonous Metals: Using Plants to Clean-Up the Environment*, 1st ed. John Wiley & Sons, pp.15-31.

Greipsson, S. (2017). *Phytoremediation | Learn Science at Scitable*. [online] Nature.com. Available at: <http://www.nature.com/scitable/knowledge/library/phytoremediatio%20n-17359669> [Accessed 28 Apr. 2017].

- Guimarães, A., Ferreira, R., Silva, W., Castro, A. and Malafaia, G. (2016). Parental exposure to tannery effluent cause anxiety-and-depression-like behaviors in mice offspring. *JSM Anxiety and Depression*, (1), p.1005.
- Hamilton, I., Gilmore, W. and Strain, J. (2000). Marginal Copper Deficiency and Atherosclerosis. *Biological Trace Element Research*, 78(1-3), pp.179-190.
- Hanlon, W. (2015). Pollution and Mortality in the 19th Century. *National Bureau of Economic Research*.
- Harmens, H., Koevoets, P., Verkleij, J. and Ernst, W. (1994). The role of low molecular weight organic acids in the mechanism of increased zinc tolerance in *Silene vulgaris* (Moench) Garcke. *New Phytologist*, 126(4), pp.615-621.
- Harter, R. (1983). Effect of Soil pH on Adsorption of Lead, Copper, Zinc, and Nickel. *Soil Science Society of America Journal*, 47(1), p.47.
- Hattab, S., Dridi, B., Chouba, L., Ben Kheder, M. and Bousetta, H. (2009). Photosynthesis and growth responses of pea *Pisum sativum* L. under heavy metals stress. *Journal of Environmental Sciences*, 21(11), pp.1552-1556.
- Hawkes, S. (1997). What Is a "Heavy Metal"?. *Journal of Chemical Education*, 74(11), p.1374.
- Hernández, L., Gárate, A. and Carpena-Ruiz, R. (1997). Effects of cadmium on the uptake, distribution and assimilation of nitrate in *Pisum sativum*. *Plant and Soil*, 189(1), pp.97–106.
- Hettiarachchi, G., Ryan, J., Chaney, R. and La Fleur, C. (2003). Sorption and Desorption of Cadmium by Different Fractions of Biosolids-Amended Soils. *Journal of Environment Quality*, 32(5), p.1684.
- Huang, J. and Cunningham, S. (1996). Lead phytoextraction: species variation in lead uptake and translocation. *New Phytologist*, 134(1), pp.75-84.
- Impellitteri, C., Lu, Y., Saxe, J., Allen, H. and Peijnenburg, W. (2002). Correlation of the partitioning of dissolved organic matter fractions with the desorption of Cd, Cu, Ni, Pb and Zn from 18 Dutch soils. *Environment International*, 28(5), pp.401-410.

- Irfan, M., Hayat, S., Ahmad, A. and Alyemini, M. (2013). Soil cadmium enrichment: Allocation and plant physiological manifestations. *Saudi Journal of Biological Sciences*, 20(1), pp.1-10.
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. and Beeregowda, K. (2014). Poisonousity, mechanism and health effects of some heavy metals. *Interdisciplinary Poisonousology*, 7(2).
- Jarup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, 68(1), pp.167-182.
- Johnston, T., Datta, R. and Sarkar, D. (2005). Phytoextraction and Phytostabilization: Technical, Economic and Regulatory Considerations of the Soil-Lead Issue. *Water Encyclopedia*.
- Jones, L., O'Reilly, M. and Morgan, A. (2007). Responses of a non-target organism to metalliferous field soils amended by a phytoremediation-promoting chelator (EDTA): The earthworm, *Eisenia fetida*. *European Journal of Soil Biology*, 43, pp.S289-S296.
- Karczewska, A., Mocek, A., Goliński, P. and Mleczek, M. (2015). Phytoremediation of Copper-Contaminated Soil. *Phytoremediation*, pp.143-170.
- Karlson, U. and Frankenberger, W. (1989). Accelerated Rates of Selenium Volatilization From California Soils. *Soil Science Society of America Journal*, 53(3), p.749.
- Karunyal, S., Renuga, G. and Kailash, P. (1994). Effects of tannery effluent on seed germination, leaf area, biomass and mineral content of some plants. *Bioresource Technology*, 47(3), pp.215-218.
- Kashem, M. and Singh, B. (2001). Metal availability in contaminated soils: I. Effects of flooding and organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn. *Nutrient Cycling in Agroecosystems*, 61(3), pp.247-255.
- Kaviani, E., Niazi, A., Moghadam, A., Taherishirazi, M. and Heydarian, Z. (2017). Phytoremediation of Ni-contaminated soil by *Salicornia iranica*. *Environmental Technology*, pp.1-12.

