EFFECTS OF LONG-TERM TREATMENT WITH SEWAGE SLUDGE ON CONCENTRATIONS OF HEAVY METALS IN SOIL AND TISSUE OF SELECTED PLANTS, POTENTIAL RISKS AND IMPLICATIONS FOR PHYTO-REMEDIATION

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ABSTRACT

Long term application of sewage has led to accumulation of heavy metals in soils, causing serious environmental problems and posing a threat to plant, human and animal health. The development of remediation strategies is thus of paramount importance in reclamation of heavy metal contaminated soils. A study was conducted to determine the concentration of heavy metals in amaranthus (*Amaranthus dubius*), tomato (*Solanum lycopersicum*), black nightshade (*Solanum nigrum*) and *Rumex pulcher* found voluntarily growing on a dedicated sewage sludge disposal land, and commercially grown turf grass at the site. A pot trial was also conducted to determine biomass yield and heavy metal uptake of Indian mustard (*Brassica juncea*), lucern (*Medicago sativa*), vetch (*Vicia sativa*), rape (*Brassica napus*), ryegrass (*Lolium perenne*), and spinach (*Spinacia oleracea*) grown on soil treated with sewage sludge for over 50 years.An additional pot experiment was conducted to determine effects of adding increasing concentration of EDTA, a chelating agent, (0, 3, 6 and 10 mmol kg^{-1}) on tissue metal composition of Indian mustard. Plants and soils were digested using a microwave in an *aqua regia* mixture. The digest was then analysed for heavy metals using the 720 varian ICP-OES. Of the plants growing on the polluted land, turf grass had the highest concentrations of all the metals under investigation with Zn (419 mg kg^{-1}), Pb (23 mg kg^{-1}),Cu (81 mg kg^{-1}),Ni (223 mg kg^{-1}) and Cr (429 mg kg^{-1}) being far above their toxicity threshold in plants. Rumex had the following metals above the limit; Cd (root- 0.7 and shoot- 1.1), Cr (shoot- 43.9 and root-77.7), Ni (shoot-96.4 and root-94.1), Pb (shoot-2.2 and root- 5.1) and Zn (shoot- 79.7 and root- 84.3). Amaranthus had the following heavy metals above the limit; Cd (shoot-0.8 and root-0.9 mg kg^{-1}), Cr (shoot-48.8 and root- 105.1 mg kg^{-1}), Ni (shoot-100.6 and root- 119.4 mg kg^{-1}), Pb (shoot- 2.9 and root-4.4 mg kg^{-1}) and Zn (shoot- 94.8 and root- 106 mg kg^{-1}).Tomato had the following heavy metals that exceeded the limit; As (shoot-0.6 and root-0.6mg kg^{-1}), Cd (shoot-1.2 and root- 1.3 mg kg^{-1}), Cr (shoot-27.4 and root-28.6 mg kg^{-1}), Ni (shoot-98.8 and root-102.2 mg kg^{-1}), Pb (shoot-0.8 and root- 2.5 mg kg^{-1}) and Zn (shoot-64 and root -83.7 mg kg^{-1}).Black nightshade had the following heavy metals above the limit; As in the roots (0.3 mg kg^{-1}), Cd (shoot-1.4 and root-1.0 mg kg^{-1}), Cr (shoot-31.4 and root- 81.6 mg kg^{-1}), Ni (shoot-85.5 and root-109.9 mg kg^{-1}), Pb (shoot-3.2 and root-8.2 mg kg^{-1}) and Zn in the roots (116.2 mg kg^{-1}).In the pot trial, mustard and rape had the highest shoot dry matter yield than other plants with 16.98 and 15.46 g pot^{-1}, respectively, on polluted soil and 11.44 and 9.71 g pot^{-1}, respectively, on the control soil. The plants grown on polluted soil accumulated higher concentrations of the heavy metals compared to the control soil. None of the plants were able to accumulate arsenic above its toxicity threshold limit of 20 mg kg^{-1}. Vetch had the highest concentration of Zn, Cu, Ni and Pb with 439, 119, 80 and 238 mg kg^{-1} which were about 3, 8, 4 and 7 times above their toxicity thresholds. Mustard had the highest uptake of Cd, Cr, Cu and Zn. The dry matter yield of mustard grown in the EDTA applied soils decreased with increase in EDTA application rate and ranged from 16.98 g pot^{-1} for the0 mmolkg^{-1} to10.55 g pot^{-1} for the 6 mmol kg^{-1}. Increasing concentration of EDTA significantly increased tissue Cd, Pb and Zn with no significant effects on As, Cr, Cu and Ni. Indian mustard in the shoots had the highest uptake level of Cd, Cr, Cu and Zn. In the roots ryegrass had the highest uptake level taking up more Cd, Cr, Cu, Ni, Zn and Pb compared to the other plants. The plants growing at the polluted site pose a health risk to humans who consume them. There is need for better controls to access to the area and to educate the local people on the risks associated with such consumption. On field phytoremediation trials of the plants that showed the most promise need to be done to evaluate the plants effectiveness.

DECLARATION

I, Awonke Mbangi declare that:

1. the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;
2. this dissertation has not been submitted in full or in part for any degree or examination to any other university;
3. this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;
4. this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. their words have been re-written but the general information attributed to them has been referenced;
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5. where I have used material for which publications followed, I have indicated in detail my role in the work;
6. this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;
7. this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed:…………………………............ Date:………………………………….

Awonke Mbangi (Candidate)

I as the candidate's supervisor have/have not approved this dissertation for submission

Signed:………………………………….. Date:…………………………………..

Professor P. Muchaonyerwa (Supervisor)

Signed:………………………………….. Date:…………………………………..

Dr. R. Zengeni (Co-supervisor)

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DEDICATION

I dedicate this work to my family,

Rhodesia, Phumla, Unathi and Esethu Mbangi

This is but a small gesture to express how much you mean to me but words and actions can never be enough compared to what you have been in my life.

TABLE OF CONTENTS

ABBREVIATIONS

DWWW: DarvillWaste Water Works

PMB: Pietermaritzburg

MPL: Maximum permissible limits

TTV: Total trigger value

DTPA: Diethylenetriaminepenta acetic acid

EDTA: Ethylenediaminetetra acetic acid

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1.1 Introduction

Sewage treatment plants across the world generate large amounts of effluents and sludge, which must be disposed of in a secure and cost effective way (Luczkiewicz, 2006). Sewage effluent can either be directed into water courses or used for irrigation and other practices. Incineration and landfills have been used for disposal of sewage sludge over the years (Murakami *et al*., 2009; Harrison *et al*., 1999). Another commonly used disposal method has been to use the waste as fertilizer (EPA, 1994). In South Africa sludge disposal practices include stockpiling of dried sludge (40%), marine disposal (2%), sludge lagoons (16%), composting (10%),instant lawn cultivation (3%),farming activities (7%) and sacrificial or dedicated land disposal (21%) (Herselman, 2001, as referenced by Marx *et al.,* 2004).

Sewage sludge is a source of valuable nutrients needed for the growth of plants and contains high amounts of organic matter (Nyamangara and Mzezewa, 1999). The organic matter increases the water holding capacity of soils, controls erosion, among other soil properties (Harrison *et al*., 2006). The challenges of using sewage sludge as a fertiliser material lies in its composition of pathogenic organisms (e.g. *Escherichia coli*) and most importantly the presence of heavy metals like Cd, Pb, As, Cu, Cr, Ni (Korboulewsky *et al*., 2002). Some of the heavy metals, a group of metals with a density greater than 6 g cm^3, are not known to have any biological function in most organisms (John *et al*., 2009) but have adverse effects on the growth of plants, animals and humans, as they affect the normal functioning of enzymes (Oancea *et al*., 2005; Chaves *et al*., 2011).The heavy metals pose the greatest challenge because they do not undergo microbial or chemical degradation but accumulate in the soil environment until they reach hazardous levels and at times leach down the soil profile to contaminate ground water (Wuana and Okieimen, 2011). However, they can change their chemical form and bioavailability depending on the environmental conditions, including pH, redox potential and soil organic matter content (Subhashini and Swamy, 2013; Wuana and Okieiman, 2012; Barazani *et al*., 2004).

Changes in the soil pH will increase or decrease the bioavailability of heavy metals. For example, at low pH(< 6.5) cationic metals like Cd^{2+} become more soluble making them more available to plants and therefore more likely to be incorporated into their tissues and ingested by humans (if consumed). With increasing acidity, the increased activity of cations is also partly due to the dissolution of hydrous oxides and their co-precipitated metals (Selim and Sparks, 2001). At high pH (>7.5) these metals are less soluble as they precipitate out of solution as oxides of iron and aluminium. Anionic species of metals like As exists as oxoanions. Where at low pH (2-6) arsenate ($H_2AsO_4^-$) is the predominant species and as the pH rises arsenite ($H_2AsO_3^-$) becomes the predominant species (Ghimire *et al*., 2003). This equilibrium exists with Cr anions as well, where in alkaline pH chromate (CrO_4^{2-}) is the prevalent form and at acidic pH, dichromate ($Cr_2O_7^{2-}$) becomes predominant. The application of sewage sludge to soil has an alkalising effect on the pH. This is because during treatment the pH of the sludge is raised to around pH8in order that metals will precipitate out of solution and are not readily available, which in turn raises the pH of acidic soils (Madyiwa *et al.,* 2004).

Other parameters like redox reactions in the soil environment also affect the chemical forms of metals. Arsenic for example generally occurs in soils in two oxidation states namely As^{+3} (arsenite) and As^{+5} (arsenate). The predominant form of As in soils is a result of the redox conditions of the soil. Under aerobic or oxic soils As^{+5} compounds are generally more predominant, and when the soil is in a reducing state As^{+3} compounds are more predominant. The As^{+3} forms are significantly more mobile and toxic in the environment than the As^{+5}

species (Sadiq *et al.,* 1995). Another soil condition that is important in the bioavailability of heavy metals is organic matter. Long term application of sewage sludge to soils has been demonstrated by a number of researchers to increase the soil organic matter by several times, of the initial percentage, depending on the duration of application (Triphathi and Misra, 2012; Katanda *et al.,* 2007; Mapanda *et al.*, 2005;Bergkvist*et al.*, 2003). The organic material in sewage sludge originates from waste water coming from urban areas, industries and agricultural waste/by products. Organic matter affects the availability, retention and mobility of metals in the soil through the formation of chelates that render the metal insoluble and unavailable for plant uptake (Tan, 2010; Daintith, 2008). The large numbers of functional groups of humic acids play a very important role in this process. (Hooda *et al*., 2000; Ayyasamy *et al.*, 2009). The higher the organic matter (>3%), the higher the chances that more metals will form chelates with humic acids that render them insoluble. The threat that heavy metals cause to plants, animals and humans determine the need to understand metal concentrations in plants growing on the polluted site and to explore approaches that can be used to remediate such soils (Williams and Brown, 2011; Lone *et al*., 2008).

Conventional methods of remediation such as soil washing, excavation and reburial are costly and impractical over large scales. They further damage the environment in that the land becomes inadequate for the growth of plants, through removal of biological activities, including useful microbes like nitrogen fixing bacteria, mycorrhiza fungi, as well as fauna (Liao and Chang, 2004; Marques *et al*., 2009; Vamerali *et al*., 2010). Phytoremediation, the use of plants to degrade, extract, contain, or immobilize contaminants from soil and water, has over the years received much attention as a cheap alternative in the fight to reclaim heavy metal polluted soils (Ghosh and Singh, 2005).The technology is site specific, depending on parameters like extend of contamination, soil type, climate and vegetation (Pulford and Watson, 2003; Lone *et al.,* 2008).Plants that have been previously used to remediate heavy metals include those in the *Brassica* family like Indian mustard (*Brassica juncea*), for nickel, cadmium, lead and zinc (Chaney *et al.,* 2007), willow trees (*Salix*) for copper and chromium (Kuzovkina *et al.,* 2004) and *Pteris vittata* for arsenic (Tu *et al.,* 2004; Gonzaga *et al.,* 2006; Salido *et al.,* 2003). A number of plants that are adapted to grow in local environments of South Africa have been shown to have potential in phytoremediation.

Ryegrass (*Lolium perenne*) has been shown to accumulate 99-500 mg Zn kg^{-1}, 2450 mg Cr kg^{-1}, 318 mg Pb kg^{-1} and 16.0 mg Cd kg^{-1} (Arienzo *et al.,* 2004; Vernay *et al.,* 2007 and Bidar *et al.,* 2009). Zaier *et al.* (2010), Marchiol *et al.* (2004) and Turan and Esringu (2007) have demonstrated that Rape (*Brassica napus*) accumulated 117-472 mg Pb kg^{-1}, 142-5983 mg Zn kg^{-1} and 309 mg Cu kg^{-1}. Grazing vetch (*Vicia sativa*) has been shown to accumulate 365 mg Zn kg^{-1}, 327 mg Cu kg^{-1} and 200-260 mg Pb kg^{-1} (Peciulyte *et al.,* 2006; Wang *et al.,* 2002). Other plants that have been shown to have phytoremediation potential for soils contaminated with heavy metals are Oriental mustard (*Brassica juncea*), Black mustard (*Brassica nigra*) (Salido *et al.,* 2003), Lucerne (*Medicago sativa*) (Gardea-Terresdeg *et al.,* 1998), spinach (*Spinacia oleracea*) (Salaska *et al.,* 2011), and Amaranthus (*Amaranthus dubius*) (Chinmayee *et al.,* 2012). Most of the plants have been tested with a small number of metals, with most of the experiments being done under hydroponic environments or where the contaminations are artificially simulated.The plants that have been used in phytoremediation are specific to a single metal or two, and no plant has been found to accumulate significant amounts of multiple metals at the same time (Pulford and Watson, 2003; Ghosh and Singh, 2005). It is essential to understand the uptake of multiple heavy metals in plants from polluted sites. Identifying plants species that can take multiple heavy metals can be helpful in remediating contaminated sites. While most plants would not be able to grow at high metal concentrations (Vamerali *et al.,* 2010) those that grow could take up high amounts of the metals. Modifying the soil environment could improve the effectiveness of these plants to accumulate metals by attempting to increase the bioavailability of metals in the soil (Alkorta *et al.,* 2004). Application of EDTA (Ethylenediaminetetra acetic acid) to polluted soil has been shown to increase bioavailability of some metals by a number of researchers (Farid *et al.,* 2013; Dipu *et al.,* 2012; Turgut *et al.,* 2004; Thayalakumaran *et al.,* 2003; Liphadzi *et al.,* 2003). According to Wu *et al*. (2004), EDTA is the most popular and effective chelating agent for reasons that it is strong, recoverable and is considered to be relatively biostable.

1.2 Background and justification

Darvill Waste Water Works (DWWW), situated east of Pietermaritzburg, receives both domestic sludge from surrounding residences and industrial waste. Over 50 years of sludge application on about 57 ha of land by the Darvill Waste Water Treatment Works has resulted in soil pollution by a myriad of heavy metals, including arsenic, cadmium, chromium, copper, nickel, lead and zinc (Mdlambuzi, 2014). The land is surrounded by residential areas like Sobantu, Hollingwood and Lincon Meade. These polluted soils pose a risk to surface and groundwater and to the surrounding community, who feed on the indigenous vegetables that grow on the land. There is need to understand the level of risks the communities are exposed to through the consumption of vegetables (both indigenous and exotic) that voluntarily grow on the site. A private company, Duzi Turf, is growing and selling turf-grass on the polluted soil, for instant lawn. It is essential to understand the levels of metals that are exported to consumers' yards, in the tissue of the turf-grass and the soil associated with the root system, when the turf grass is sold.

Phytoremediation could offer a cost effective alternative compared to the aforementioned expensive and impractical conventional methods. However most phytoremediation work has focussed on one or two heavy metals at a time, yet the area at DWWW would require an approach that addresses multiple heavy metal uptake. There is need to test a number of plant species for their ability to accumulate heavy metals from a soil that is polluted with multiple metals.

1.3 Objectives

The aim of this study was to determine concentrations of multiple heavy metals in selected plant species after long term application of sewage sludge in Darvill Waste Water Works.

