Vegetable farms in Cape Town: water quality and possible remediation techniques.

By

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Declaration

I declare that: “Vegetable farms in Cape Town: water quality and possible remediation techniques” is my own work, that it has not been submitted before for any degree or examination in any other university, and all the sources I have used or quoted have been indicated and acknowledged as complete references.

Annamarie Guinnevere Martin

March 2013

Signed . . . . . .

Keywords

Cadmium

Lead

EDTA

Turnip

Spinach

Irrigation water

Mitigation

Heavy metals

Accumulation

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Abstract

Vegetable farms in Cape Town: water quality and possible remediation techniques

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MSc thesis, Department of Biodiversity and Conservation Biology, University of the Western Cape

Heavy metal contamination tends to be a problem in inner city agricultural areas and gardens. High levels of certain heavy metals have been found in the soil and vegetables in the Cape Town Metropolitan area. The aim of this project was twofold. Firstly to ascertain whether water (ground or surface) was responsible for the heavy metal problem found in vegetables in the Philippi and Kraaifontein-Joostenbergvlakte farming areas in Cape Town; and secondly to evaluate the efficacy of two possible remediation methods, namely chelation (with EDTA) and precipitation (using phosphate), aimed at tackling the problem. In order to achieve this a water survey and greenhouse experiment were conducted. The water survey involved collecting a number of samples; both from surface dams and boreholes, from the two farming areas. Results showed minimal heavy metals in both, and therefore ruled this out as the source of the heavy metal problem. The greenhouse sand culture experiment tested the effects of the two remediation methods on the growth, development and elemental content of turnip and spinach plants treated with two cadmium and lead concentrations. In summary, Cd reduced growth more than Pb; Cd accumulated in roots and leaves, and Pb in roots. Several treatments, both metal and mitigation

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enhanced the chlorophyll content. The difference between the EDTA and phosphate mitigation treatments were not significant in the case of cadmium but in the case of lead, high phosphate resulted in increased growth. The large variation of results in this study, and indeed those of the available literature, indicate that the remedial treatments investigated here are not necessarily the most effective and that other treatments should be investigated to control the uptake of either cadmium or lead, as agricultural soils in future become more contaminated with either or both of these heavy metals.

November in the year of Our Lord MMXII

Maker of the Universe and of me, I am grateful for the ability and preservation to complete this, and the people given me along the way who made it possible,

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Chapter 1: The nature of heavy metal accumulation in the environment

1.1 Sources of heavy metals

1.1.1 General

Heavy metals are naturally occurring persistent elements which cannot be removed from the environment. They occur at varying levels depending on the parent rock from which they originate; they are increased exponentially by a range of anthropogenic activities, improper management of metal industrial wastes and mining wastes (Thawornchaisit and Polprasert, 2009) which then have a number of outlets and pathways through which these metals reach the soils where they become bound (Greger, 1999). Cd and Pb are toxic heavy metals and continuous exposure to even the most minute quantities may lead to health problems (Teemu *et al.*, 2008) with food and water being the primary sources of exposure to non-smokers.

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Earlier research has placed emphasis on physicochemical indicators of soil quality. Biological indicators such as enzyme activities, microbial biomass, basal and substrate induced respiration, mineralizable N, structural and functional biodiversity, and so on, are becoming increasingly used due to their being more sensitive to changes in the soil as well as to their capacity to provide information that integrates many environmental factors (Epelde *et al.*, 2008). Contamination by heavy metals can affect soil ecology, quality and productivity in agriculture, which both contaminate water and affect human health (Thawornchaisit and Polprasert, 2009). Soils may be contaminated with Cd due to disposal of municipal and industrial wastes, irrigation with sewage effluent, application of phosphorus fertilizers, and atmospheric

deposition. Cadmium is then taken up into the food chain by plants (Yang *et al.*, 2009; Teemu *et al.*, 2008).