Kidd, P., Barceló, J., Bernal, M., Navari-Izzo, F., Poschenrieder, C., Shilev, S., Clemente, R. and Monterroso, C. (2009). Trace element behaviour at the root–soil interface: Implications in phytoremediation. *Environmental and Experimental Botany*, 67(1), pp.243-259.

Kim, S. (2001). Monitoring of electrokinetic removal of heavy metals in tailing-soils using sequential extraction analysis. *Journal of Hazardous Materials*, 85(3), pp.195-211.

Konotop, Y., Mészáros, P., Spieß, N., Mistríková, V., Piršelová, B., Libantová, J., Moravčíková, J., Taran, N., Hauptvogel, P. and Matušíková, I. (2012). Defense responses of soybean roots during exposure to cadmium, excess of nitrogen supply and combinations of these stressors. *Molecular Biology Reports*, 39(12), pp.10077-10087.

Kos, B., Greman, H. and Lestan, d. (2003). Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant Soil and Environment*, 49(12), pp.548-553.

Krishnamurti, G., Huang, P. and Kozak, L. (1999). SORPTION AND DESORPTION KINETICS OF CADMIUM FROM SOILS: INFLUENCE OF PHOSPHATE. *Soil Science*, 164(12), pp.888-898.

Kuppens, T., Van Dael, M., Vanreppelen, K., Thewys, T., Yperman, J., Carleer, R., Schreurs, S. and Van Passel, S. (2015). Techno-economic assessment of fast pyrolysis for the valorization of short rotation coppice cultivated for phytoextraction. *Journal of Cleaner Production*, 88, pp.336-344.

Lasat, M. (2002). Phytoextraction of Poisonous Metals. *Journal of Environment Quality*, 31(1), p.109.

Li, Z., Wu, L., Hu, P., Luo, Y., Zhang, H. and Christie, P. (2014). Repeated phytoextraction of four metal-contaminated soils using the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. *Environmental Pollution*, 189, pp.176-183.

Lone, M., He, Z., Stoffella, P. and Yang, X. (2008). Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. *Journal of Zhejiang University SCIENCE B*, 9(3), pp.210-220.

- Lu, S., Bai, S. and Xue, Q. (2007). Magnetic properties as indicators of heavy metals pollution in urban topsoils: a case study from the city of Luoyang, China. *Geophysical Journal International*, 171(2), pp.568-580.
- Luo, C., Shen, Z. and Li, X. (2005). Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere*, 59(1), pp.1-11.
- Maguire, D., Goodchild, M. and Rhind, D. (1991). *Geographical information systems. Principles and applications*. 1st ed. Harlow: Essex Longman XXIX, p.Chapter 1.
- Maluckov, B. (2015). Bioassisted Phytomining of Gold. *JOM*, 67(5), pp.1075-1078.
- Mangabeira, P., Ferreira, A., de Almeida, A., Fernandes, V., Lucena, E., Souza, V., dos Santos Júnior, A., Oliveira, A., Grenier-Loustalot, M., Barbier, F. and Silva, D. (2011). Compartmentalization and ultrastructural alterations induced by chromium in aquatic macrophytes. *BioMetals*, 24(6), pp.1017-1026.
- Manu, M., Băncilă, R., Iordache, V., Bodescu, F. and Onete, M. (2017). Impact assessment of heavy metal pollution on soil mite communities (Acari: Mesostigmata) from Zlatna Depression – Transylvania. *Process Safety and Environmental Protection*, 108, pp.121-134.
- Maric, M., Antonijevic, M. and Alagic, S. (2012). The investigation of the possibility for using some wild and cultivated plants as hyperaccumulators of heavy metals from contaminated soil. *Environmental Science and Pollution Research*, 20(2), pp.1181-1188.
- Marquard, R., Böhm, H. and Friedt, W. (1990). Untersuchungen über Cadmiumgehalte in Leinsaat (*Linum usitatissimum* L.). *Fett Wissenschaft Technologie/Fat Science Technology*, 92(12), pp.468-472.
- McGrath, S. and Zhao, F. (2003). Phytoextraction of metals and metalloids from contaminated soils. *Current Opinion in Biotechnology*, 14(3), pp.277-282.
- McGrath, S., Zhao, J. and Lombi, E. (2002). Phytoremediation of metals, metalloids, and radionuclides. *Advances in Agronomy*, pp.1-56.
- Mei, B., Puryear, J. and Newton, R. (2002). Assessment of Cr tolerance and accumulation in selected plant species. *Plant and Soil*, 247(2), pp.223–231.