The specific objectives were to determine the:

i. Concentration of heavy metals in turf grass with associated soil after long term application of sewage sludge.
ii. Concentration of heavy metals in tissue of selected vegetables (amaranthus, rumex, black nightshade and tomato) growing on polluted site.
iii. Distribution of heavy metals between the root and shoot tissues in selected plant species (mustard, lucerne, vetch, rape, ryegrass and spinach) grown on contaminated soils.
iv. Effects of increasing EDTA concentration in soil on tissue metal composition of Indian mustard grown on polluted soil.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The water supply of a community after being polluted by a variety of uses constitutes wastewater. It is a combination of water-carried wastes from industry, residences, institutions and commercial establishments along with storm water (Nebel and Wright, 1996). This waste water contains sewage that carries pathogenic organisms able to transmit diseases to animals and humans. The bacterium present in wastewater has the ability to reduce the oxygen content of water and can therefore be harmful if applied to fresh water as it would render it anaerobic (Garcia-Delgado *et al.,* 2007). The presence of organic material producing unpleasant odour is also a problem associated with wastewater. It also holds nutrients that can cause toxicity (at certain levels of accumulation) in ecosystems through processes like eutrophication and most importantly accumulation of toxic heavy metals like cadmium, lead and mercury among others (Fernandez *et al*., 2009; Chen *et al.,* 2004;). The immediate collection, removal and treatment before disposal is therefore imperative to the sanitation and health of society. Sewage sludge is the by product that is produced in waste water treatment plants after the different process of treatment have taken place (Fernandez *et al*., 2009; Wang, 1997).

A number of methods are used to dispose sewage sludge these include application to forestry and farm land, landfilling, incineration, ocean dumping and lagooning. In South Africa on-site disposal methods still lead as a remedy employed by wastewater treatment plants (Snyman, 2011). These include direct land application and pilling of sludge in stocks on site. The disposal of sewage sludge is a key environmental problem in countries all around the world. It is however more of a concern in developing countries where municipalities often operating under limited resources, are expected to handle large quantities of sewage sludge. This is made worse by the exponential increase in population, urbanization and industries

(Nyamangara and Mzezewa, 1999). This results in the disposal of poorly treated or untreated sewage sludge to the environment. The consequence of this is the accumulation of contaminants that are found in sewage sludge. These include amongst others heavy metals. The long term application of sewage sludge on land has been found by a number of researchers to result in accumulation of heavy metals (Bhattacharyya *et al.,* 2008; Walter and Cuevas, 1999; Nyamangara and Mzezewa, 1999; Obasi *et al*., 2013). All metals at certain levels of concentration are toxic to living organisms. They can affect biodiversity of plant species and cause chronic illness to animals and humans (Ukpong *et al.*, 2013). Unlike organic contaminants heavy metals are non-biodegradable, as they do not undergo microbial or chemical degradation. They persist in the soil environment for a long time after their addition. They can however change in their chemical form and also bioavailability depending on the environmental conditions (Subhashini and Swamy, 2013; Wuana and Okieiman, 2011; Barazani *et al*., 2003).

The imminent danger caused by the contamination of soils by heavy metals necessitates remediation, to prevent them from entering the food chain. Phytoremediation is a technology that has been found to be cheap, environmentally friendly and feasible compared to the conventional methods of remediation. It involves the use of higher plants in the uptake of the metals from the soil system (Memon and Schroder, 2009; Gonzalez and Gonzalez-Chavez, 2006; Yoon *et al.,* 2006).

2.2 Sewage sludge production and management in South Africa.

Large volumes of waste water are received by treatment plants all around South Africa. The resultant sewage sludge after the treatment process largely depends on the characteristics of the waste water received together with the efficiency of the treatment process (Linder and Lundehn, 2002).

The current treatment and management of sewage sludge generated in South Africa is poor and as such poses a threat to the environment and subsequently human health (Keirungi, 2006; Umgeni water, 2014). According to Marx *et al*., (2004) the quantity of untreated sewage sludge to be disposed of daily from the 900 registered sewage treatment plants in South Africa is estimated at 1750 t DS/d for undigested sludge and 1 375 TDS/D for digested sludge. This places tremendous pressure on the environment receiving these amounts.

This problem is not unique to South Africa but is a trend in all developing countries (Zavodska, 2009). The root causes of this are the high rate of industrialization and exponential increase in population without the subsequent upgrade of these facilities, resulting in treatment plants operating well over capacity. The Darvill Waste Water treatment plant for example has a current biological treatment capacity of 65 Mℓ/day but the average daily inflow from November 2007 to November 2013 was approximately 81 Mℓ/day which is more than the plant's capacity (Umngeni water, 2014). This is the trend across South Africa, according to Linder and Lundehn (2002), Shornvile sewage treatment works situated in the Eastern Cape (King Williams Town) is at least three years beyond its designated life due to the accelerated growth of the town. This is made worse by the fact that the plant was originally built to cater for domestic sewage, but handles about 550 m^3 /day of industrial effluent. The major constraint to addressing waste management in developing countries like South Africa is simply the cost involved in the process (Keirungi, 2006). This result in the use of low cost technology that does not effectively manage the waste received. Other contributing factors to this problem are the lack of skilled human resources with the technical expertise necessary for waste management planning and operation (Zavodska, 2009). In addition, the low priority attached to the waste management sector in developing countries means that the levels of services required for protection of public health and the environment are often not attained (Keirungi, 2006).

2.2.1 Production of sewage sludge

Sewage sludge flowing into wastewater treatment plants contains substantial amounts of materials like paper, wood, faeces and heavier solid objects that could possibly cause blockage of pipes or even damage to machinery. These have to be removed before the actual treatment occurs (Smith, 2009). This is done by passing the sludge through a series of strainers and small quantities of residuals are generated. This pre-treatment also produces settleable solids and these are removed by primary sedimentary tanks (Bergheim *et al.*, 1998). Untreated sewage sludge has high levels of pathogens that can cause diseases and is very unstable (decomposable). This results in the generation of odors that make it attractive to disease-carrying vectors such as insects, rodents and birds. Further treatment of sludge is therefore important to reduce the pathogens. This further treatment is typically based on application of high temperature, chemicals, reduction of volatile organic content or removal of moisture from the sludge (Linder and Lundehn, 2002). These sewage sludge treatment processes are discussed below. These include thickening, stabilizing, dewatering and conditioning.

2.2.2 Thickening

Thickening is the process of increasing the solid content of the sludge. This is done by removing a portion of the liquid content. Gravity thickening is the most used internationally, as it is regarded to be simple and least expensive in consolidating waste sludge (Carrondo *et al.*, 1978). This process results in significant reduction in volume and this subsequently decreases the cost of operations. Thickening can also be accomplished by gravity floatation and centrifugation methods (Hong *et al.,* 2009).

2.2.3 Stabilisation

The stabilisation process involves the destruction of volatile organic material. This is done to minimise unpleasant odours and to reduce the number of pathogens like Helminth eggs. Chemicals are added to achieve this process, resulting in unfavourable conditions for the survival of the unwanted organisms. Heat is also used in killing of pathogens, though it is unpopular in South Africa and other developing countries because of high operation and maintenance costs (Kouloumbus *et al.,* 2008; Fytili and Zaboniotou, 2008).The destruction of some organic solids in this process causes a reduction in volume. This process improves sludge dewatering characteristics (Bergheim *et al*., 1998).

2.2.4 Dewatering

The process of dewatering is a physical operation that seeks to reduce the water content of the dilute sludge. This is achieved by separating the liquid content from the solid content of the sludge. This can be done naturally or mechanically. Natural techniques of dewatering include the drying beds or drying lagoons. Mechanical techniques include centrifuging, vacuum filter and filter press. The choice of technique to be employed is informed by a number of factors. These include the type of sludge to be dewatered, moisture content required for disposal and also the space available (Spinosa *et al., 2011*).

2.2.5 Conditioning

Conditioning is the improvement of the dewatering characteristics of the sludge. This involves chemical or physical treatment of the sludge (Tao *et al.,* 2006). Chemical conditioning results in the formation of semisolids with the liquid medium through the process of coagulation. Solids are coagulated and the absorbed water is released (Kouloumbus *et al.,* 2008). Different chemicals are used in this process e.g. sulphuric acid, calcium oxide, ferrous chloride, etc. The chemical addition results in changes in pH, which can be an increase or decrease depending on the chemical added. This causes small particles to coagulate into larger particles and water in the sludge solids is given up readily. The pH of sludge depends on its chemical composition (Bishop, 1995).

Physical conditioning of sludge involves heat treatment and freezing and thawing. When sludge is heated, the temperature causes water to escape from the sludge. Heat treatment releases water within the cell structure of the sludge, this then result in the improvement of the dewatering characteristics of the sludge (Parker *et al.,* 1971). The freeze-thaw method rests on the fact that during the process of freezing water, solid particles are excluded ahead of the ice that is formed (Kouloumbus *et al.,* 2008). The alternate freeze and thaw is able to convert the consistency of the residuals to a granular-type that is easy to drain than before. The time of freezing and the temperature at which the sludge is frozen are regarded as important parameters that are to be considered in order to optimize the process (Lue-Hing *et al.*, 1998).

From the sludge generated by wastewater treatment process it can be observed that there is no reduction in the concentration of contaminants like heavy metals. Heavy metals that are present in the sewage sludge flowing into the wastewater treatment plants tend to accumulate in the generated sludge (Stylianou *et. al.*, 2007). Heavy metal levels have been found to be mostly concentrated in the sludge compared to the soil environment. The long term application of sludge containing elevated concentrations then results in the accumulation of these heavy metals in the soil environment over time. They can also result in the contamination of water bodies if the effluent is discharged in waterways (Karvelas *et. al.,* 2003).The reduction or removal of heavy metal from sludge is done in certain cases where the sludge is to be sold for agricultural purposes. This is enforced by guidelines and laws that are put in place for protection of consumers and the imminent threat of these metals entering the food chain. These include the Permissible Utilisation and Disposal of Sewage Sludge (PUDSS) document (WRC, 1997). This reduction can be achieved either by source control of industrial and domestic discharges to sewage plants or by removing metals from sludge. Source control has been a very difficult process as identification is usually a great challenge (Qi-teng *et al.,* 1998).

After sludge has been generated through the treatment process it is then disposed. The different methods employed in disposal are discussed below. According to Herselman *et al*., (2005) the existing methods of disposal are becoming increasing unacceptable in relation to the health of the environment. These are affected by a number of factors and they will be discussed in greater detail in the sections to follow.

2.3 Sewage sludge disposal methods

Increasing volumes of sludge generated by sewage treatment plants in South Africa puts tremendous pressure on municipalities to use environmentally sound disposal practices. Safe disposal of sewage sludge must be:

- Environmentally acceptable;
- Cost effective;
- Minimise the environmental pollution;
- Politically/socially acceptable, and;
- Operationally feasible (Van Niekerk, 2004).

The conventional methods used in sewage sludge disposal are 1) dumping in water bodies, 2) composting, 3) incineration and 4) land disposal (Linder and Lundehn, 2002; Van Niekerk, 2004). These methods are discussed in greater detail below.

2.3.1 Disposal in water systems

The disposal of waste both treated and untreated to rivers, oceans and other water bodies has been a practice that has been done for a long time in countries all around the world (Bishop, 1995). Discharge of partly treated or untreated sludge is as a result of different factors. These include decayed infrastructure, malfunctioning of facilities, heavy rainfall events which overwhelm systems that operate on combined sewer and storm water drains (DEM, 2011). This can also be caused by exponential growth in population and expansion of industries without the necessary upgrading of the treatment plants that are meant to receive the waste (Kosobucki *et al.,* 2000). This causes limitations in resources and affects the operation process (DEFRA, 2012).

Ocean dumping has been prohibited by many first world countries since its banning by the United States according to the 'Ocean dumping ban act of 1988' (Copeland, 1999). In South Africa disposal of waste to the ocean is still permissible (MARX *et al.,* 2004; WRC, 1997). Waste material dumped in water systems can contain different levels of contaminations. These include heavy metals, organo-halogen compounds, oil products, acids, bases pesticides, etc. The nature of these materials has great potential to cause harm to these aquatic ecosystems and they pose a threat to the general public (Bishop, 1995; Ternes, 1998). Nutrient releases from organic material entering these water systems have the potential to stimulate a process called eutrophication. This occurs as a result of excess nutrient level which causes the growth of algae and phytoplankton. When these die they sink to the bottom where they are decomposed and the nutrients contained in organic matter are converted into inorganic forms by bacteria. The process of decomposition consumes oxygen, thereby depriving fish and other organisms (Baronti *et al.*, 2000).

The presence of heavy metals also results in contamination of water in rivers and other water bodies where disposal occurs. Heavy metals poise a greater threat than organic contaminants as they cannot be biologically degraded and will accumulate in the sediments of rivers and streams, until they are taken up by aquatic plants and ingested by fish (Ayas et *al.,* 2007). A study was done by Ayas *et al.* (2007) to determine the extent of heavy metal contamination of an aquatic ecosystem covering a waste reservoir and an internationally recognized bird area known as Nallihan Bird Paradise (NBP). The results showed that these metals were found widespread throughout the study area, but metal concentrations in the water samples were below detection limits. Lead, cadmium, copper and nickel contamination were found to have accumulated in the sediments and fish tissue. The low heavy metal concentration in water was as a result of alkaline pH that was tested which affected solubility and also the adsorption and accumulation of metals by suspended solids. Fishery in the reservoir has a commercial importance and locals consume considerable amount of fish caught in the reservoir, this puts them at risk of ingesting the metals.

The discharge of waste water to streams and rivers is a practice that is of beneficial effect to the recycling of water provided proper treatment has been done to ensure insignificant levels of heavy metals, pathogens and other contaminants. This practice is most critical in areas of arid to semi-arid climatic conditions, like South Africa (WRC, 1997; Herselman *et al*., 2005).

2.3.2 Composting

Composting is a method that entails the biological decomposition of the organic constituents of the sewage sludge under a regulated environment (Amir *et al.,* 2005). The end product being a humus like product with undetectable levels of pathogens that is easy to handle, store or use. Depending on the heavy metal concentration this can be sold as an organic fertilizer to home owners, landscapers and farmers. The composting system makes use of oxygen, temperature and technological approaches. Three different types of composting methods are commonly used namely aeratal static pile, windrow and in vessel. A detailed description of these methods can be found in the following reference (Kosobucki *et al.,* 2000).

2.3.3 Incineration

Incineration is a sludge disposal method that involves heating of sewage sludge at high temperatures in a concealed structure. This technology has high cost implications and is currently used in first world countries (Murakami *et al.,* 2009). The system reduces the sludge to ash that is considerably less in volume than the original. This disposal method is favoured in treatment plants of large cities where there is a high rate of sludge generation and limited space for disposal (Werther and Ogada, 1999). Incineration removes pathogens and toxic organic constituents, but the metals remain in the ash and will require disposal. Incineration therefore is a means of reduction in volume and does not fully answer the questions of final disposal (Otero *et al.,* 2002).

2.3.4 Land disposal

In South Africa this is the most widespread method of disposal used by waste water treatment plants (WRC, 1997). A survey was done on 40 waste water treatment plants in South Africa, to determine the extent of which land dedicated for disposal was being used. The findings showed that stockpiling was the most used disposal method either alone or until it is utilized by farmers or municipalities on recreational grounds and landfills. It accounted for 40 percent of the disposal method. Liquid sludge application also accounted for 40 percent, these included practices like irrigation, flooding, sludge ponds and instant lawn irrigation (Herselman *et al.*, 2005).