Soil remediation may be necessary to avoid exposure of plants, humans and livestock to heavy metals. Soil contaminated with Zn, Ni and Cu caused by mine wastes is also known to be phytotoxic to sensitive plants and the greatest threat to human health is that of Pb contamination which can cause seizures, mental retardation and behavioral disorders (Prasad, 1999). Lead forms strong bonds with soil components once its introduced to the soil environment and is generally retained in the surface soil layer, therefore the adverse effects on both plants and the humans who consume them persist for extended periods.

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The mobility of metals within soils is dependent on absorption, which is in turn affected by speciation, pH, ionic strength, composition of the soil solution and the clay and organic matter content (Ross, 1994). Soils consist mainly of inorganic clay minerals and organic substances, and due to the hydroxyl groups and oxygen electron pairs in the clay, and the carboxyl and phenolic groups in organic substances, the soil colloids are negatively charged and the positively charged metal ions are attracted to them. The same is true for water where metals also bind to the negatively charged small molecules (Greger, 1999).

Low pH increases metal availability. The H^+ ion has a higher affinity for the colloids than the metal ions, they then compete for these sites and this releases the metals. High organic content immobilises metals while high cation exchange capacity (CEC) increases the possibility of binding to negative charges. A rule of thumb is that the

higher the clay/organic material content of the soils and the pH, the tighter bound the metals in the soil (Greger, 1999).

Metals like Zn, Cu, Pb and Ni are strongly absorbed by a wide variety of soils, a ‘time bomb effect’ is also proposed by earlier literature where plant uptake and leaching of heavy metals would increase over time due to the decomposition of organic matter. This may lead to phytotoxic effects, transfer into the food chain, and then possibly contamination of the groundwater (Gove *et al.,* 2001).

The problem of trace elements in groundwater has become one of major concern over the past few years, as the amount of surface water per person available on earth decreases rapidly. Any element, especially a heavy metal that threatens the quality of the groundwater supply, is now of major concern, as this is fast becoming our primary source of water. Groundwater is a major source of irrigation waters and this has resulted in an increased heavy metal content of our crops. The steady increase in the inputs of Ag, Cd, Cr, Cu, Hg, Pb, Sb, Sn and Zn in waters over the past century has been accredited to an increase in anthropogenic activities and are now found in amounts that cannot through natural processes be safely disposed of (Wilson, 1979). South Africa’s water resources need to be adequately managed, in both quantity and quality, as it has been predicted that the demand will outweigh the supply by the year 2025 (Oberholster *et al.,* 2008).

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The heavy metal content of crops may also be affected by other factors such as the application of fertilizers, sewage sludge or irrigation with wastewater (Chary *et al.,* 2008).

The factors influencing the solubility and plant availability of metals include the leaching rate, pH, cation exchange capacity (CEC), redox potential, soil texture, clay content, and amount of organic matter present in the soil. Soil temperature has also been postulated as a factor explaining varying metal accumulation in crops (Greger, 1999).

Growth is often used as a measure of the effects of various stressors on plants; any changes in growth are normally the most direct and visible signs of a plant under stress, and the organs that have first contact with any toxic substances, normally the roots, display rapid responses in their growth patterns. Three categories of plants are most frequently studied under trace metals; crops of commercial interest grown on nutrient deficient or polluted soils; metallophytes - plants with evolved metal resistance, and trees (Prasad, 1999). This study falls into the first of the three, and concentrated on Cd and Pb stress.

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Cadmium; a pollutant whose sources include fertilizers, sewage sludge and industrial emissions, has been emitted into the environment for decades. Cadmium is a toxin with no known physiological function in plants and toxicity includes necrosis, wilting and stunted growth. Previous studies that have addressed the effect of Cd on the growth of crops have given the following results. The roots of various monocot cereal plants grew even when exposed to 60 µM Cd, with only slight growth reductions being recorded. Root growth in dicots, however, was severely affected by Cd concentrations of 10-30 µM. Cd stress also induced the sequestration of a Cd-binding complex containing phytochelatin into the roots of most cereals, which has been hypothesised as the reason for the resistance of monocot roots to Cd (Prasad, 1999).