- Memon, A. and Schröder, P. (2008). Implications of metal accumulation mechanisms to phytoremediation. *Environmental Science and Pollution Research*, 16(2), pp.162-175.
- Méndez, A., Paz-Ferreiro, J., Araujo, F. and Gascó, G. (2014). Biochar from pyrolysis of deinking paper sludge and its use in the treatment of a nickel polluted soil. *Journal of Analytical and Applied Pyrolysis*, 107, pp.46-52.
- Mendez, M. and Maier, R. (2007). Phytoremediation of mine tailings in temperate and arid environments. *Reviews in Environmental Science and Bio/Technology*, 7(1), pp.47-59.
- Micó, C., Recatalá, L., Peris, M. and Sánchez, J. (2006). Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. *Chemosphere*, 65(5), pp.863-872.
- Miltoncreekmemories.co.uk. (2018). *Milton Creek Memories | Milton Creek Sittingbourne Kent | » Bricks*. [online] Available at: <http://miltoncreekmemories.co.uk/bricks/> [Accessed 13 Aug. 2018].
- Mühlbachová, G., Šimon, T. and Pechová, M. (2005). The availability of Cd, Pb and Zn and their relationships with soil pH and microbial biomass in soils amended by natural clinoptilolite. *Plant Soil and Environment*, 51(1), pp.26-33.
- Mulligan, C., Yong, R. and Gibbs, B. (2001). Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology*, 60(1-4), pp.193-207.
- Murakami, M., Ae, N. and Ishikawa, S. (2007). Phytoextraction of cadmium by rice (*Oryza sativa* L.), soybean (*Glycine max* (L.) Merr.), and maize (*Zea mays* L.). *Environmental Pollution*, 145(1), pp.96-103.
- Murry, R., Miller, D. and kryc, k. (2000). *Analysis of Major and Trace Elements in Rocks, Sediments, and Interstitial Waters by Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES)*. ODP Tech. Note.
- Nagajyoti, P., Lee, K. and Sreekanth, T. (2010). Heavy metals, occurrence and poisonosity for plants: a review. *Environmental Chemistry Letters*, 8(3), pp.199-216.

National Planning Policy Framework 2012, c.11. Available at <https://www.gov.uk/guidance/national-planning-policy-framework/11-conserving-and-enhancing-the-natural-environment#para120> (Accessed: 8 August 2018)

Nazir, A., Malik, R., Ajaib, M., Khan, N. and Siddiqui, M. (2011). Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi. *Pakistan Journal of Botany*, 43(4), pp.1925-1933.

Ni, W., Yang, X. and Long, X. (2002). Differences of cadmium absorption and accumulation in selected vegetable crops. *Journal of Environmental Sciences*, 14(3), pp.399-405.

Nicholson, F. and Chambers, B. (2008). SP0547: Sources and Impacts of Past, Current and Future Contamination of Soil. Appendix 1 : Heavy metals.

Oves, M., Khan, M., Zaidi, A. and Ahmad, E. (2012). Soil Contamination, Nutritive Value, and Human Health Risk Assessment of Heavy Metals: An Overview. *Poisonousity of Heavy Metals to Legumes and Bioremediation*, pp.1-27.