According to Herselman *et al.*. (2005) these land disposal sites can be distinctly classified into two, namely those of beneficial use and those of non-beneficial use. Dedicated land disposal sites of non- beneficial use (sacrificial land) are pieces of land often on the outskirts of cities and towns, specifically set aside for the disposal of sewage sludge. These lands receive sludge at different application rates dependent on the rate of generation by specific treatment plant. The quality of the sludge applied ranges from type A-B sludge given in Table 2.1 . This type of sludge is of the lowest quality and is characterised by odour nuisances, fly-breeding, pathogenic organisms and variable amounts of inorganic/ organic constituents (WRC, 1997). The nature of the sludge applied onto these lands opens up great potential for environmental problems. These include leaching of contaminants like heavy metals and nutrients like nitrogen, phosphorous, and calcium in ground water. This becomes more pronounced in cases where there are no restrictions on application rates of sewage sludge. This is most prevalent in soils with a sandy texture having low clay content, where the water holding capacity is low and material move along the profile with ease (Wang, 1997). This material can also be transported by runoff water under high rainfall conditions to nearby water bodies. Depending also on the slope of the dedicated land site, erosion can also contribute to the transportation of the applied sludge (WRC, 2002). Indigenous vegetables like amaranthus have been found to spontaneously grow on these lands. These plants take up different levels of heavy metals and if they are to be harvested for consumption they pose a threat to the communities consuming them (Mellem *et al*., 2009; Gonzalez and Gonzalez-Chavez, 2006). Heavy metals are known to be health hazards even at low concentrations when ingested by humans (Ukpong *et al.*, 2013). These disposal sites offer little advantage from an environmental point of view, but offer cheap disposal over large area for waste water treatment plants, and they save money on drying equipment where liquid sludge is applied (Epstein *et al.,* 1999).

Table 2.1: Classification of sewage sludge to be used or disposed of on land.

Type A	Unstable sludge with an unstable odour; it contains a high level of pathogenic organisms. Primary/raw sludge falls into this group.
Type B	Stable sludge with a low and less offensive odour; it contains a reduced level of pathogenic organisms. Humus, waste activated and anaerobically digested sludge's falls into this group.
Type C	Stable sludge with an insignificant odour; it contains an insignificant level of pathogenic organisms. Anaerobically digested sludge when preceded or followed by pasteurization falls into this group.
Type D	Similar to type C; but has a specified maximum concentration of heavy metals and other elements.

(*Source:WRC, 1997)

2.3.5 Land disposal sites of beneficial use

The objective of these disposal techniques is to maximize on the beneficial effect that sewage sludge offers. These include appreciably amounts of organic material, which when applied to soil is able to increase cation exchange capacity, water holding capacity, improve soil structure aggregation and reduce or prevent erosion (Singh and Agrawal, 2008; Herselman *et al.*, 2005;). Sewage sludge also contains nutrients in varying amounts depending on the nature of the sludge, and when applied to soil these are able to support plant growth. Addition of sludge to soils in general brings appreciable gains in chemical and physical properties of the soils environment (Harrison *et al.,* 1999).

Land disposal methods of beneficial use include growing vegetables, tobacco, sugar cane, etc, use in public gardens and beautification, recreational facilities, instant lawn cultivation, crops for grazing and other uses. These beneficial uses are not without limitations as the amount of pathogenic organisms, odour and contaminants like heavy metals are present in different degrees depending on the type of sludge (Wang *et al.,* 2008; Sanchez-Monedero *et al.,* 2004). There is also the reluctance from the public to accept that reuse of sludge can be beneficial. Therefore the use of the methods as means of disposal requires that sludge undergo biological, chemical, heat or other treatment processes that will reduce odour, fly breeding, contaminants and pathogens to insignificant levels (Epstein *et al.,* 1999).

2.4 Heavy metals

In all the challenges facing the disposal of sewage sludge, heavy metal concentration is by far the most significant (Shamuyarira and Gumbo, 2014). This is because heavy metals do not undergo biological degradation and will persist in the soil environment long after their application (Ghosh and Singh, 2005). Therefore continuous application of sludge containing heavy metals to soil will result in an accumulation of the metals, thereby increasing the threat to ground water contamination through processes like leaching. These can also pose a threat to plant species diversity and also the food chain as a whole, because when present in soil they can be taken up by plants, and inevitably by animals and humans if such plants are consumed (Pulford and Watson, 2002). For these reasons guidelines have been developed to assist treatment plants to promote safe handling, disposal and utilization of sewage sludge (Snyman *et al.,* 2000). These guidelines were introduced in South Africa in 1991 and were revised in 1997 with amendments to heavy metal loading and usage restrictions. Table 2.2 shows these guidelines both those of 1991 and 1997. These guidelines represent the metal content that should be contained in sludge aimed for unrestricted use. Heavy metal concentrations higher than those given below should be investigated and monitored (Snyman, and Herselman, 2006).

Table 2.2: Guidelines on element permissible limits on the utilization and disposal of sewage sludge of 1991 and 1997.

Metal	1991 Limit (mg kg^{-1} of dry sludge)	1997 Limit (mg kg^{-1} of dry sludge)
Cd	20	15.7
Co	100	100
Cr	1750	1750
Cu	750	50.5
Hg	10	10
Mo	25	25
Ni	2750	200
Pb	400	50.5
Zn	2750	353.5
As	15	15
Se	15	15
B	80	80
F	400	400

Snyman *et al*., 2000; Herselman and Moodley, 2009.

Monitoring soils receiving sewage sludge with heavy metals is very important because of their effect on the environment and living organisms. Table 2.3 gives the permissible limits in soil extracted with *aqua regia*. These limits have been set to safeguard soil quality from degrading to such a degree that major mediation is required to restore soil functionality. The total trigger value (TTV) is a limit that when exceeded indicates to the producer that the ability of the soil to take sludge at high volumes is approaching its limit and that additional management requirements are to be implemented (Herselman and Moodley, 2009). These include improvement of sludge quality through source control, application of lime to limit mobility of metals in the soil profile. MPL stands for maximum permissible limits in soil. When heavy metals exceed the MPL set by Herselman and Moodley (2009) for aqua regia digestion, then sewage sludge application is not permissible in which case a remediation plan should be implemented.

Table 2.3: Maximum permissible total metal content in soil.

Metal	TTV (mg kg^{-1})	MPL (mg kg^{-1})
Cd	3	5
Cr	350	450
Cu	120	375
Hg	1	9
Ni	150	200
Pb	100	150
Zn	200	700
As	2	20

TTV stands for total trigger value and MPL stands for maximum permissible limit (Herselman and Moodley 2009).

The source of metals in sewage sludge is through the sewage system, as the system receives domestic wastewater, urban runoff and industrial waste. Sewage sludge from industrial discharge contains the most concentration of heavy metals (Linder and Lundehn, 2002), however domestic sludge is also a significant source of certain metals for example copper and zinc. This can be from anything like batteries to the corrosion of drinking water pipes. Other heavy metals like lead originate from urban runoff coming from the exhaust pipes of vehicles (Alloway, 1990), though this should have declined a lot in the last few years with the introduction of unleaded gasoline. Other contributions to lead include pesticides, paint, plastic rain gutters and pollution from mining and smelting.

2.5 Adaptability of plants to heavy metal polluted soil

A number of plants have been documented to grow in heavy metal polluted soils through various anthropogenic sources like mine tailings, dumping, atmospheric deposition leaded gasoline via exhaust fumes and sewage sludge disposal among others (Mehes-Smith *et al*., 2013). These plants vary from wild plants like *Polygonum aviculare* growing around mining waste, able to accumulate Zn up to 9236 $mgkg^{-1}$_as shown by Gonzalez and Gonzalez-Chavez (2006), to vegetables like amaranthus growing in dumpsites (Adewuyi *et al*., 2010). The effect that heavy metals have on plants varies and so too is their response. Some heavy metals play important roles in plants like DNA transcription (Zn), photosynthesis (Mn) and hydrolysis of urea into ammonia and carbon dioxide (Ni) (Vamerali *et al*., 2010). However, at high concentrations all metals become toxic (Kramer, 2005).

Some plants are able to resist or cope with elevated levels of heavy metals. This is done by avoiding the metals from entering their cytoplasm, or by detoxifying metal ions that have crossed their membranes (Mehes-Smith *et al*., 2013). Three categories can be used to classify the strategies employed by plants growing on heavy metal polluted soils; excluders, accumulators/indicators and hyperaccumulators (Mehes-Smith *et al*., 2013; Vamerali *et al*., 2010; Kumer *et al*., 1995). Excluders are those plants that limit the translocation of metals to the above ground parts of the plants regardless of how much metals are in the soil environment. Indicators are those plants that accumulate metals in the above ground biomass usually at concentrations that are comparable to metal levels in the soil. Hyperaccumulators refer to those plants that increase internal sequestration, translocation and accumulation of metals in their above ground biomass to levels that far exceed those found in the soil (Mehes-Smith *et al*., 2013).

Edible plants that concentrate heavy metals into their tissues can be very detrimental if they are consumed as metals can cause illnesses, like cardiovascular diseases (Pb and Cd), cancer (Cu and As) and liver and kidney problems (Oliver, 1997). The maximum allowable limits of some metals in edible plants are; 0.2 (As), 0.2 (Cd), 2.3 (Cr), 40 (Cu), 10 (Hg), 30 (Ni), 0.3 (Pb) and 60 (Zn) mg kg^{-1}(Bempah *et al*., 2012; Codex Alimentarius Commission, 2001). Heavy metals also affect the general health of the soil, Smejkalova and Boruvka (2003) documented that an increase in Cd, Pb and Zn pollution resulted in a decrease in soil microbial activity, $C_{biomass}$:C_{ox} ratio and inhibition of enzymatic activities. It is necessary to recognize plants that are able to deal with excess metals in soil.

2.6 Phytoremediation technology

Phytoremediation is a technology that uses the ability of some plants species to accumulate metals at concentrations exceeding toxic levels in plants (Poresbta and Oastrawska, 1999). Reeves and Brooks (1983) were among the first researchers to demonstrate the high level of metal uptake by plants. They determined the concentration of lead and zinc in *Thlaspirotundi foliumsubsp*. *Caprifolium* and *Alyssum wulfenianum* growing on mine tailings. Lead levels of up to 8200 μg g−1 (0·82%) and zinc levels of up to 17 300 μg g−1 (1·73%) were discovered in dried leaves of *Thlaspirotundifolium* and 860 and 2500 μg g−1 were found in *Alyssum wulfenianum.* This technology has since been further investigated by researchers all around the world (Chaney, 1983; Cunningham *et al.*, 1995; Comis, 1996; Dushenkov *et al.*, 1997).

The conventional methods that have been used in heavy metal remediation include soil washing, chemical extraction, isolation and containment, excavation and landfill. These have been found to have high cost implications compared to phytoremediation and they contribute to further environmental degradation (Pulford and Watson, 2002). Processes like excavation results in destruction of soil structure, loss of fertility and recontamination elsewhere during landfill (Lone *et al.,* 2008). Phytoremediation on the other hand is innovative, cost-effective and environmentally friendly. It has to however be mentioned that it does have its limitations, these include time consuming, can only remediate as far as the rooting depth, dependent on seasons for plants to grow (Ghosh and Singh, 2005; Pulford and Watson, 2002; Raskin and Ensley, 2000). A contrast between advantages and disadvantages is given in table 2.4.

Table 2.4: Advantages and Disadvantages of Phytoremediation.

Advantages	**Disadvantages**
Low cost	Time consuming
Applicable to different contaminants	High levels maybe toxic to plants.
Have economic gains as metals can be recycled.	Site specific.
The treatment is permanent.	Plant growth dependent on season and climatic condition.
Remediation is done in situ thereby avoiding destruction of soil properties.	Not capable of 100% reduction.
Environmentally friendly.	Can only remediate as far as the rooting depth.

Ghosh and Singh, 2005; Pulford and Watson, 2002; Raskin and Ensley, 2000**.**

2.6.1 Types of phytoremediation technology

Phytoremediation can be classified into 5 techniques depending on the process employed, these are as follows.

2.6.1.1 Phytoextraction

This form of phytoremediation involves the uptake of heavy metals from sediments, water and soil by plant roots into the harvestable plant part. This technique is the focus of the proposed research and is the most commonly recognized. Incineration of the harvested plant dramatically reduces the volume of the material needing disposal. Valuable metals can be extracted from the metal rich ash and serve as a source of revenue, thereby offsetting the expense of remediation. Phytoextraction is a long term remediation solution requiring alot of cropping cycles to reduce the metal concentrations to below acceptable levels. Time of remediation is dependent on extent of metal contamination, efficiency of plant in heavy metal removal, length of growing season, bioavailability of metal in soil, it is however estimated to take anything between 1 to 20 years. (Ahmadpour *et al.,* 2014; Barcelo and Poschenrieder, 2003; Pulford and Watson, 2002; Blaylock and Huang, 2000).

2.6.1.2 Phyto-degradation

This technique of remediation involves uptake, metabolizing and degradation of contaminants within the plant, or degradation of contaminants in the soil, ground water or any medium in question by enzymes produced and released by the plant (Pivetz, 2001). These enzymes include nitroreductase, dehalogenase, peroxidase, nitrilase and laccase. They are associated with transformations of phenols, chlorinated compounds, munitions and herbicides (Pivetz, 2001). According to Newman and Reynolds (2004), Shang and Gordon (2002) demonstrated that the groundwater contaminant trichloroethylene (TCE) taken up by suspension cell cultures of hybrid poplar becomes part of the non-volatile, un-extractable portion of the cells. Thompson *et al*. (1998) also demonstrated the transformation of 2,4,6-trinitrotoluene (TNT) by hybrid poplar trees to 4-amino-2,6-dinitrotoluene (4-ADNT), 2-amino-4,6-dinitrotoluene (2-ADNT), and other unidentified compounds in laboratory hydroponic and soil experiments. This technique however would be ineffective on heavy metals as they cannot be degradable (Safronova *et al*., 2011; Pulford and Watson, 2002).

2.6.1.3 Rhizofilration

This technique makes use of aquatic and terrestrial plant species to adsorb or precipitate heavy metals onto plant roots from contaminated surface water, waste water or aquatic environment (Barcelo and Poschenrieder, 2003; Ghosh and Singh, 2005). Root exudates produce biogeochemical conditions resulting in the precipitation of contaminants onto the roots. The contaminant either remains on the root or translocated within the root or other parts of the plant depending on the plant species, the nature and concentration of the contaminant. Rhizofiltration is similar to phytoextraction as both results in the accumulation of the contaminant in the plant. It is however different in that phytoextraction requires a significant amount of the contaminant to be translocated to the above ground part of the plant. In addition the contaminant in rhizofiltration is initially in water rather than soil (Pivetz, 2001). It is also different from phytostabilisation where the contaminant only remains in the root zone. An example of this technique is the use of sunflower to remove radionulcides from a small pond near the Cherobyl reactor in Ukraine. The sunflowers were grown in a floating raft on a pond for eight weeks. Bioaccumulation results indicated that sunflowers could remove cesium and strontium from the pond (Pivetz, 2001).

2.6.1.4 Phytostabilization

In this technique, plants are used to decrease the activity or bioavailability of heavy metals, thereby preventing their transmission to the broader environment. This can be done by sorption, precipitation, complexation or metal valence reduction. In addition, plants can reduce water and wind erosion of the soil, thus preventing migration of the contaminant in runoff or dust emissions, and may reduce or prevent leachate generation. An example of the application of this technique is the Keating tailings site an abandoned mine land in Montana, USA. The Bureau of Land Management (BLM) applied this technique in a period of 3 years from 2003 to 2006. Positive results were realized in that applicable, relevant and appropriate requirements were met with regards to ground cover that can reduce migration of metals to air, surface water and ground water (Neuman and Ford, 2006).

The disadvantage of this technique is that the contaminants remain in the soil and therefore need to be examined closely (Jadia and Fulekar, 2009; Ghosh and Singh, 2005).

2.6.1.5 Phytovolatization

Phytovolatization uses plants to take up contaminants from the original medium (ground water or soil water) and convert them into volatile forms then transpire them into the atmosphere. During this, metabolic processes within the plant might alter the form of the contaminant to even less toxic forms. A point in case would be the study done by Moreno *et al*. (2008), who investigated the removal of Hg from solutions by Indian mustard in hydroponic conditions with solutions containing Hg concentrations from 0 to 10 mgL^{-1}. The plants were enclosed in gastight volatization chambers to assess the effect of Hg concentrations on plant transpiration, accumulation and volatilization. Volatilization was found to increase linearly as a function of Hg concentrations in solutions.