Lead contamination of soils, and subsequently vegetables, is a most serious health threat to humans, so it was deemed important for this study. Exposure can occur through multiple pathways, including inhalation, ingestion in food, water, soil or dust. Pb fortunately occurs as a soil precipitate, a less bioavailable form (Prasad, 1999). Pb has limited solubility in soil environment due to complexation with various organic and inorganic soil colloids, sorption on oxides and clays, and precipitation as carbonates, hydroxides and phosphates. The mobilized or soluble fraction of Pb is often very low, typically less than 1%, even though total soil Pb concentrations are high in many contaminated sites (Sakar *et al.,* 2008).

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The toxicity of Pb is often hidden in plants due to the fact that accumulator plants can absorb large amounts of Pb without showing any visible symptoms of Pb toxicity. Lead has been shown to accumulate in plants from several sources including soil but the reports on accumulation of the Pb within plants are variable, with large differences in Pb deposition being reported in different plant species. On leaf surfaces, Pb deposition is known to cause adverse physiological effects, either by blocking the stomata or by disrupting metabolic pathways after entering into the leaf (Gopal and Rizvi, 2008).

The cleanup of Pb contaminated soils requires the use of various techniques, among others phytoremediation, i.e., the use of plants for environmental remediation is an emerging cleanup technology for metal contaminated sites. The success of Pb phytoextraction however depends primarily on the phytoavailability of Pb as it must be in either soluble or exchangeable form for plant uptake to occur (Kayser *et al.,*

2000). The term ‘mobilised Pb’ refers to the soluble and exchangeable forms released from the soil solid phase to soil pore water in the presence of chelating agents.

Plants have the ability to only extract soluble or free forms of Pb, therefore mobilized Pb fraction is a limiting factor for Pb phytoremediation (Lasat, 2002). Increasing soluble and exchangeable Pb concentrations in the soil solution are key factors in phytoremediation of Pb-contaminated soils. Recent research has focused on artificially inducing Pb desorption from complex soil matrices to enhance Pb phytoextraction (Kayser *et al.,* 2000).

Although much information is available on the effects of Pb on leaf growth, it can also affect the leaves via changes in other morphological parameters like leaf length and width as well as thickness of leaf blade, cuticle, spongy and palisade mesophyll cells, and changes in shape, number and size of stomata. Photosynthesis is considered as one of the most sensitive metabolic processes to Pb toxicity and various studies have reported inhibition of photosynthesis in different plants grown under Pb stress. The Pb-induced reduction in the photosynthesis may also be due in part to stomatal closure, damage to chloroplast ultrastructural organization, alteration in the metabolites of photosynthesis, replacement of ions like Mg and Mn by Pb in the chloroplast and inhibited synthesis or degradation of the photosynthetic pigments (Prasad *et al.,* 2001).

Remediation is a general term with a number of techniques falling under the umbrella.

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Categories of phytoremediation (from Alkorta et al 2004)

Term	Definition
Phytoextraction	The use of plants to remove pollutants (mostly, metals) from soils.
Phytofiltration	The use of plants roots (rhizofiltration) or seedlings (blastofiltration) to absorb or adsorb pollutants (mostly, metals) from water.
Phytostabilization	The use of plants to reduce the bioavailability of pollutants in the environment.
Phytovolatilization	The use of plants to volatilize pollutants.
Phytodegradation Phytotransformation	The use of plants to degrade organic pollutants
Phytostimulation	The use of plant roots in conjunction with their rhizospheric microorganisms to remediate soils contaminated with organics

Enhanced rhizosphere degradation .
Rhizodegradation
Plant-assisted bioremediation
Plant-asssisted degradation
Plant-aided `in situ` biodegradation

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This study centered specifically around phytoextraction through chelation and immobilization by precipitation. Phytoextraction (Prasad *et al.,* 2001) by definition is use of plants to take up contaminants (most commonly metals) from soil into their roots, to later be translocated into shoots or other organs, which can be enhanced by the use of chelators (in this study EDTA). Cd is amongst the most readily bio-available metals, while Pb falls in the moderate category. Pb can be taken up by certain plants, but uptake is enhanced by chelators. Phytostabilization is the use of plants or the compounds they secrete to stabilize low levels of contaminants in the soil (by adsorption or precipitation) to prevent them leaching and endangering public health (Kumar *et al.*,1995; Prasad 1999). Phytoextraction could potentially be an environment-friendly and cost-effective technology compared to conventional remediation techniques (Neugschwandtner *et al.,* 2008) and may also be achieved through chemical means (chemical stabilization) as is the case in this study (using phosphate).