Oxbrow, A. and Moffat, J. (1979). Plant frequency and distribution on high-lead soil near Leadhills, Lanarkshire. *Plant and Soil*, 52(1), pp.127-130.

Pamukcu, S. and Wittle, J. (1992). Electrokinetic removal of selected heavy metals from soil. *Environmental Progress*, 11(3), pp.241-250.

Panwar, B., Ahmed, K. and Mittal, S. (2002). *Environment, Development and Sustainability*, 4(1), pp.1-6.

Parmar, P., Dave, B., Panchal, K. and Subramanian, R. (2013). Identification of Potential Species *Croton bonplandianum*, Sedges and ; *Balanitis aegyptiaca* for the Application of Phytoremediation. *American Journal of Plant Sciences*, 04(06), pp.1246-1251.

Passant, N., Peirce, M., Rudd, H., Scott, D., Marlow, I. and Watterson, J. (2002). *UK Particulate and Heavy Metal Emissions from Industrial Processes*. [online] Department for Environment, Food & Rural Affairs, the National Assembly for Wales, the Scottish Executive and the Department of the Environment in Northern Ireland, pp.107-108,137-138. Available at:

https://ukair.defra.gov.uk/assets/documents/reports/empire/AEAT6270Issue2finaldraft_v2.pdf [Accessed 13 Aug. 2018].

Paz-Alberto, A. and Sigua, G. (2013). Phytoremediation: A Green Technology to Remove Environmental Pollutants. *American Journal of Climate Change*, 02(01), pp.71-86.

Phippen, B., Horvath, C., Nordin, R. and Nagpal, N. (2008). Ambient water quality guidelines for iron: overview. *Ministry of Environment Province of British Columbia*.

Pillai, S., Girija, N., Williams, G. and Koshy, M. (2013). Impact of organic manure on the phytoremediation potential of *Vetiveria zizanioides* in chromium-contaminated soil. *Chemistry and Ecology*, 29(3), pp.270-279.

Pilon-Smits, E. (2005). Phytoremediation. *Annual Review of Plant Biology*, 56, pp.15-39.

Pinto, A., Mota, A., Devarenes, A. and Pinto, F. (2004). Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. *Science of The Total Environment*, 326(1-3), pp.239-247.

Porębska, G. and Ostrowska, A. (1999). Heavy Metal Accumulation in Wild Plants: Implications for Phytoremediation. *Polish Journal of Environmental Studies*, 8(6), pp.433-442.

Prasad, M., Greger, M. and Landberg, T. (2001). *Acacia nilotica* L. Bark Removes Poisonous Elements from Solution: Corroboration from Poisonousity Bioassay Using *Salix viminalis* L. in Hydroponic System. *International Journal of Phytoremediation*, 3(3), pp.289-300.

Prince, W., Kumar, P., Doberschutz, K. and Subburam, V. (2002). CADMIUM POISONOUSITY IN MULBERRY PLANTS WITH SPECIAL REFERENCE TO THE NUTRITIONAL QUALITY OF LEAVES. *Journal of Plant Nutrition*, 25(4), pp.689-700.

Rabelo, L., Costa e Silva, B., de Almeida, S., da Silva, W., de Oliveira Mendes, B., Guimarães, A., da Silva, A., da Silva Castro, A., de Lima Rodrigues, A. and Malafaia, G. (2016). Memory deficit in Swiss mice exposed to tannery effluent. *Neuropoisonousology and Teratology*, 55, pp.45-49.

Raskin, I., Kumar, P., Dushenkov, S. and Salt, D. (1994). Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology*, 5(3), pp.285-290.

Raskin, I., Smith, R. and Salt, D. (1997). Phytoremediation of metals: using plants to remove pollutants from the environment. *Current Opinion in Biotechnology*, 8(2), pp.221-226.

Rattan, R., Datta, S., Chhonkar, P., Suribabu, K. and Singh, A. (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. *Agriculture, Ecosystems & Environment*, 109(3-4), pp.310-322.

Rauch, J. and Pacyna, J. (2009). Earth's global Ag, Al, Cr, Cu, Fe, Ni, Pb, and Zn cycles. *Global Biogeochemical Cycles*, 23(2), p.n/a-n/a.