The limitation of this technique is that the metals can be recycled and re-deposited into the soil water system, therefore repeating the process of contamination, though not necessarily on the site of origin (Jadie and Fuleka, 2009). It is also only possible to volatile metals like mercury.

2.7 Plants for remediation

The suitability of a species of plant to be used in phytoremediation essentially depends on its ability to accumulate heavy metals in its tissue, rate at which it can grow and plant density (U.S.EPA, 2000). The plants should be able to take up metals at rates that would otherwise be considered toxic to ordinary plants. A special group of plants able to accumulate heavy metal levels over 1000 $mgkg^{-1}$ have been identified (Raskin and Ensley, 2000). These are termed hyper-accumulators and there are about 400 known species in this group of plants (U.S.EPA, 2000; Malik and Biswas, 2012). A substantial amount of these hyper-accumulators are found in the *Brassica* family like Indian mustard (*Brassica juncea*), black mustard (*Brassica nigra*), turnip (*Brassica campestris*), rape (*Brassica napus*), and kale (*Brassica oleracea*). They have been found by a number of researchers to accumulate different metals like cadmium, copper, lead, chromium, nickel and zinc (Sheng and Xia, 2006; Belimov *et al.*, 2005; Dell'Amico *et al*., 2008).

According to Dzantor and Beauchamp (2002) the full scale deployment of these hyper-accumulators in metal remediation has lagged behind because of their specialized nature. They are uniquely adapted to certain environments, putting a restriction on optimum growth which invariably affects metal uptake. This then affects their reproducibility on different sites of contamination in various environmental conditions (Ghosh and Singh, 2005).

A different group of plants often called moderate accumulators have also been used in phytoremediation of metal contamination. These plants are able to tolerate high metal concentrations and have been demonstrated to accumulate high concentrations of metals in their tissues, though not at the concentration required to be called hyper-accumulators

(Vamerali *et al*., 2010). These plants include grasses like vetiver grass, agricultural crops like maize, indigenous vegetables and many others like sunflower, oats, barley, vetch, etc. (Rahimi *et al.,* 2013; Pulford and Watson, 2003; Safronova *et al.,* 2011; Chen *et al.,* 2004; Huang *et al.,* 1997).

Selection of plants for phytoremediation largely remains site specific. Factors like climate, physiochemical soil properties, extent of contamination still hold great importance in terms of selecting species to be used in phytoremediation. Consideration should be given to the above factors and sites ought to be treated as separate entities with regard to the factors mentioned above (Ghosh and Singh, 2005; Pulford and Watson, 2003).

2.8 Concluding remarks

The application of sewage sludge over a long period of time can result in heavy metal pollution of soils. Heavy metals can be detrimental to plants, animals, humans and even to the health of the soil at elevated levels. Soils polluted with heavy metals need to be remediated because of the danger they pose to society. Phytoremediation has been found to be a cheap and environmentally friendly alternative compared to the conventional methods that have been used previously. Soil parameters like pH play a very important in the availability of heavy metals to plants. Certain plants have been demonstrated to have a unique ability of tolerating elevated levels of heavy metals in the soil. These need to be examined for potential use in phytoremediation strategies of polluted soils.

CHAPTER 3

MATERIALS AND METHODS

3.1 Heavy metal concentration in tissue of plants growing on sewage sludge disposal site.

3.1.1 Description of Study Area

The study was carried out at the Darvill Waste Water Works (DWWW) (latitude 29.60250^{o}S to 29.61139^{o}S and from longitude of 30.43390^{o}E to 30.43861^{o}E), at an altitude of 596 m above sea level, on the eastern boundary of the city of Pietermaritzburg in KwaZulu-Natal Province (Figure 3.1). Pietermaritzburg receives around 695 mm of rainfall annually and has mean maximum temperatures of 26^{o} C and mean minimum temperatures of 20^{o} C. Most of the rainfall is received during the summer season (October-February). The site is on a 78 ha plot, on the western bank of Msunduzi River, and 57 ha is irrigated with sewage sludge using sprinkler system. It is divided into five transects. The sixth transect upslope (where no sludge has been applied) was used as the control.

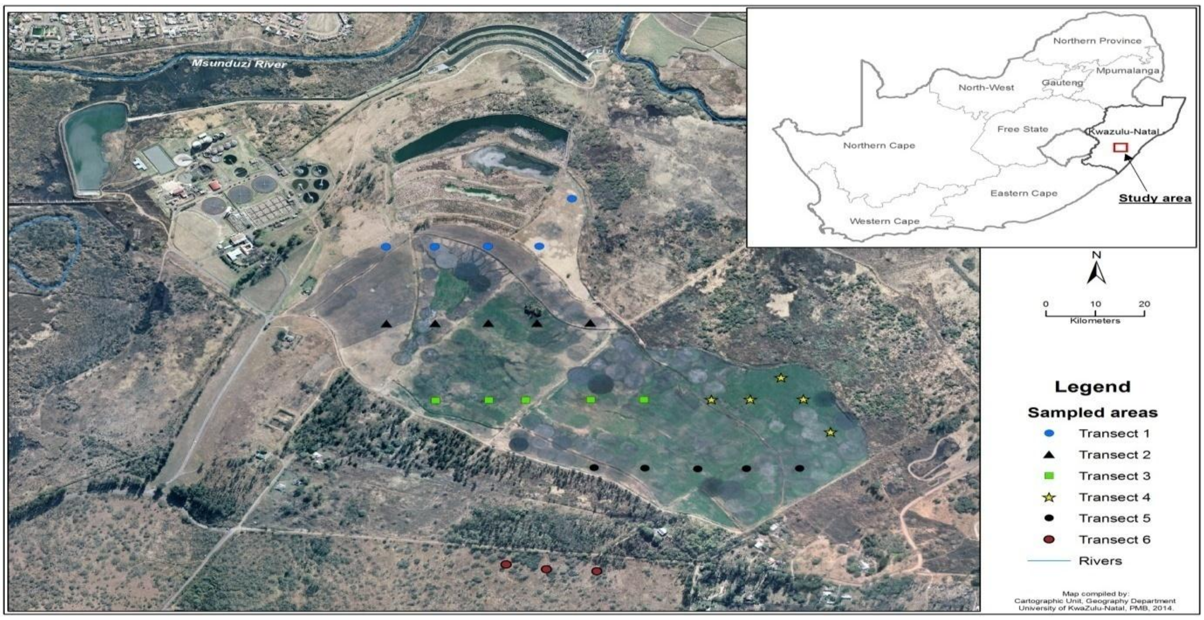

Figure 3.1: Map of Darvill wastewater works dedicated disposal site showing sampling points. Blue circles indicate transect one, black triangles represent transect two, green squares represent transect three, yellow stars represent transect four, black circles represent transect five and red circles represent transect six (control).

3.1.2 Soil pH

The pH (KCl) results adapted from Mdlambuzi (2014) from the top 30 cm of the soil are given below in Table 3.1. These will give an indication of the extent of bioavailability of the heavy metals to the plants for uptake. Soil pH (KCl) in transects 1 and 6 is 4.1 or lower in all three depths, while it is between 4.6 and 5.5 units at all depths for all the other transects. The surface soils (0-10 cm) have the highest pH for most transects except 2 and 3.

Table 3.1: The pH (KCl) in the top 30 cm of the soil at the Darvill sewage disposal land

Transects	Depth (cm)	pH (KCl)
1	0-10	4.1
	10-20	3.8
	20-30	4.1
2	0-10	4.7
	10-20	4.9
	20-30	4.6
3	0-10	4.6
	10-20	5.3
	20-30	4.6
4	0-10	5.5
	10-20	5.1
	20-30	4.8
5	0-10	5.8
	10-20	5.6
	20-30	4.8
6	0-10	4.1
	10-20	4.0
	20-30	4.0

Adapted from Mdlambuzi (2014)

3.1.3 Heavy metal concentration in soils

Table 3.2 gives heavy metal concentrations in the 0- 30 cm depths of the soil on all transects. Maximum permissible limits (MPL) above which disposal of sludge is prohibited are also given. Cadmium, Cr, Pb and Zn were approaching or greater than the MPL in the sludge treated soils (Table 3.2). Arsenic, Cu, Ni and Hg were well below the MPL in all transects (Table 3.2).

Table 3.2: Heavy metal concentrations in the 0-30 cm of the soil at the Darvill sewage disposal land.

	Metal concentration (mg kg^{-1})							
Transect	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
MPL	20	5	450	375	9	200	150	700
1	11.7	8.0	430	116	2.1	86	140	394
2	6.1	9.7	602	186	2.2	37	125	832
3	9.3	2.3	851	147	2.2	25	83	660
4	4.6	9.3	929	168	3.2	30.9	114	657
5	3.6	5.6	875	181	2.3	26.7	115	672
Control	1.6	1.9	180	24	2.2	9.2	23	31

Adapted from Mdlambuzi (2014), MPL stands for maximum permissible limit as given by Snyman *et al.*, 2000; Herselman and Moodley, 2009.

3.1.4 Plant sampling

Plants were sampled in May of 2014. A 1m^2 quadrant was used for sampling plants in triplicates for all transects. Both the shoots and roots were sampled. Some plants occurred in only some transects and not others, at the time of sampling. Amaranthus (*Amaranthus dubius*) and rumex (*Rumex pulcher*) were sampled from transects 3, 4 and 5 while tomato (*Solanum lycopersicum*) and black nightshade (*Solanum nigrum*) were sampled from transects 3 and 5. The plants were rinsed with distilled water to remove foreign material and then oven dried at 65^{o}Cto constant weight (about 5 days). The plant samples were then ground to < 0.5 mm using Fritsch Pulverisette mortar grinder and stored before analysis.

3.1.5 Analysis of plant tissue metal concentrations

Plant samples (0.5 g) were weighed into a 250 ml conical flask and 18 ml of Nitric and Perchloric acid mix (4:1 ratio) was added, and digestion was done on a block set at 120 ^{o} C until solution was clear and white fumes could be seen, as described by Hseu (2004) and

Odu *et al.* (1986). Cooled samples were transferred to a 25 ml volumetric flask and filled to the mark with deionised water. The sample solutions were then analysed for heavy metals using the 720 Varian inductively coupled plasma optical emission spectrometer (ICP-OES). Plant tissue heavy metal concentrations were compared to limits set by Bempah *et al.* (2012). These limits determine the amount of heavy metals that should be present in plants that will be consumed by animals and humans, above which serious harm can be caused. These are different from those given in section 3.2, where the tolerance levels of most metals in plants are given above which most plants would not survive.

3.1.6 Amaranthus leaves sold at the local market.

Leaves of amaranthus (*Amaranthus dubius*) sold at the local market, harvested by vendors at Darvill and other areas where no sludge is applied were bought for R12 a bunch weighing an average of 70 g and analysed for heavy metals. Two samples of amaranthus were analysed. The analysis was done following the same procedure as the plants above.

3.1.7 Sampling of turf grass and soil attached to the root system

Turf grass grown on transects 1, 2 and 3 sold to the community, by Duzi Turf, a private company, was purchased and sampled using a $1m^2$ quadrant. A machine which cuts out the turf together with the top ±5 cm of the soil was used. Turf samples were taken to the lab and the soil attached to the rooting system was separated. The turf tissue samples were cleaned with distilled water to remove foreign material and oven dried at 65 ^{o}C to constant weight, and ground (< 0.5 mm) before digestion and analysis. The soil samples separated from the roots were air-dried, sieved (<2mm) and stored before digestion and analysis.

3.1.8 Analysis of heavy metals in soil attached to turf roots

Soil samples were digested using a microwave assisted acid digestion procedure as following the EPA method 3051 (EPA, 1998). Soil (0.5g) was weighed into digestion vessels to which 16 ml of *aqua regia* (12 ml of 32% HCl and 4 ml of 55% HNO_3) solution were added before digestion with a microwave digester (EPA 3051H-HP500). After cooling, the digests were transferred into 50 ml volumetric flask and filled to the mark with deionised water, before analysis of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) using the 720 Varian ICP-OES. Results obtained from ICP analysis were in mgL^{-1} and were converted to mg kg^{-1}

3.2 Heavy metal concentrations in tissue of plant species grown on polluted soil after long-term application of sewage sludge.

3.2.1 Soil Sampling and analysis

The polluted soil used in this study was treated with sewage sludge for over 50 years and no sludge had been applied to the control soil, at the Darvill Sewage Disposal site as indicated in Figure 3.1 .The polluted soil was sampled from 28 points (0-30cm depth) using a spade, and mixed together to form one large composite sample. The control soil was also sampled from the reference site and mixed the same way. The two soils were, air-dried for five days, sieved (< 2mm) and analysed for pH, total carbon, bases and heavy metal concentrations.

3.2.2 Analysis of pH, total carbon, nitrogen and exchangeable bases

Soil pH was measured in 1M KCl solution at a 1:5 soil: solution ratio using a Radiometer PHM 210 meter. Total C and N were determined with TruMac CNS/NS Carbon/ Nitrogen/ Sulfur Determinator using the Leco machine. Phosphorus was extracted with 0.25 M ammonium bicarbonate, EDTA disodium salt and 0.01 M ammonium fluoride (AMBIC) solution. Exchangeable bases (Na, Mg, K, and Ca) were extracted with 1M NH_4Cl (pH 7) as outlined by the Non-Affiliated Soil Analysis Work Committee (1990) and analyzed using the atomic absorption spectrophotometer (Varian AA 280 FS).

3.2.3 Total heavy metal analysis in soil

The soil samples were digested following the EPA 3051 method (EPA, 1998) and analysis of heavy metals was done using the 720 Varian Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Soil (0.5g) was weighed and 16 ml of *aqua regia* (12ml concentrated (32%) HCl and 4 ml concentrated (55%) HNO_3) solution was added into Teflon™ vessels HP500 and placed in a MARS 5 microwave oven (Microwave Accelerated Reaction System, manufactured by CEM Corporation, USA). The samples were digested at 175^{o}C for 10 minutes (EPA 3051H-HP500). After cooling, the digests were quantitatively filtered into 50 ml volumetric flasks using What man No. 40 filter paper and filled to the mark with deionised water, before analysis of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) using the ICP-OES in mg L^{-1}.The results were converted to mg kg^{-1} of soil.

3.2.4 Diethylenetriaminpentaacetic acid (DTPA) extractable heavy metals in soils

Plant available heavy metals were extracted with diethylenetriaminepentaacetic acid (DTPA).The soils (25 g) were extracted with 50 ml of solution containing 0.005 M DTPA, 0.01 M $CaCl_2$ and 0.10 M triethanalamine (TEA) (1.97 g diethylenetriaminepenta acetic acid, 1.47 g calcium chloride dehydrate, 14.92 g triethanalamine and 6.8 g hydrochloric acid in 1.0 L de-ionised water, pH 7.3). The soil samples were shaken for two hours at 25^{o}C, at a speed of 40 rpm, and then filtered with Whatman no. 2 filter paper, and the pH measured immediately, before storing the samples at 4^{o}C until analysis. Extractable Zn, Cu, Ni, Cr, Pb, Cd and As in the extracts, were measured with a 720 Varian ICP-OES.

3.2.5Effects of plant species on tissue metal concentrations

The plant species used in this study were selected based on rapid growth and high biomass production (Marchiol *et al.*, 2004; Kramer, 2005), ability to extract/tolerate elevated levels of heavy metals (based on literature reviewed), the ability to grow in the climatic conditions of KwaZulu-Natal and availability of seed in South Africa. The sixplant species used were Indian mustard (*Brassica juncea*), lucerne (*Medicago sativa*), vetch (*Vicia sativa*), Rape (*Brassica napus*), ryegrass (*Lolium perenne*), and spinach (*Spinacia oleracea*).

The study was conducted through a pot experiment carried out in a glasshouse at the University of KwaZulu-Natal (UKZN) in Pietermaritzburg, with mean maximum and minimum temperatures of 26^{o} C and 20^{o} C, respectively.