Plants that are capable of absorbing very high concentrations of heavy metals have compounds that bind with the heavy metals in the plant body, and detoxify them. One such compound is the polypeptide phytochelatin that renders cadmium innocuous when it binds with it. Nickel has been known to be detoxified by binding with histidine and citric acid in the same way. Lead detoxification has been achieved through binding with oxalic acid (Shinmachi *et al.*,2003; Liphadzi and Kirkham, 2005; Wahla and Kirkham, 2007)

Plants growing on polluted metal-contaminated soils take these metals up into their organs, and therefore contribute to environmental decontamination, provided the plant material is removed. At the very bottom of many food chains, metal-accumulating plants are directly or indirectly responsible for the largest proportion of the dietary uptake of toxic metals by animals, including humans.

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Plants for phytoextraction should have a high tolerance threshold for the metal, be able to accumulate reasonably high levels of the metal in their above-ground tissues, have rapid growth rates, a profuse root system, and produce reasonably high biomass in the field. In the last few years, a number of metal-tolerant and metal-accumulating plants have been identified. Hyperaccumulators are plants that have the capacity to accumulate large quantities of metals from the surrounding soil, and the term was first used to describe plants that contain greater than 0.1% nickel (Ni) in their dried leaves. Since then, threshold values have also been established for other metals such as zinc (Zn), lead (Pb), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe) and manganese (Mn). (Herna´ndez-Allica *et al.*, 2008).

In recent years, a number of plant species have been identified which can tolerate and accumulate elevated amounts of heavy metals in their tissues and could be used in the phytoremediation of metal contaminated soils. *Elsholtzia argyi* is a newly reported Cu as well as Pb tolerant plant species, which exists widely in the ancient Pb/Cu-mining areas of Southeast of China and can grow up to 160 cm high with a large biomass. This plant species is ideal for the phytoremediation of Cu and Pb contaminated soils and the volatile constituents in its flowers give a possible utilization of plant resources after they are being used for phytoremediation purposes. Islam *et al.* (2008) also found a contrasting ecotype of *E. argyi* growing in the normal agricultural soils of Hangzhou suburbs Zhejiang Province of China that has much lower metal concentrations as compared to the plants from the Pb/Cu-mining sites (Islam *et al.*, 2008).

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However, if metals are not available to plants, their abilities to tolerate, take up and accumulate metals are irrelevant to phytoextraction. Hopkins and Huner (2009) penned pollutant availability to plants as the *sine qua non* of phytoremediation, just as important as the ability of plants to deal with pollutants. For a certain plant to be considered for metal phytoextraction, it is essential to test its phytoextraction potential in the specific soil needing remediation. Herna´ndez-Allica *et al.* (2008) conducted studies to determine the potential of fast-growing, high biomass crop plant species to phytoremediate metal polluted soils. Initially a hydroponic screening method was used to identify fast-growing, high biomass crop plant species with the ability to accumulate heavy metals (Pb, Zn, and Cd) in their shoots. A pot experiment followed, using metal enriched compost, to establish the metal tolerance and shoot metal

accumulation of those cultivars that had shown the greatest capacity for shoot metal accumulation in the hydroponic study. These cultivars were tested for the phytoremediation of soil, collected from the vicinity of an abandoned mine, polluted with moderate levels of total Pb, Zn, and Cd (Herna´ndez-Allica *et al.,* 2008).