Reeves, R., Brooks, R. and Macfarlane, R. (1981). Nickel Uptake by Californian *Streptanthus* and *Caulanthus* with Particula Reference to the Hyperaccumulator *S. polygaloides* Gray (Brassicaceae). *American Journal of Botany*, 68(5), p.708.

Robertson, S. (2011). Direct Estimation of Organic Matter by Loss on Ignition: Methods. SFU Soil Science Lab, pp.1-11.

Robinson, B., Anderson, C. and Dickinson, N. (2015). Phytoextraction: Where's the action?. *Journal of Geochemical Exploration*, 151, pp.34-40.

Roccoliello, E., Serrano, H., Mariotti, M. and Branquinho, C. (2015). Nickel phytoremediation potential of the Mediterranean *Alyssoides utriculata* (L.) Medik. *Chemosphere*, 119, pp.1372-1378.

Rodríguez Martín, J., Arias, M. and Grau Corbí, J. (2006). Heavy metals contents in agricultural topsoils in the Ebro basin (Spain). Application of the multivariate geo-statistical methods to study spatial variations. *Environmental Pollution*, 144(3), pp.1001-1012.

Rodríguez, M., Barsanti, L., Passarelli, V., Evangelista, V., Conforti, V. and Gualtieri, P. (2007). Effects of chromium on photosynthetic and photoreceptive apparatus of the alga *Chlamydomonas reinhardtii*. *Environmental Research*, 105(2), pp.234-239.

Romney, E., Wallace, A., Wood, R., El-Gazzar, A., Childress, J. and Alexander, G. (1977). Role of soil organic matter in a desert soil on plant response to silver, tungsten, cobalt, and lead. *Communications in Soil Science and Plant Analysis*, 8(9), pp.719-725.

Rose, F., O'Reilly, C., Smith, D. and Collings, M. (2006). *The wild flower key*. London: Frederick Warne.

Salihaj, M., Bani, A. and Echevarria, G. (2016). Heavy metals uptake by hyperaccumulating flora in some serpentine soils of Kosovo. *Global NEST Journal*, 18.

Samantaray, S., Rout, G. and Das, P. (1998). Role of chromium on plant growth and metabolism. *Acta Physiologiae Plantarum*, 20(2), pp.201-212.

Sas-Nowosielska, A., Kucharski, R., Pogrzeba, M., Krzyżak, J., Kuperberg, J. and Japenga, J. (2008). Phytoremediation Technologies Used To Reduce Environmental Threat Posed By Metal-Contaminated Soils: Theory And Reality. *Simulation and Assessment of Chemical Processes in a Multiphase Environment*, pp.285-297.

Sassa.org.uk. (2017). Analytical Methods : Lab Mag Sus - SASSA. [online] Available at: http://www.sassa.org.uk/index.php/Analytical_Methods:Lab_Mag_Sus [Accessed 30 Aug. 2017].

Satarug, S., Garrett, S., Sens, M. and Seas, D. (2011). Cadmium, environmental exposure, and health outcomes. *Ciência & Saúde Coletiva*, 16(5), pp.2587-2600.

Sauerbeck, D. (1991). Plant element and soil properties governing uptake and availability of heavy metals derived from sewage sludge. *Water, Air, and Soil Pollution*, 57-58(1), pp.227-237.

Schmidt, A., Yarnold, R., Hill, M. and Ashmore, M. (2005). Magnetic susceptibility as proxy for heavy metal pollution: a site study. *Journal of Geochemical Exploration*, 85(3), pp.109-117.

Serrano, H., Cotrim, H., Pinto, M., Martins-Loução, M. and Branquinho, C. (2016). Metal hyperaccumulation patterns within *Plantago* phylogeny (Plantaginaceae). *Plant and Soil*, 411(1-2), pp.227-241.

Shakoor, B., Ali, S., Hameed, A., Farid, M., Hussain, S., Yasmeen, T., Najeeb, U., Aslam Bharwana, S. and Hasan Abbasi, G. (2014). Citric acid improves lead (pb) phytoextraction in brassica napus L. by mitigating pb-induced morphological and biochemical damages. *Ecopoisonousology and Environmental Safety*, 109, pp.38-47.