The pot experiment was laid out in a randomized complete block design with six plant species and two soil pollution levels (polluted and control), replicated three times. Pots with inner diameter of 20 cm and height of 17 cm were filled with 3kg of soil. Fertilizer was added to the soil before planting as per crop requirement following recommendations of a soil test, so that the plants could grow to their optimum and any stress would be as a result of heavy metal toxicity. No fertilizer was added to the polluted soils where lucerne and vetch were sown as according to the recommendations the soil had enough nitrogen (N), phosphorous (P) and potassium (K) for their maximum yield. The control soil where lucerne and vetch were grown was fertilised with 43 kg of P ha^{-1}as required and 175 kg of K ha^{-1}, respectively. This was supplied in the form of superphosphate and potassium sulphate. No nitrogen fertilization was applied, as the plants could fix N. Spinach, mustard and rape were fertilised with 100 kg N ha^{-1}(required for maximum yield) in both the polluted soil and the control. This was supplied in the form of LAN (Lime Ammonium Nitrate). No P and K fertilizer were required for the polluted soils, while the control soil was fertilised with 200 kg P ha^{-1} and 225 kg K ha^{-}

^{1}, supplied in the form of single superphosphate and potassium sulphate, respectively. Rye grass was fertilised with 160 kg N ha^{-1}, supplied in the form of LAN for both the polluted and control soils. No P and K were required in the polluted soil, whereas 45 kg P and 31 kg K ha^{-1} were added to the control soil, as superphosphate and potassium sulphate, respectively.

Plant seeds were sown at the optimum rates for each plant species. Lucerne and vetch were sown at 25 kg ha^{-1}, mustard and rape were sown at 6 kg ha^{-1}, rye was sown at 30 kg ha^{-1} and spinach was sown at 280 000 plants ha^{-1}. Replicates that had different emergence rates were thinned to be the same number in both the contaminated and the control soil. The locations of the pots in each block were rotated periodically to ensure uniform light intensity to all pots. Plants were watered with distilled water, to replenish water loss through evapotranspiration. Weeds were removed manually during the duration of the experiment.

The experiment was run for six weeks. Shoots were harvested by cutting with a scissors at the soil surface. The pots were then emptied of the soil and the roots separated. The plant materials were cleaned immediately after harvesting by rinsing in distilled water. Both the shoots and roots were oven dried at 70^{o}C for three days, and weighed to determine dry matter yield, and ground to <2mm before analysis for heavy metals, with ICP-OES after microwave digestion. The uptake of the plants was calculated by multiplying the dry matter yield and the concentration of the heavy metals for each plant.

Plant tissue heavy metal concentrations were compared with toxicity threshold limits which according to Vamerali *et al.*, 2010, should not be exceeded since most plants cannot survive in such elevated metal concentrations. These are different from those used in section 3.1 for the plants harvested at the site for consumption purposes by neighbouring communities. The limits were used to give a reflection of tolerance to heavy metal toxicity the different plants were able to accommodate. These thresholds limits were 20 (As), 5 (Cd), 2 (Cr), 40 (Cu), 10 (Hg), 15 (Ni), 10 (Pb) and 150 (Zn) mg kg^{-1}.

3.2.6 Effects of EDTA application rate on metal concentrations in Indian mustard tissue

This trial was conducted with Indian mustard grown on the heavy metal polluted soil only in a pot trial. Indian mustard seeds were grown in 12 pots with 3 kg soil, without fertiliser addition. The plants were grown for four weeks in order to allow them to establish before addition of EDTA to the soil. Increasing concentrations of EDTA (0, 3, 6 and 10 mmol kg^{-1}) were added to the soil and each rate was replicated three times. The plants were grown for a further two weeks before termination of the experiment. Details of glasshouse conditions and management of the trial were similar to those of the plant species pot trial. The same procedures described in the plant species experiment, for harvesting and sampling of the plants, sample preparation and analyses were used.

3.3 Statistical analysis and data handling

Analysis of variance (ANOVA) was carried out using Genstat 14thedition to determine the effects of plant part (shoots and roots) on tissue heavy metal concentrations on volunteer plants, and to determine differences across species and between shoots and roots of plant grown for their phytoremediation potential. The least significant difference test ($LSD_{0.05}$) was used for multiple comparisons between the treatment means.

CHAPTER 4

RESULTS

4.1 Heavy metal concentration in tissue of plants growing on sewage sludge disposal site.

All the plants growing on land after long-term treatment with sludge had Cd, Cr, Ni and Pb concentrations well above their maximum permissible limits of 0.2 mg kg^{-1} for Cd and Cr, 30 mg kg^{-1} for Ni and 2.3 mg $kg^{-1}$for Pb (Table 4.1). Amaranthus, rumex and *S. nigrum* had the highest Cr concentration. Chromium in amaranthus shoots was 20 times while in the roots it was 45 times the limit. Cadmium concentration in the shoots is the same in all the plants except *S. nigrum* which is higher than rumex, with the concentration in the roots relatively the same in all plants. Nickel concentration in plants was relatively the same in both shoots and roots. All the plant shoots had relatively the same Pb concentration whereas *S. nigrum* had the highest Pb concentration in the roots. The concentration of Zn was above its limit of 60 mg kg^{-1} in the roots and shoots with the exception of *S. nigrum* in the shoot. Root tissue of all the plants has relatively the same As concentration, while tomato had the highest shoots concentration. Copper and mercury were well below the limit in both the shoots and roots for all the plants.

Table 4.1: Mean concentrations of heavy metals in plants growing in the study area.

Species	*Plant part*	Heavy metal concentration (mg kg^{-1})							
		As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
#*MPL*		0.2	0.2	2.3	40	10	30	0.3	60
Rumex	*Shoot*	0.0	0.7	43.9	14.0	1.6	96.4	2.2	79.7
	Root	0.2	1.1	77.7	16.6	1.6	94.1	5.1	84.3
Amaranthus	*Shoot*	0.1	0.8	48.8	33.5	2.0	100.6	2.9	94.8
	Root	0.2	0.9	105.1	13.3	2.4	119.4	4.4	106
Tomato	*Shoot*	0.2	1.2	27.4	17.9	3.0	98.8	0.8	64
	Root	0.6	1.3	28.6	19.5	1.8	102.2	3.5	83.7
S. nigrum	*Shoot*	0.1	1.4	31.4	10.0	4.0	85.5	3.2	53.1
	Root	0.3	1.0	81.6	19.5	2.1	109.9	8.2	116.2
LSD		0.40	0.67	31.81	17.58	1.56	29.15	2.62	48.04

#MPL Source: Bempah *et al.*, 2012; Hg limit based on FAO, Ni based on Vamerali *et al.*, 2010.

4.1.2 Amaranthus leaves sold at the local market

Amaranthus leaves harvested from the study (polluted) site had higher As, Cd, Cr, and Zn than the limit, while only Cr and Zn were at or above the limit for the control leaves(Table 4.2). The amaranthus leaves from the study site had higher concentrations than the MPL by a factor of 4 for As and Cd, and 16 for Cr, while Pb and Zn were at or slightly above the MPL.

Table 4.2: Heavy metal concentrations (mean± standard deviation) in amaranthus leaves sold to the local people at the market place**.**

Metal	MPL	Leaf tissue metal concentration (mg/kg)	
		polluted	control
As	0.2	0.8 ±0.2	0.0±0.0
Cd	0.2	0.8±0.1	0.1±0.2
Cr	2.3	37.7±3.3	4.0±1.1
Cu	40	11.2±0.8	1.4±0.4
Hg	10	-	-
Ni	30	25.5±2.4	9.7±2.3
Pb	0.3	0.3±0.2	0.0±0.0
Zn	60	69.1±6.6	59.3±3.6

Heavy metal limits are based on Bempah *et al.*, 2012 and WHO/FAO. Limit for Ni is based on Vamerali *et al.*, 2010 # Hg-no result #

4.1.3Turf grass and soil associated with roots.

Concentration of Cr, Cu, Ni, Pb and Zn in the turf grass tissue was higher than their respective MPL (Table 4.3).Chromium and Zn were the most highly concentrated, however Ni is 13 times its MPL. The concentration of As, Cd and Hg were still well below the limits. Concentrations of Cr on soils associated with the roots of turf grass was higher than the MPL (Table 4.3). Concentrations of Cu, Hg, Ni, As, Zn and Pb were lower than the MPL.

Table 4.3: Concentration of heavy metals (mean±standard deviation) in turf grass and soil associated with the roots of the grass.

Metal	Tissue metal concentration (mg kg^{-1})		Soil metal concentration ($mgkg^{-1}$)	
	*MPL	Turf tissue	#MPL	Root-associate soil
As	20	7.1±5.0	20	8.8±4.4
Cd	5	2.0±1.2	5	4.9±0.8
Cr	2	403.7±54.5	450	563.1±89.6
Cu	40	66.5±14.8	375	87.4±7.8
Hg	10	4.0±2.1	9	3.0±0.9
Ni	15	197.3±39.4	200	105.1±10.6
Pb	10	20.7±3.7	150	68.5±33.3
Zn	150	414.7±21.8	700	470.3±53.8

*MPL (*Source: Vamerali *et al.*, 2010); #MPL (#Source: Herselman and Moodley, 2009).

4.2 Heavy metal concentrations in tissue of plant species grown on polluted soil after long-term application of sewage sludge.

4.2.1Soil analysis

The soil parameters analysed in this study are all higher in the polluted soil compared to the control (Table 4.4). The polluted soil had over 20 times more P than the control. The pH of the polluted soil was 5.9, almost one pH unit higher than the, control with pH5.0. The total C, exchangeable Ca and K in the polluted soil was 7 times higher than the control. Magnesium also follows the same trend, with the polluted soil having twice the concentration in the control.

Table 4.4: Soil pH, total C, P, K, Ca and Mg (mean ± standard deviation) in the soils used in the study.

Parameter	Polluted soil	Control soil	LSD
pH(KCl)	5.9± 0.16	5.0± 0.07	0.28
Total C (%)	16.0± 0.18	2.2± 0.37	1.21
P ($cmol_{(+)}kg^{-1}$)	2.23± 0.30	0.1± 0.02	1.49
K ($cmol_{(+)}kg^{-1}$)	2.0± 0.49	0.3± 0.04	1.20
Ca ($cmol_{(+)}kg^{-1}$)	29.2± 4.21	4.4± 0.10	10.53
Mg ($cmol_{(+)}kg^{-1}$)	5.6± 2.42	2.2± 0.07	6.06

The polluted soil has had higher concentration for all pseudo-total heavy metals compared to the control (Table 4.5). Cadmium, Cr, Pb and Zn in the polluted soil had concentrations that were above the maximum permissible limits. Nickel, As and Cu concentration were above the total trigger values. In the control soil all heavy metals were well below their respective maximum permissible limits, with As above the total trigger value and Pb approaching the total trigger value.

Table 4.5: Concentrations of heavy metals in soils used in this study.

	Concentration in mg kg^{-1}				
Heavy metals	Polluted soil	Control soil	LSD	*TTV	*MPL
Arsenic (As)	11.6±5.62	4.0±4.80	11.2	2	20
Cadmium (Cd)	7.4±0.36	1.9±1.25	3.1	3	5
Chromium (Cr)	898±152.4	38±5.2	366	350	450
Copper (Cu)	264±15.5	20±1.1	38	120	375
Nickel (Ni)	188±11.5	63±10.0	53	150	200
Lead (Pb)	221±36.2	74±20.9	72	100	150
Zinc (Zn)	792±45.4	183±13.6	146	200	700

± Std.dev; TTV stands for total trigger value, MPL stands for maximum permissible limits in soil (*Source: Herselman and Moodley, 2009).

4.2.2DTPA extracted heavy metals in soils.

The concentrations of heavy metals extracted with DTPA were higher in the polluted soil than the control for all heavy metals tested, except As where the control had0.3 mg kg^{-1}compared to 0.1 mg kg^{-1}found in the polluted soil (Table 4.6). In the polluted soil available Cr, Ni and Pb concentrations, were over 3 times, Cu was over 5 times, and Zn was over 10 times the concentration of the control.

Table 4.6: Heavy metal concentrations extractable with DTPA.

Heavy metals	Soil metal concentration (mg kg^{-1})		
	Polluted	Control	LSD
Arsenic (As)	0.1±0.01	0.3±0.01	0.04
Cadmium (Cd)	1.2±0.04	0.1±0.01	0.06
Chromium (Cr)	6.0±1.07	1.7±1.33	1.99
Copper (Cu)	15.1±0.86	2.7±0.12	1.26
Nickel (Ni)	32.1±0.63	8.9±2.03	3.56
Lead (Pb)	11.2±0.72	3.3±0.06	1.19
Zinc (Zn)	38.5±0.63	3.0±0.58	4.21

4.2.3 Dry matter yield

There were no differences in shoot dry matter between polluted and control soils for individual plant species, except mustard and rape which had higher drymatter yield in the polluted soil (Table 4.7). The shoot dry matter yields were in the order; mustard = rape > rye > spinach= lucerne = vetch in both the polluted and control soil. In the case of root dry matter, only rye had a significantly higher yield in the control than the polluted soil (Table 4.8). The root dry matter yields were in the order; rye >mustard = rape > spinach= lucerne = vetch in both the polluted and control soil.

Table 4.7: Shoot dry matter of plant species grown in contaminated and control soil.

	Shoot dry matter (g pot^{-1})	
Plants	Contaminated soil	Control soil
Rye	7.41	7.31
Lucerne	4.59	3.95
Vetch	4.04	4.24
Mustard	16.98	11.44
Spinach	4.96	4.92
Rape	15.46	9.71
LSD	**2.218**	

Table 4.8: Dry matter results for roots of winter plants grown in contaminated and control soil.

	Root dry matter (g pot^{-1})	
Plants	Contaminated soil	Control soil
Rye	1.54	2.05
Lucerne	0.65	0.37
Vetch	0.36	0.44
Mustard	0.96	0.98
Spinach	0.33	0.30
Rape	1.01	0.78
LSD	**0.351**	

The dry matter yield of mustard grown in the EDTA treated soils decreased with increase in EDTA application rate in both the shoot and root (Table 4.9), and the plants grown in soil treated with 10mmol EDTA kg^{-1} wilted and died within a week of application.

Table 4.9: Shoot and root dry matter of mustard grown in EDTA treated soils.

EDTA rate (mmol kg^{-1})	Shoot dry matter (g pot^{-1})	Root dry matter (g pot^{-1})
0	16.98	0.96
3	11.19	0.82
6	10.55	0.72

Plant grown at 10mmol EDTA kg^{-1}wilted and died within a week of application

4.2.4 Heavy metals in plants

Concentrations of both Zn and Cu (Table 4.10) were higher in the plants grown in the contaminated soil compared to the control (both root and shoots). Lucerne and vetch had the highest concentration of Zn in the shoots, while vetch and rye had the highest in the roots. Lucerne and vetch also had the highest shoot Cu concentration, while rye had the highest root concentration.

Table 4.10: Zinc and copper concentration (mg kg^{-1}) in shoot and root tissue of selected plants grown on polluted and control soils.

	Zn		Cu	
Species	Polluted	Control	Polluted	Control
Shoots				
Indian Mustard	169.1	53.4	56.4	10.9
Lucerne	426.2	180.2	103.2	14.8
Vetch	439.6	138.3	119.2	16.7
Rape	164.6	56.3	26.2	6.8
Rye	167.3	67.2	42.5	7.0
Spinach	119.3	66.8	42.1	15.8
LSD	70.45		13.32	
Roots				
Indian Mustard	116.5	69.8	35.1	7.6
Lucerne	181.8	98.6	39.7	9.1
Vetch	482.5	70.6	41.2	7.2
Rape	233.4	120.4	32.4	5.6
Rye	297.5	224.4	96.9	11.9
Spinach	106.0	63.0	21.9	8.8
LSD	62.25		10.99	

Maximum limits are 150 and 15 mg kg^{-1} for Zn and Cu, respectively (Source: Vamerali *et al.*, 2010).

Chromium and Ni (Table 4.11) were higher in the plants grown in the contaminated soils than the control. Chromium concentrations in both the shoots and the roots were above the limit in all plants grown on both soils. Lucerne, vetch and rape had the highest shoot Ni concentration while rye had the highest concentration in the roots.