The concept of using hyperaccumulators to remediate metal polluted soils was first introduced by Chaney (1983). Research in the area over the past few decades has revealed that even hyperaccumulators would need many years to effectively remediate metal contaminated sites. The use of natural hyperaccumulators presents several problems. Firstly no well-known cultivation, pest management or harvesting practices exist, second these plants are relatively small and they have slow rates of biomass accumulation. Consequently faster-growing, high biomass crop plant species that accumulate moderate levels of metals in their shoots are being tested for their phytoremediation potential. The genus *Brassica*, includes several of such species that also display a significant heavy metal tolerance, as do many grasses such as maize, barley, oat and ryegrass (Kumar *et al.*,1995). Some authors feel a greater shoot biomass can more than compensate for a lower shoot metal concentration. For example, Ebbs and Kochian (1997) reported that *Brassica juncea* removed fourfold more Zn than the hyperaccumulator *Thlaspi caerulescens* from a soil contaminated with >11 000 mg Zn kg^{-1} soil. This was due primarily to the fact that, in 6 weeks, *B. juncea* produced 10 times more biomass than *T. caerulescens* (Herna´ndez-Allica *et al.,* 2008).

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Phytoremediation revolves very much around the chemistry of metal interactions with the soil matrix, and sorption to soil particles reduces the activity of metals in the

system. The higher the cation exchange capacity (CEC) of the soil, the greater the sorption and immobilisation of the metals. The phytoextraction efficiency of a plant is dependent on the heavy metal contents in the biomass and the biomass production. Increasing metal–chelant complexes in the soil promotes the uptake by plants, the translocation of heavy metals from roots to shoots and their accumulation in the harvestable parts of the plants (Grčman *et al.,* 2001; Schmidt, 2003). Because of the lack of large-scale cultivation techniques for metal-hyperaccumulating plants and their low biomass, research has been focused on the use of chelating agents and high yielding agricultural crops that can be cultivated using established agronomic practices. Some agricultural crops (e.g., *Brassica napus L., Brassica juncea L., Cannabis sativa L., Helianthus annuus L., Phaseolus vulgaris L., Sinapis alba L. and Zea mays L.*) were found to be effective in removing metals under model conditions but their evaluation in field conditions is sorely lacking (Komárek *et al.,*2007; Neugschwandtner *et al.,*2008).

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In the growth of strawberries (*Fragaria* x *ananassa*, Rosaceae), Cd was added to the soil up to 60 ppm.). Roots accumulated the highest Cd of all plant organs. Under Cd stress leaf weights were actually reduced more than root weights though more than 90% of the Cd absorbed was in the roots. This led the authors to the conclusion that leaf dry weights are the best indicators of Cd toxicity in strawberries (Prasad, 1999).

Wheat plants (*Triticum aestivum*) were grown in nutrient solutions with Cd concentrations up to 1 mM, and at the highest concentrations elongation of plants was reduced to 28-40% of the control. Increased Cd concentrations were also accompanied by decreasing Fe, Mg, Ca and K concentrations. It has also been suggested that the Cd effects on the essential nutrient content of the plant led to the

growth inhibition, reduced chlorophyll content and inhibition of photosynthesis that resulted from the experiment. Premature senescence has also been attributed to Cd stress. In another experiment bean (*Phaseolus vulgaris*) and tomato (*Lycopersicon esculentum*) plants were subjected to a Cd concentration range from 0-50 µM for seven days and the inhibitory effect on growth was greater in bean then in tomato (Prasad, 1999).

While metal-binding low molecular weight compounds (phytochelatins) have consistently been punted as integral in the resistance of plants to toxic trace metals; the resistance of the plants depends mostly on the resistance of their roots, which must absorb balanced amounts of essential nutrients (Prasad, 1999).

1.2 Problem statement

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Through preliminary work conducted by other students in the research group (Meerkotter 2012; 2003, Sogayiso 2003) it has come to our attention that a heavy metal problem exists in the Philippi and Kraaifontein-Joostenbergvlakte vegetable farming areas of Cape Town area, with the most common problem elements being cadmium, copper, lead and zinc, appearing in concentrations exceeding the legal limits set by South African regulations and guidelines (DWAF, 1996).