Shallari, S. (1998). Heavy metals in soils and plants of serpentine and industrial sites of Albania. *The Science of The Total Environment*, 209(2-3), pp.133-142.

Sharma, P. and Dubey, R. (2005). Lead poisonosity in plants. *Brazilian Journal of Plant Physiology*, 17(1), pp.35-52.

Sheilsflynn (2011). *Milton Creek - Parklands Project Monitoring and evaluation of environmental and socio-economic criteria*. sheilsflynn.

Sheoran, V., Sheoran, A. and Poonia, P. (2016). Factors Affecting Phytoextraction: A Review. *Pedosphere*, 26(2), pp.148-166.

Sipahi, T., Karakurt, C., Bakirtas, A. and Tavail, B. (2002). Acute iron ingestion. *The Indian Journal of Pediatrics*, 69(11), pp.947-949.

Sousa, A., Caçador, I., Lillebø, A. and Pardal, M. (2008). Heavy metal accumulation in *Halimione portulacoides*: Intra- and extra-cellular metal binding sites. *Chemosphere*, 70(5), pp.850-857.

Stern, B. (2010). Essentiality and Poisonosity in Copper Health Risk Assessment: Overview, Update and Regulatory Considerations. *Journal of Poisonousology and Environmental Health, Part A*, 73(2-3), pp.114-127.

Štrba, T., Turisová, I. and Aschenbrenner, Š. (2014). Flora and Vegetation of a Copper Mine Heap in Richtárová (The Starohorské Vrchy Mts., Slovakia). *Annales UMCS, Biologia*, 69(1).

Sukreeyapongse, O., Holm, P., Strobel, B., Panichsakpatana, S., Magid, J. and Hansen, H. (2002). pH-Dependent Release of Cadmium, Copper, and Lead from Natural and Sludge-Amended Soils. *Journal of Environment Quality*, 31(6), p.1901.

Svoboda, L., Zimmermannová, K. and Kalač, P. (2000). Concentrations of mercury, cadmium, lead and copper in fruiting bodies of edible mushrooms in an emission area

of a copper smelter and a mercury smelter. *Science of The Total Environment*, 246(1), pp.61-67.

Tangahu, B., Sheikh Abdullah, S., Basri, H., Idris, M., Anuar, N. and Mukhlisin, M. (2011). A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation. *International Journal of Chemical Engineering*, 2011, pp.1-31.

Tariq, S. and Ashraf, A. (2016). Comparative evaluation of phytoremediation of metal contaminated soil of firing range by four different plant species. *Arabian Journal of Chemistry*, 9(6), pp.806-814.

Temminghoff, E., Van der Zee, S. and de Haan, F. (1997). Copper Mobility in a Copper-Contaminated Sandy Soil as Affected by pH and Solid and Dissolved Organic Matter. *Environmental Science & Technology*, 31(4), pp.1109-1115.

The Soil Thematic Strategy (2006). The EU Commission

Tobler, W. (1970). A Computer Movie Simulating Urban Growth in the Detroit Region. *Economic Geography*, 46, p.234.

Tóth, G., Hermann, T., Da Silva, M. and Montanarella, L. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. *Environment International*, 88, pp.299-309.

Tremlová, J., Vašíčková, I., Száková, J., Goessler, W., Steiner, O., Najmanová, J., Horáková, T. and Tlustoš, P. (2016). Arsenic compounds occurring in ruderal plant communities growing in arsenic contaminated soils. *Environmental and Experimental Botany*, 123, pp.108-115.

Tye, A., Young, S., Crout, N., Zhang, H., Preston, S., Zhao, F. and McGrath, S. (2004). Speciation and solubility of Cu, Ni and Pb in contaminated soils. *European Journal of Soil Science*, 55(3), pp.579-590.

Valko, M., Morris, H. and Cronin, M. (2005). Metals, Poisonousness and Oxidative Stress. *Current Medicinal Chemistry*, 12(10), pp.1161-1208.