Table 4.11: Chromium and nickel concentrations (mg kg^{-1}) in shoot and root tissue selected plants grown on polluted and control soils.

	Cr		Ni	
Species	Polluted	Control	Polluted	Control
Shoots				
Indian Mustard	69.8	4.4	27.7	15.3
Lucerne	99.1	8.3	74.2	24.3
Vetch	40.8	5.5	80.2	13.6
Rape	28.2	7.7	70.9	18.6
Rye	98.6	21.3	38.7	14.3
Spinach	24.2	5.6	22.5	6.9
LSD	18.45		12.31	
Roots				
Indian Mustard	99.2	15.0	34.1	20.0
Lucerne	82.7	18.0	44.3	17.7
Vetch	232.4	12.1	50.5	22.7
Rape	139.7	20.6	40.7	4.6
Rye	236.1	81.4	99.7	10.4
Spinach	53.1	15.4	14.2	1.9
LSD	27.42		12.64	

Maximum limits are 2 and 20 mg kg^{-1} for Cr and Ni, respectively (Source: Vamerali *et al.*, 2010).

Rape and mustard had the highest shoot Cd concentration, with vetch, lucerne and rye having the highest concentration in the roots (Table 4.12). The plants grown in the control soil had lower concentrations of Cd in both the shoots and the roots with respect to the limit. Shoot Pb concentration was highest in vetch, rye and spinach, with rye and vetch having highest concentrations in the roots. Spinach in the control had shoot and root Pb concentrations well above the limit of 20 mg kg^{-1}as well as rye in the roots, while all the other plants were below the limit.

Table 4.12: Cadmium and lead concentrations (mg kg^{-1}) in shoot and root tissue of selected plants grown on polluted and control soils.

	Cd		Pb	
Species	Polluted	Control	Polluted	Control
Shoot				
Indian Mustard	9.0	0.6	43.7	6.8
Lucerne	4.8	0.8	21.2	6.9
Vetch	6.7	0.6	138.0	3.6
Rape	14.4	0.6	56.6	4.6
Rye	3.9	0.8	113.1	7.3
Spinach	4.0	0.9	131.9	88.3
LSD	1.29		23	
Root				
Indian Mustard	4.6	0.6	83.5	9.6
Lucerne	17.6	1.0	169.9	17.2
Vetch	23.3	0.9	265.7	12.7
Rape	3.5	0.4	171.4	13.9
Rye	16.7	1.3	597.7	74.8
Spinach	3.8	0.7	72.4	35.8
LSD	2.06		22.06	

(Maximum limits are 2 and 20 mg kg^{-1} for Cd and Pb, respectively (Source: Vamerali *et al.*, 2010)

Arsenic concentration in plants tissue is shown below in Table 4.13. Arsenic was well below the limit in all the plants grown in both soils, and did not significantly differ between species in both shoot and root As concentrations.

Table 4.13: Arsenic concentrations (mg kg^{-1}) in shoots and root tissue of selected plants grown on polluted and control soils.

	Shoot		Root	
Plant	Contaminated	Control	Contaminated	Control
Indian Mustard	0.57	0.00	0.34	0.00
Lucerne	0.74	0.87	3.00	0.51
Vetch	1.10	0.41	3.03	0.00
Rape	0.83	0.00	2.09	0.00
Rye	0.43	0.67	1.62	1.40
Spinach	0.74	0.00	1.60	0.23
LSD	1.074		1.4347	

Maximum limit is 20 mg kg^{-1}(Source: Vamerali *et al.*, 2010).

4.4.5 Uptake of heavy metals by plants

The uptake level of heavy metals given in Figures 4.1-7 is a product of the dry matter results multiplied by the heavy metal concentration. The uptake of arsenic (Figure 4.1) showed no significant differences between the plants from the polluted soils and those of the control soil. Rape and mustard (from the polluted soil) had the highest shoot Cd uptake while rye had the highest root Cd followed by lucerne and vetch (Figure 4.2). Mustard (from the polluted soil) has the highest shoot Cr uptake and rye has the highest root uptake (Figure 4.3). The shoot Cu uptake (from the polluted soil) in Figure 4.4 was highest in mustard whiles rye had the highest root uptake compared to the other plants. Rape and rye had the highest shoot and root Ni uptake, respectively (from the polluted soil) shown in Figure 4.5. Root Pb uptake in Figure 4.6 was highest in ryeand all the plant species had the same shoot Pb uptake except for lucern (from the polluted soil). On Figure 4.7, mustard, lucern, vetch and rape had highest shoot Zn uptake, and rye had the highest rootuptake (from the polluted soil).

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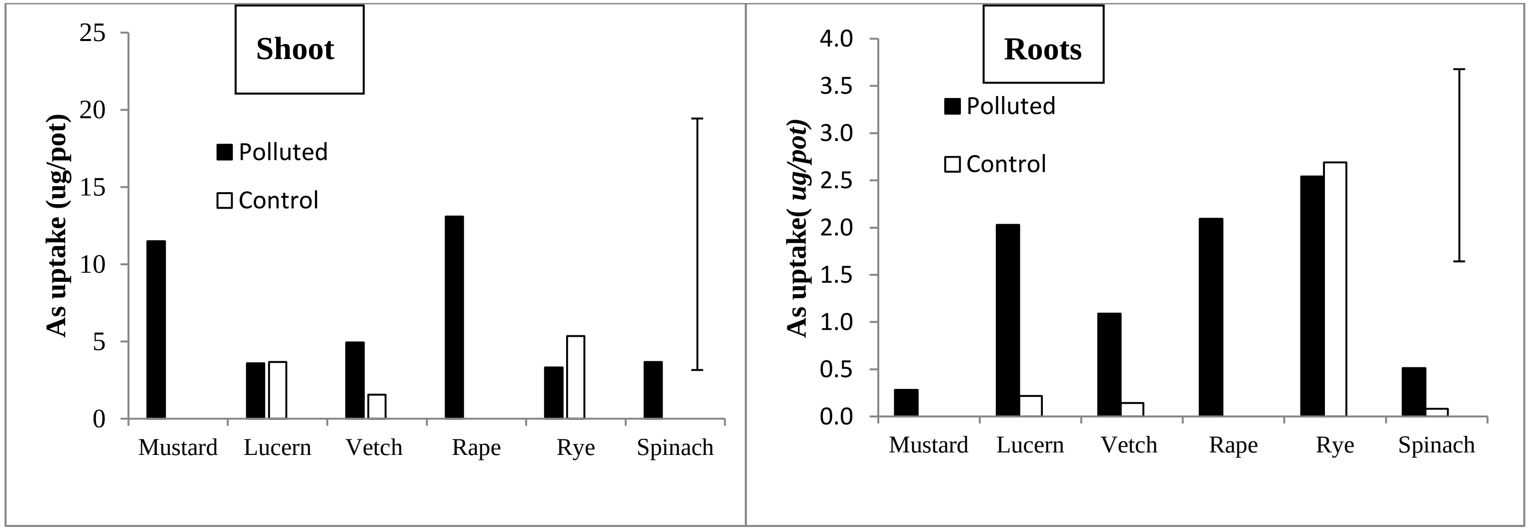

Figure 4.1: Arsenic uptake of plant shoots and roots from polluted and control.

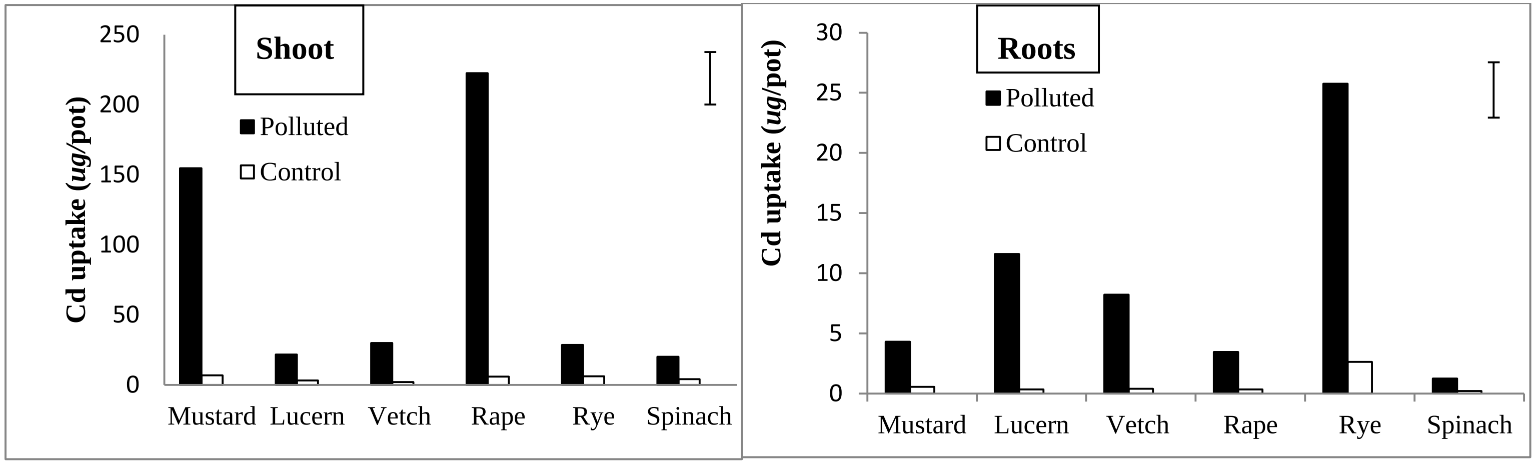

Figure 4.2: Cadmium uptake of plant shoots and roots from polluted and control soil.

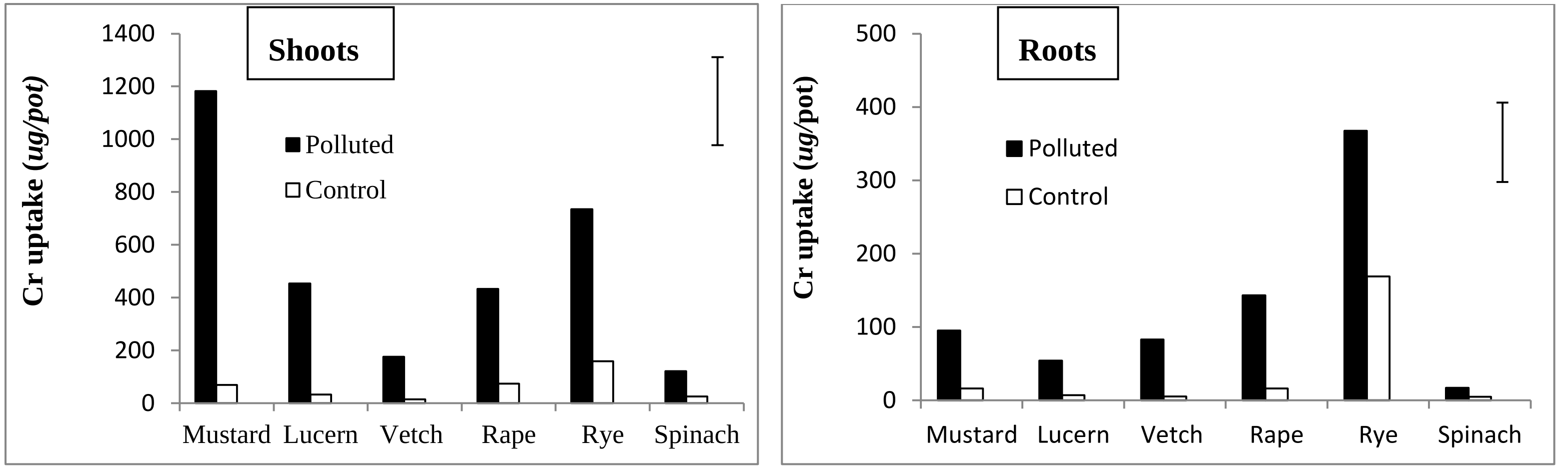

Figure 4.3: Chromium uptake of plant shoots and roots from polluted and control soil.

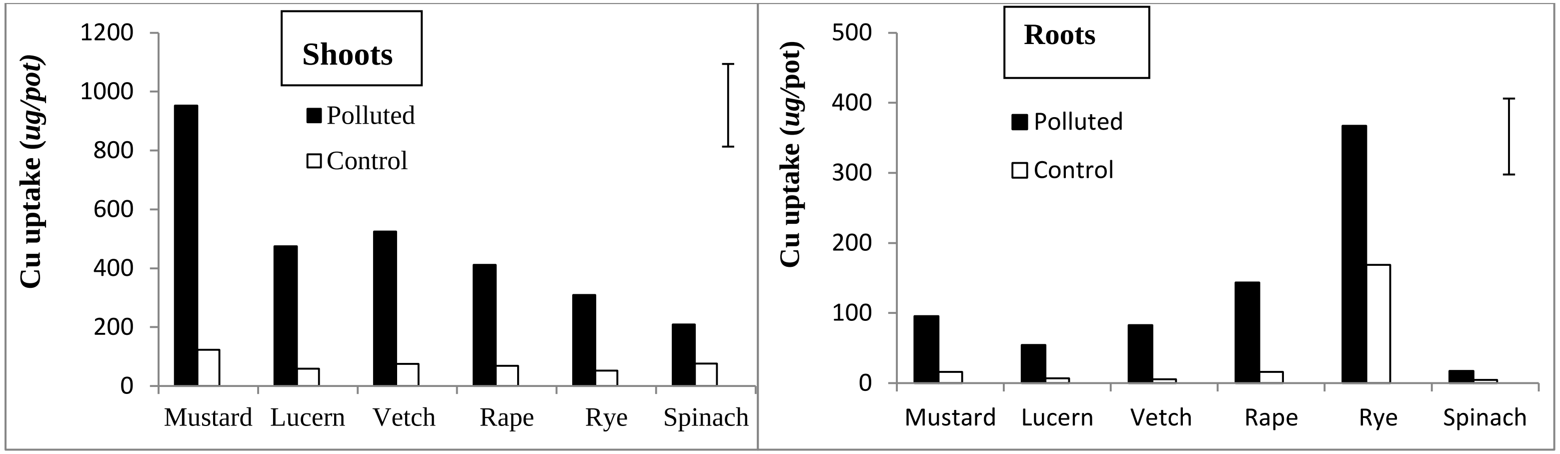

Figure 4.4: Copper uptake by plant shoots and roots from polluted and control soil.

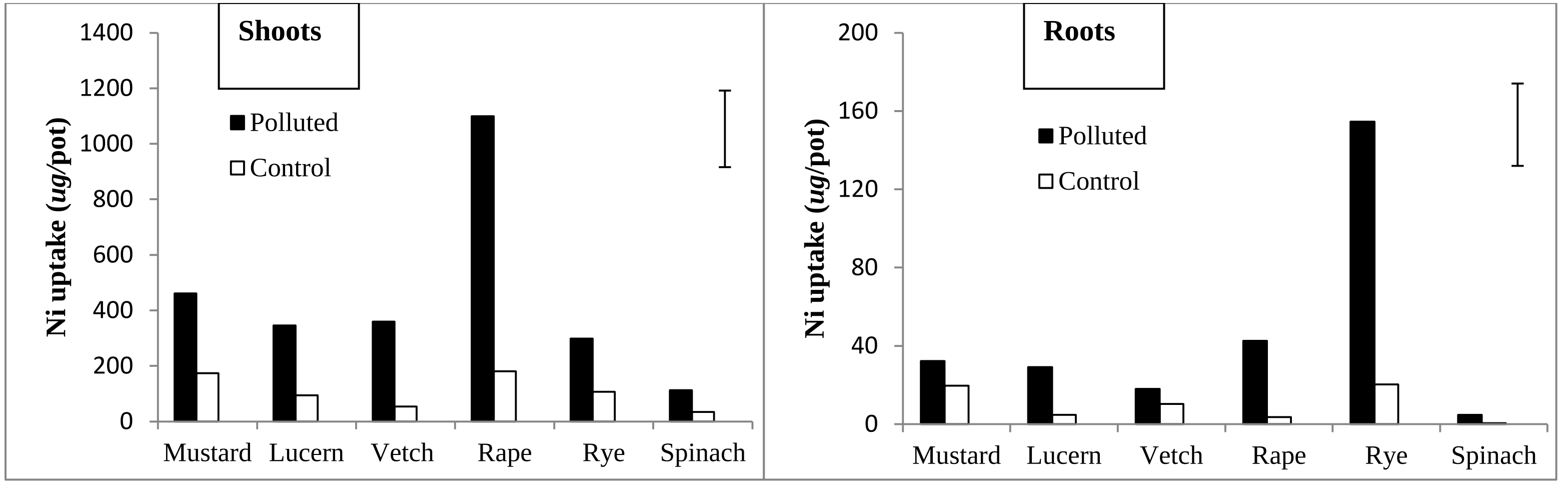

Figure 4.5: Nickel uptake by plant shoots and roots from polluted and control soil.