- Vara Prasad, M. and De Oliveira Freitas, H. (1999). Feasible biotechnological and bioremediation strategies for serpentine soils and mine spoils. *Electronic Journal of Biotechnology*, 2(1).
- Verbruggen, N., Hermans, C. and Schat, H. (2009). Mechanisms to cope with arsenic or cadmium excess in plants. *Current Opinion in Plant Biology*, 12(3), pp.364-372.
- Vuori, K. (1995). Direct and Indirect effects of iron on river eco systems. *Annal Zoo Fennici*, 32, pp.317-328.
- Wallace, A., Romney, E., Cha, J., Soufi, S. and Chaudhry, F. (1977). Nickel phytotoxicity in relationship to soil pH manipulation and chelating agents. *Communications in Soil Science and Plant Analysis*, 8(9), pp.757-764.
- Wang, A., Angle, J., Chaney, R., Delorme, T. and Reeves, R. (2006). Soil pH Effects on Uptake of Cd and Zn by *Thlaspi caerulescens*. *Plant and Soil*, 281(1-2), pp.325-337.
- Wang, J., ZHANG, D., STABNIKOVA, O. and TAY, J. (2005). Evaluation of electrokinetic removal of heavy metals from sewage sludge. *Journal of Hazardous Materials*, 124(1-3), pp.139-146.
- Wang, Q., Dong, Y., Cui, Y. and Liu, X. (2001). Instances of Soil and Crop Heavy Metal Contamination in China. *Soil and Sediment Contamination*, 10(5), pp.497-510.
- Wang, Q., Liu, X., Cui, Y., Dong, Y. and Christie, P. (2002). Responses of legume and non-legume crop species to heavy metals in soils with multiple metal contamination. *Journal of Environmental Science and Health, Part A*, 37(4), pp.611-621.
- Wang, X. and QIN, Y. (2005). Correlation between magnetic susceptibility and heavy metals in urban topsoil: a case study from the city of Xuzhou, China. *Environmental Geology*, 49(1), pp.10-18.
- WebMD. (2017). *Iron Poisoning Basics*. [online] Available at: <http://www.webmd.com/a-to-z-guides/iron-poisoning#1> [Accessed 21 Aug. 2017].
- Welch, R. (1995). Micronutrient Nutrition of Plants. *Critical Reviews in Plant Sciences*, 14(1), pp.49-49.

- Wenzel, W., Bunkowski, M., Puschenreiter, M. and Horak, O. (2003). Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. *Environmental Pollution*, 123(1), pp.131-138.
- Wu, L., Bradshaw, A. and Thurman, D. (1975). The potential for evolution of heavy metal tolerance in plants III. *Heredity*, 34(2), pp.165-187.
- Xiaoli, C., Shimaoka, T., Xianyan, C., Qiang, G. and Youcai, Z. (2007). Characteristics and mobility of heavy metals in an MSW landfill: Implications in risk assessment and reclamation. *Journal of Hazardous Materials*, 144(1-2), pp.485-491.
- Yang, X., Baligar, V., Martens, D. and Clark, R. (1996). Cadmium effects on influx and transport of mineral nutrients in plant species. *Journal of Plant Nutrition*, 19(3-4), pp.643-656.
- Yang, X., Feng, Y., He, Z. and Stoffella, P. (2005). Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. *Journal of Trace Elements in Medicine and Biology*, 18(4), pp.339-353.
- Yoon, J., Cao, X., Zhou, Q. and Ma, L. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of The Total Environment*, 368(2-3), pp.456-464.
- Yruela, I. (2009). Copper in plants: acquisition, transport and interactions. *Functional Plant Biology*, 36(5), p.409.
- Zayed, A. and Terry, N. (2003). Chromium in the environment: factors affecting biological remediation. *Plant and Soil*, 249(1), pp.139–156.
- Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F. and Zhang, G. (2011). The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environmental Pollution*, 159(1), pp.84-91.
- Zhang, Y. (2012). Effect of Cadmium on Element in the Root of Mung Bean Seedlings. *Advanced Materials Research*, 550-553, pp.1200-1203.

Zhou, Z., Niu, Y., Zhang, S., Huang, D. and Yuan, X. (2016). Applied Study on the Spatial Interpolation Method for the Evaluation of Soils with Heavy Metals in Mountainous Tea Planting Regions. *Journal of Residuals Science & Technology*, 13(7).