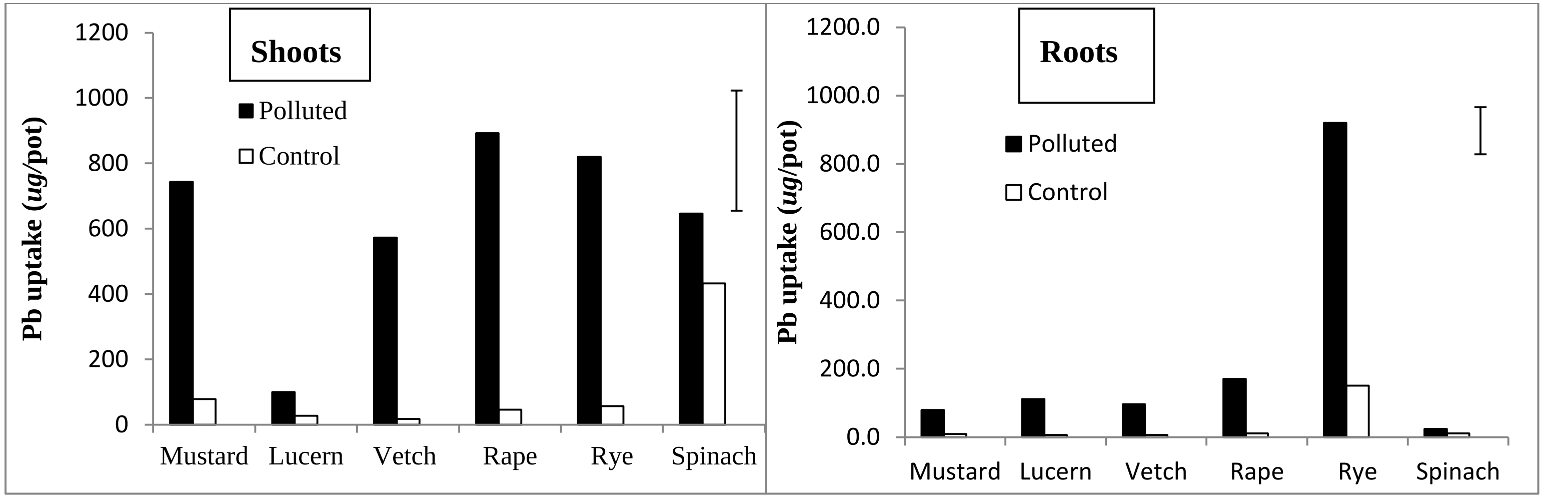

Figure 4.6: Lead uptake by plant shoots and roots from polluted and control soil.

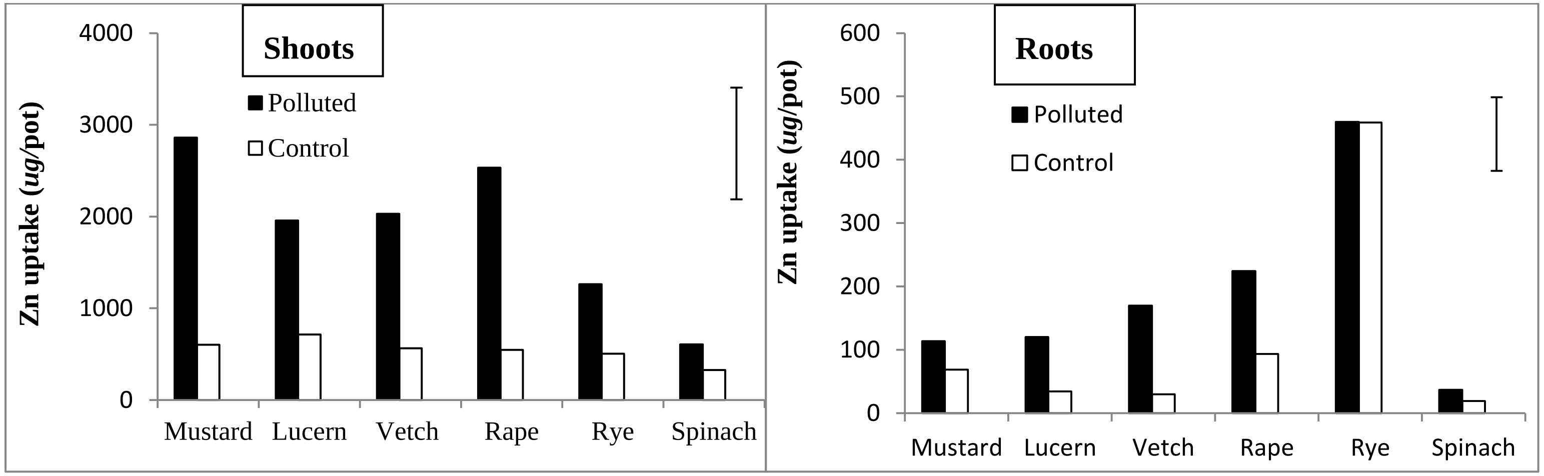

Figure 4.7: Zinc uptake by plant shoots and roots from polluted and control soil.

4.4.6 Effects of EDTA application on heavy metal concentrations in Indian mustard tissue

Concentration of heavy metals in mustard plants grown in the polluted soils with different application rates of EDTA is shown in (Table 4. 14). Concentration of Cd, Pb and Zn increased with increase in application rate of EDTA, Cu increases at the 3 mmol concentration in both shoot and root, while shoot As decreased at 3 mmol concentration but increases in the roots at this same concentration. No symptoms of stress or toxicity were observed in the plants during the first four weeks of growth. However after the application of EDTA (2-3 days) signs of stress were observed in the leaves of all except the 0mmol kg^{-1} pots. Brown spots were observed and yellowing began and increased, eventually at the 10 $mmolkg^{-1}$application rate plants died. The other plants continued to grow until six weeks.

Table 4.1: Heavy metal concentrations (mg kg^{-1}) in shoot and root tissue of mustard grown on polluted soil amended with increasing concentrations of EDTA.

	0 mmol EDTA kg^{-1}	3 mmol EDTA kg^{-1}	6mmol EDTA kg^{-1}	LSD	Limit#
As					
Shoots	0.57	0.00	0.28	1.14	
Roots	0.34	0.60	0.11		20
Cd					
Shoots	9.0	102.6	172.0	41.78	2
Roots	4.5	83.6	174.6		
Cr					
Shoots	69.8	46.3	25.6	45.19	2
Roots	99.2	121.7	78.4		
Cu					
Shoots	56.4	138.7	87.8	52.87	15
Roots	35.1	108.0	29.7		
Ni					
Shoots	27.7	31.2	47.1	19.50	20
Roots	34.1	26.5	19.4		
Pb					
Shoots	43.7	285.9	745.3	46.91	20
Roots	83.5	166.7	251.0		
Zn					
Shoots	169.1	490.6	781.6	74.53	150
Roots	116.4	367.4	176.2		

#Source: Vamerali *et al*., (2010).

CHAPTER 5

DISCUSSION AND CONCLUSION

The elevated level of heavy metals, in soils applied with sewage sludge, compared to the control, was a result of long term application of the sludge. The accumulation of heavy metals in soils applied with sewage sludge has been demonstrated by a number of researchers (Madyiwa *et al.,* 2004; Mapanda *et al*., 2002; Katanda *et al.,* 2007; Bergkvist *et al.,* 2003). This observation was consistent with a survey done by Snyman *et al.* (2004), which showed that 61% and 44 % of sludge's surveyed exceeded the Ni and Zn limits and 35 % exceeded one or two metals. Based on the limits (Herselman and Moodley, 2009), sludge disposal should be ceased at Darvill and a remediation plan be implemented, since Cd, Cr, Pb and Zn have exceeded MPL while As, Cu and Ni had exceeded the respective total trigger values.

The higher organic carbon percentage in the contaminated soil (Table 4.4)has a vital role in the mobility of heavy metals and their bioavailability in the soil for plant uptake. Long term application of sewage sludge to soils has been demonstrated by a number of researchers to increase the organic carbon of the soil by several times the initial percentage depending on the duration of application (Triphathi and Misra, 2012; Katanda *et al.,* 2007; Bergkvist *et al.,* 2003; Mapanda *et al.,* 2004). After application, the sewage sludge is decomposed by microorganisms in the soil resulting in the release of nutrients with higher levels of P, K, Ca, Mg and heavy metals in the contaminated soil compared to the control.

Soil pH is of great importance in the solubility and bioavailability of heavy metals, with the acidic pH of the control enhancing solubility of cationic metals. The higher pH in the contaminated soil is caused by the alkalising effect of sewage sludge, which originates from processing, where pH is deliberately increased to pH 8 for precipitation of most heavy metals.

The observation was consistent with Madyiwa *et al.* (2004), who found that sewage sludge increased the pH of a sandy soil by at least 1 pH unit compared to the control in Zimbabwe.

5.1 Heavy metal concentration in tissue of plants growing on sewage sludge disposal site.

The high levels of metals (above MPL), except Cu and Hg, in all the four plants growing on the sewage disposal land could be a health hazard to the people consuming them. The elevated levels of heavy metals in plants growing on sewage sludge applied soils agreed with findings by Tripathi and Misra (2012), who reported that sewage sludge disposal resulted in heavy metal pollution of soil and their accumulation in plant species, particularly *Nepeta hindostana,* which accumulated 29 ppm Pb (96 times the limit),26 ppm Cu and 56 ppm Cd (280 times the limit).Unlike *N. hindostana*, three of the plants examined in this study namely amaranthus, tomato and *S. nigrum* are edible and consumption of amaranthus and *S. nigrum* leaves and tomato fruits can cause a disruption to the normal functioning of enzymes, kidney and liver failure among other illnesses, in animals and humans (Oliver, 1997).

The heavy metal concentrations of amaranthus, *Solanum nigrum* and tomato plant in this study were consistent with those found by Mdlambuzi (2014) on the same site and Mellem (2009) at Northern Waste Water Works in Durban. Mdlambuzi (2014) found whole plant of amaranthus contained 207-368 mg Zn kg^{-1}, 126-288 mg Cr kg^{-1}, 201-372 mg Cu kg^{-1}, 9-12 mg Ni kg^{-1}, 0.9-1.2 mg Cd kg^{-1}, 0.3-1.1 mg Hg kg^{-1} and 9-12 mg Pb kg^{-1}. Mellem (2009) found that whole plants of *Amaranthus dubius* growing on a site irrigated with sewage sludge contained53 mg Cr kg^{-1}, 64 mg Cu kg^{-1}, 27mgNi kg^{-1}, 7.8 mg Hg kg^{-1}, 26 mg Pb kg^{-1} and 3.9 mg As kg^{-1}. In *S. nigrum,* the concentrations of Cr, Cu and Zn were lower whereas those of Cd, Hg, Ni and Pb were higher than in the study by Mdlambuzi (2014), with whole plants. Tomato was found to have the following concentration of heavy metals in the whole plant;

Zn (87.3-395 mg kg^{-1}), Cr (11-145 mg kg^{-1}), Cu (71-198 mg kg^{-1}), Ni (2.3-17.4 mg kg^{-1}), Cd (0.3-1.5mg kg^{-1}), Hg (0.6-1.0mg kg^{-1}) and Pb (10-17 mg kg^{-1}). Tomato shoots in this study had lower Zn, Pb, Cu and Cr and higher Cd, Hg and Ni than in Mdlambuzi's study.

The results of tissue heavy metal concentrations in all the plants could be explained by the low soil pH (4.1-5.8), high organic matter and high concentrations in the soil, with Cd, Cr, Pb and Zn approaching or greater than the MPL while As, Cu, Ni and Hg were below the MPL but higher than the control soil (Mdlambuzi, 2014).The low pH contributes greatly in increased bioavailability of the heavy metal to plants for uptake (Wuana and Okieiman, 2012). Cationic heavy metals, like $Cd^{+2}$are more soluble at low pH and therefore more available to plants for uptake, and as pH increases to neutral or alkaline they are less soluble (Wuana and Okieiman, 2012).

Amaranthus plant poses the greatest risk for the introduction of these metals to the food chain as it has the highest demand and it is the most consumed by the locals. The high concentrations with respect to the permissible limits of As (0.8 mg kg^{-1}), Cd (0.8 mg kg^{-1}), Cr (37.7 mg kg^{-1}), Pb (0.3 mg kg^{-1}) and Zn (69.1 mg kg^{-1}) in amaranthus leaves sold at the market (harvested at the study site) means that the local people are at risk of accumulating these metals. Concentration of heavy metals (Cd, Cr, Cu, Zn, Ni, As and Pb) in amaranthus leaves (harvested from the study area and sold) were within the same range with those in shoots of amaranthus sampled from the different transects in the study area. Amaranthus leaves harvested elsewhere and sold at the same market could pose a lesser threat than from the study site, except that they contained greater Cr, and this supported the view that the metals originated from long-term application of sewage sludge (Katanda *et al.,* 2007; Bergkvist *et al.,* 2003).

The distribution of heavy metals between the shoots and roots of amaranthus plant from the study area varies from one metal to another. Arsenic distribution in all the plants (rumex, amaranthus, tomato and *Solanum nigrum*, Table 4.1) is the same in both the roots and the shoots. According to Ali *et al.* (2009) there is great variability between species in terms of As sensitivity and transportation, but generally in most plants only a fraction of the arsenic is translocated to shoot tissue with most found in the roots. However, this was contrary to As hyper-accumulaters like *Pteris vittata* (Chinese brake fern), where the majority of As is deposited into the shoot. The low As concentration in shoots, with respect to the permissible limit of 0.2 mg kg^{-1},suggested that not much As is transferred to the shoots and minimizes the risk of As toxicity to the people who feed on amaranthus, tomato and *S. nigrum*. The concentration of As in the roots will contribute in the retention of the metal in the root which minimizes metal leaching to contaminate ground water.

The similar Cd concentration in amaranthus roots and shoots was consistent with the findings of Sola *et al.* (2003), who found that Cd concentration in the roots, stems and leaves of amaranthus grown on designated refuse landfill soils in Ibadan Nigeria, showed no specific trend of accumulation of Pb and Cd. The same observations were made for rumex, tomato and *S. nigrum* in this study. The different growth stages of the plants at the time of sampling could also explain the differences in Cd concentration in the shoots and roots, and thiswas supported by the findings of Lopez-Millan *et al*. (2009) on the Cd toxicity in tomato plant grown in hydroponics, with increasing Cd concentrations. Elevated concentrations of Cd in the edible parts of tomato, *S. nigrum* and amaranthus could result in Cd accumulation in the kidney and livers resulting in hypertension and cardiovascular diseases, together with causing renal damage and osteoporosis in humans (Jarup *et al.*, 1998).

The higher concentration of Cr (amaranthus, rumex, and *S. nigrum*) in the roots compared to the shoots could be because Cr is immobilized in the vacuoles of the root cells, thereby rendering it less toxic, which is a natural toxicity response of plants (Hossner *et al.,* 1998). This is supported by the findings of Mangebeira *et al.* (2006),who reported greatest Cr concentration in the roots and progressively less in the stems and leaves of plants, which demonstrated the poor mobility of Cr. Chromium is a non-essential element to plants and because of this plants do not possess specific mechanisms for its uptake (Shanker *et al.,* 2005). Although more Cr is found in the roots, all the plants had Cr concentrations that were at least 13 times the permissible limit of 2.3 mg kg^{-1}in the shoots. Whereas Cr is known to enhance the action of insulin which is critical to the metabolism and storage of carbohydrates, fat and protein in animals and humans, elevated levels have been found to cause cancer to the lungs, gastrointestinal system and central nervous systems (Costa and Klein, 2006). The less toxic Cr (III) is the expected form of Cr found in the plantsbecause of the acidic conditions and high organic matter and Fe (II), in soil solution, which result in easy reduction of the toxic Cr (VI) to Cr (III) (Shanker *et al.,* 2005; Hossner *et al.,* 1998)

Nickel concentration in all the plants (rumex, amaranthus, tomato and *Solanum nigrum*)is the same in both the shoots and the roots. According to Seregin and Kozhevnikova (2006), Ni unlike other metals that do not form part of plant enzymes, is a constituent of urease and is essential (an element is considered essential when plants cannot complete their life cycle in its absence and it cannot be replaced by any other element) in small quantities for some plants. They indicate that its distribution in plants is not uniform as it largely depends on the growth stage of the plant. It is taken up by the plant via passive diffusion and active transport, after it binds with ligands in the soil like carboxylate ion, imidazole, sulfhydryl group, and aliphatic amine are the most important. The high accumulation of Ni in the shoot with regard to the MPL of 30 mg kg^{-1}, poses a risk to the people who would consume the plants, as just like other heavy metals it still remains toxic at high concentrations. This however has positive implications with regard to phytoremediation as this means the plant shoots can be easily removed.

Similar to Ni, Zn is also a heavy metal that is essential in plants, it is in fact a constituent of numerous enzymes than Ni (Seregin and Kozhevnikova, 2006). It is for this reason that we find its concentration in the shoots greater than the MPL of 60 mg kg^{-1} for most of the plants.

Lead is mostly concentrated in the roots for most of the plants, the only exception being tomato. However all the plants have exceeded the MPL and will pose a risk if consumed. The high concentration of lead in the roots compared to the shoots could pose a challenge in phytoremediation with regards to harvesting the plants. However its retention in the roots could prevent it from leaching down and contaminate ground water. Unlike Ni and Zn, lead (Pb) is not known to have any biological function in organisms including plants. According to Pourrut *et al.*. (2011) plants have a number of defensive mechanisms of coping with lead toxicity, most of which involve its sequestration in the roots by complex formation, binding of lead by glutathione, phytochelatins, and amino acids.

Copper and mercury present the least toxicity to plants and subsequently to humans and animals feeding on the edible parts of the plants, because none of the plants accumulated the metals above the limit in their shoots. The higher Cu concentration in amaranthus shoots than the roots was consistent with the findings of Rahma *et al.* (2013) and Ziaratio and Alaedini (2014), with 200 amaranthus species. While the accumulation of the heavy metals in amaranthus, rumex, *S. nigrum* and tomato may pose a risk to human and animal health, these plants may have potential to remediate the pollution as they can take significant quantities of the metals. Their effectiveness could lie in the ability to grow these plants for high biomass production to maximise uptake. However, other plants, like turf-grass, that have other value and are not edible could be more ideal.

The higher concentration of all heavy metals in turf grass than all the four plants harvested from the study area, suggested that turf grass accumulates more metals without being negatively affected, indicating a greater potential for removal of heavy metals from the site. The ability of turf grass to accumulate heavy metals in its tissues was also demonstrated by Onder *et al.* (2007) and Qu *et al.* (2003).Duo *et al.* (2010) reported that turf-grass grown on municipal solid waste compost, following the application of EDTA, took up 24 ppm Ni, 218 ppm Cu, 2015 ppm Zn, 1.74 ppm Cd and 81 ppm Pb. The concentrations of Cd and Ni were higher, and those of Pb, Zn and Cu were significantly lower, in this study than the findings by Duo *et al.* (2010).

From a phytoremediation perspective, findings indicate that turf grass has the greatest potential of all the plants examined in this study. This is because it was able to accumulate a number of the heavy metals (Cr, Cu, Ni, Pb and Zn) far above the normal toxicity threshold (Bempah *et al.,* 2012; Vamerali *et al*., 2010). While none of the plants were able to accumulate metals at a rate to be classified as a hyperaccumulator, the characteristics of fast growth and strong regeneration capacity of turf grass allowing it to be mowed many times in 1 year makes it highly suitable for phytoremdediation. However, where the sale of turf-grass for instant lawn is practiced, large quantities of the heavy metals could be transferred to other sites as part of the tissue and soil associated with the root system.

The 211×, 5×, 11×, 2 × and 3 times the toxicity threshold limit of Cr, Cu, Ni, Pb and Zn, respectively, suggested that such large concentrations are transferred to new sites that include residential areas, golf courses and other sites. In addition to the metals transferred as part of plant tissue, the high levels of Cd and Cr in the soil attached to the turf grass roots also pose a threat of cross contamination to the soils where the turf grass, purchased from the study site, is used as instant lawn. The level of pollution off-site may largely depend on the size of the land and the frequency of replacement of the lawn. Small properties receiving turf grass on a yearly basis would be in greater risk of accumulating these metals in their soil than sport fields or recreation grounds with large area.

5.2 Heavy metal concentrations in tissue of plant species grown on polluted soil after long-term application of sewage sludge.

The low concentration of arsenic in all the plants with regard to its toxicity threshold limit of 20 mg kg^{-1}(Vamerali *et al.*,2010) can be attributed to the fact that none of the plants have been previously shown to inherently accumulate arsenic specifically at elevated concentrations. The similar concentrations in plants between plants grown in polluted than the control soil can be attributed to similar soil As concentrations which were both between the TTV and MPL level (Herselman and Moodley, 2009). This implies that the control soil may not the best reference with regards to As, possibly due to an As rich parent material or to other anthropogenic activities apart from sludge application.

The higher concentration of Pb in roots compared to shoots of the plants was consistent with Koeppe (1981),Sharma and Dubey (2005) and Tangahu *et al.* (2011) who observed that larger amounts of Pb are bound to the roots of most plants exposed to Pb-contaminated media than the above-ground. The Pb in the soil would be immobilized through adsorption and accumulation in plant roots or precipitation within the root zone preventing its migration in soil as well as movement by erosion (Tangahu *et al*., 2011). The higher translocation of Pb (shoots>roots) in the spinach from both the polluted and control soil was an exception, and this was supported by work done by Singh *et al.*. (2012), where, of all vegetables tested, spinach accumulated more metals in the leaves (67% of total) than the roots. This is however contrary to the findings of Alia *et al.* (2015), reported that spinach exposed to different Pb treatments had higher Pb concentration in the roots than the shoots, and the difference could be because of using artificially polluted soil to simulate real life contamination, compared to actual pollution.

Cadmium concentration is higher in the plants grown on polluted soil compared to the control. This is caused by the significantly higher cadmium concentration in the polluted soil which is greater than the MPL of 5 mg kg^{-1}. Rape had the highest concentration of cadmium in the shoots followed by Indian mustard. Both these plants are in the *Brassicaceae* family. According to Babula *et al.*. (2012) the *Brassicaceae* family is well known in accumulating a wide range of heavy metals especially cadmium and zinc. In the roots vetch had the highest concentration of Cd followed by lucerne and rye. The high levels of Cd in the two legumes can be attributed to their high activity in the roots, where symbiotic relationship exists with some micro-organisms in fixation of nitrogen. These interactions in the roots alter the rhizosphere environment such that the pH of the soil is reduced and some elements are solubilized making them more available for uptake e.g phosphorous (Liu *et al.*, 2012). This alteration of the rhizosphere environment is such that Liu *et al*., (2012) demonstrated that co-cultivation with legumes increased Cd accumulation in adjacent crops. Maize co-planted with legumes accumulated more Cd in its leaf, stem and roots. Cadmium concentration in grains of a wheat crop was also found to be highest when grown immediately after a crop rotation cycle with lupins planted before (Mench, 1998).

Chromium concentration concentration in both the polluted and control plants is above its MPL of 2 mg kg^{-1}. This poses a risk such plants were to be consumed, however it has positive implications with regards to phytoremediation. Lucerne and rye are the most promising contenders with the highest concentration accumulated. Vetch and rye have the highest concentration in the roots and would be the most effective in the retention of the metals in the root zone thereby preventing their leaching to ground water. Copper, Ni and Zn concentration for the polluted plants is above the limit, and like Cd, Pb and Cr if the shoots are consumed they will have adverse effects, they however present a good opportunity for phytoremediation. Nickel and Zn, unlike Cu are needed in plant metabolism as they are constituents of some enzymes, which will explain their accumulation in the shoots. Vetch and lucerne showed the highest concentration of Zn and Cu in the shoots meaning that they could be considered in phytoremediation strategies involving the two metals.

The importance of high biomass in phytoremediation as stated by a number of researcher's like Sun *et al*. (2011) can be seen in the high uptake of metals in Figures 4.2-4.7 by mustard in the shoots compared to the other plants (polluted). Mustard did not accumulate the highest concentration of Cd, Cr, Cu and Zn, but its superior dry matter resulted in it having taken up the most of these heavy metals (Cd was the same with rape and Zn was the same with lucerne, vetch and rape). The same can be seen with rye in the roots, its superior dry matter in the roots compared to other plants resulted in it having a high uptake for most of the heavy metals (Cd, Cr, Cu, Ni, Zn and Pb). This observation reinforces the need for any phytoremediation strategy to include high yielding biomass plants for its effectiveness.

The increase of lead concentration with increase in EDTA concentration is consistent with the results obtained by Wu *et al*. (2004), which showed a significant increase in Pb concentration with increase in EDTA concentration. While the results of this study showed higher tissue Zn and Cd with increase in EDTA, Wu *et al*. (2004) did not observe similar trend. The increase in Zn concentration can be supported by the findings of Ebbs and Kochian (1998), where after EDTA application Zn concentration in barley, Indian mustard and oat increased significantly, and of Marques *et al*. (2009) in that EDTA application increased the concentration of Zn up to 231%, 93%and 81% in leaves stems and the roots of *S. nigrum*. Cadmium accumulation at mature stages in plants is also said to be boosted by EDTA application according to Farid *et al.* (2013). The ability of EDTA to induce the uptake of metals in plants can be explained by co-ordination chemistry, where metals bind with ligands in aqueous solutions. These ligands are ions or compounds that have active lone pairs of elections in the outer energy level, used to form co-ordination bond with the metal ion. A few of these ligands have the ability to form more than one bond to a single metal atom. They hold the metal so strongly such that they effectively remove the metal and prevent them from reacting with any other substance. These are described as polydentate, which means ‘many teethed’ (Skoog *et al*., 2013; Oxtoby *et al.,* 2015). EDTA is known as a hexadentate ligand as it has six pairs of unbounded electrons. Molecules that bind at multiple points tend to be more thermodynamically stable and they displace monodentates in co-ordination complexes in solution, thereby increasing their uptake. This is in part due to the spontaneity of entropy-favouring reactions and their frequent occurrence (Rahman *et al.,* 2003).

All the plants treated with 10 mmol kg^{-1} wilted and died a week after EDTA application, indicating that excess concentrations of salts are harmful to plants. The harm caused by EDTA could have been both an osmotic influence and metal toxicity, as wilting and reduction in size were observed including brown spots and yellowing of leaves which are signs of disruptions in the normal functioning of enzymes in plant which can be caused by high concentrations of heavy metals (Cheng, 2003).Cells in the roots of plants normally have a greater concentration of solutes (organic compounds and sugars found in cells) than the surrounding soil solution and water flows as a result of this concentration gradient from the soil through the partially-permeable cell membranes into the root cells, and water is taken up by the plant. As the salt concentration in the soil rises, this difference is reduced and water does not move as freely into the plant resulting in stress (Brady and Weil, 2008). Chelating agents like EDTA have been demonstrated to improve the uptake of metals like lead cadmium and zinc, and at certain levels of concentration these can greatly affect the growth of plants (Ghani, 2010). The reduction in dry matter reduces the potential uptake of the metals.

Of all the plants in this study, turf grass grown in Darvill was found to concentrate substantial amounts of As, Cr, Cu, Ni and Zn. This is attributed to the extensive root system of the turf which is able to explorea larger surface area for adsorption/absorption of the heavy metals into the turf compared to other plants. Because of this the turf grass is the most suitable candidate for any phytoremediation strategy to be implemented in Darvill. EDTA can also be tested with turf grass to find out if it can have the same effects that were shown in the mustard experiment. Heavy metal enhancement following EDTA application can reduce the time needed to remediate a contaminated soil by more than half the time. Handling of heavy metal laden plants after remediation and the possibly leaching of metals following EDTA application in soil still remain a challenge in phytoremediation (Sas-Nowosielska *et al*., 2004).

The uptake levels of the plants are relatively low with respect to how much heavy metals are in the soil. This is because most plants were not able to emulate some of their highest recorded heavy metal concentrations in this study. For example rye grass has been shown to accumulate up to 500 mg of Zn kg^{-1},2450 mg Cr kg^{-1}, 318 mg Pb kg^{-1}yet it could only take up

167, 98 and 113 mg $kg^{-1}$of Zn, Cr and Pb in its shoots. Another plant that greatly under performed in this study is Indian mustard. It has been extensively used to extract toxic metals from contaminated soils worldwide, including Cd, Cr, ^{137}Cs, Cu, Ni, Pb, U and Zn (Kumar *et al*., 1995; Blaylock *et al*., 1997; Weerakoon and Somaratne, 2003; Zhu *et al*., 1999). Even with the addition of EDTA heavy metal levels did not increase to the levels showed by other researchers. This is largely due to the limited availability of the metals in the soil for uptake by the roots of plants as shown in the results of the DTPA extractiion.

5.3 Conclusion

Indigenous and exotic plants that grew on the heavy metal polluted soils, particularly amaranthus, had tissue Cr, Zn, Ni, Cd, Pb concentration higher than the limits.

Turf-grass tissue had 211, 5, 11, 2 and 3 times the toxicity threshold limit of Cr, Cu, Ni, Pb and Zn, respectively, while the root associated soil had Cr and Cd at the MPL.

The concentrations of most metals in the shoots of species tested in the pot trial were well above the toxicity threshold and pose a threat to consumers of the vegetables among the plants. Lucerne and vetch have shown the most promise in multiple heavy metal uptake, between them they had the highest concentration of Zn, Cu, Ni and Pb. The *Brassica* species, mustard and rape, had the greatest drymatter and therefore, the greatest uptake of most of the metals studied.

The distribution of the metals between the roots and the shoots of the plants is vastly different, As was highly concentrated in the roots of tomato while all the other plants had the same concentration. Cadmium and Ni concentration was the same in all the plants. Chromium had higher concentrations in the roots for rumex, amaranthus and *S. nigrum*, while tomato had the same concentration in both the roots and the shoot. Copper had higher concentration in the shoot and all the other plants had similar concentrations. *Solanum nigrum* had higher Hg concentration in the shoot and all the other plants had the same concentration. Lead was mostly distributed in the roots for most of the plants except amaranthus. Zinc had the same concentration in both plants tissues except for *S. nigrum* where it had higher concentrations in the roots.

Addition of increasing concentrations of EDTA to soil increased mustard tissue metal concentration particularly for Pb, Cd and Zn but decreased drymatter yield with the highest concentration (10 mmol of EDTA) killing the plants.

RECOMMENDATIONS

Indigenous vegetables, especially amaranthus, growing at the polluted sites pose a health risk to humans who consume them. There is need for better controls to access to the area and to educate the local people on the risks associated with such consumption.

Mustard, lucerne, vetch and rape have shown potential for removal of large amounts of heavy metals, especially when EDTA had been added to the soil (mustard only tested). However, this effectiveness needs to be tested under field conditions and the use of these plants needs serious controls as their consumption could be a disaster to human health.

The high concentrations of heavy metals in turf grass can be used to phyto-remediate the heavy metal contamination in the soils. More work should include testing the effects of adding EDTA on metal uptake by turf-grass, while taking into consideration the management of the plant material to minimise off-site pollution.

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