The overall aim of the project is firstly to see if the water supply could be the source of the specific heavy metals that are problematic in the area, as found by the two aforementioned students, then compare two methods of remediation of the heavy metal problem, namely precipitation and chelation, in a pot experiment with silica

soils, so as to assist the farmers in the area as to the best way to combat any future heavy metal problems.

1.3 Heavy metals in vegetables in Cape Town

The Cape Flats Aquifer on which the two farming areas of Kraaifontein-Joostenbergvlakte (K-J) and Phillippi (P) occur is a sand unit of Cenozoic age, deposited on top of impervious Malmesbury Shales and Cape Granite. Farmers abstract from both the primary (unconfined) and secondary aquifers (of Malmesbury shales meta-sediment). Due to its unconfined nature, the primary Cape Flats aquifer is more exposed to possible pollution from above ground activities, than the secondary Malmesbury aquifer, but cross contamination may occur (Fraser and Weaver, 2000; Saayman and Adams, 2002). Farmlands in and around cities are often used for growing vegetables, and are therefore acutely vulnerable to metal contamination (Yang *et al.*, 2009) from industrial sources.

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It is therefore important to assess the risks of heavy metals exceeding the national limits in various crops, and advise farmers accordingly. A vast knowledge base still needs to be gathered on the accumulation of the metals in crops under various soil conditions, which govern their bioavailability.

The Philippi and Kraaifontein-Joostenberg Vlakte farming areas were chosen for the water survey, firstly as they are the major supplier of vegetables the Cape's vegetable markets and chain stores, secondly due to their proximity to residential and industrial areas. Farmers were visited during March-May of 2007 and water samples collected from the boreholes and dams used for irrigation purposes.

1.3.1 Chelation: a possible solution for farms in Cape Town

The low solubility and bioavailability of some toxic metals (e.g., Pb) is the major limiting factor in induced phytoextraction as the Pb exchangeable fraction is very low in most soils. Several synthetic chelating/complexing agents, such as ethylenediaminetetraacetic acid (EDTA), ethylenetrinitrilopentaacetic acid (DTPA), N-hydroxy- ethylenediaminetriacetic acid (HEDTA), nitrilotriacetate (NTA) and a natural chelant like ethylenediaminedisuccinate (EDDS) have been used to enhance metal solubility during phytoextraction (Sakar *et al.,* 2008). Synthetic chelating agents have the potential to remobilise metals and to form strong soluble complexes. Ethylenediaminetetraacetic acid (EDTA) proved to be the most effective chelating agent among several tested in increasing Pb desorption from soils (Neugschwandtner *et al.,* 2008). EDTA is non-selective agent that can form strong complexes with a variety of metals including alkaline–earth cations and target heavy metals (Saifullah *et al.,* 2009).

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While EDTA, NTA, oxalate, malate, citrate have all successfully been used as chelators to increase the mobility and uptake of metals by plants from contaminated soils, the use of synthetic chelators significantly increased Pb and Cd uptake and translocation from roots to shoots. Enhanced phytoextraction of heavy metals using chelating agents and agricultural crops is widely discussed as a remediation technique for agricultural soils contaminated with low mobile heavy metals (Neugschwandtner *et al.*,2008).

The main disadvantage of EDTA is its low biodegradation capacity and persistence in the environment which poses a high risk of metal leaching due to the rapid mobilisation of metals and the subsequent slow decrease of metal mobility in the soil solution. The harvested biomass containing high concentrations of heavy metals would have to be removed from the site and stored at a landfill. Therefore, there is a need to estimate the movement of metals through the soil profile and to optimise agronomic practices to maximise the cleanup potential of remediative plants and to minimise risks to humans and the environment (Israr and Sahi, 2008; Neugschwandtner *et al.*, 2008;). EDTA redistributes surface contamination down the soil profile, causing a reduction in the concentration near the soil surface and spreads the heavy metals through the entire root zone for uptake (Wahla and Kirkham, 2007).

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Phytoremediation using chelating agents is thought to have great potential in cleaning up polluted soils in developed countries where the soils have been allowed to degrade (e.g., the former satellites of the Union of Soviet Socialist Republics) and in developing countries (Liphadzi and Kirkham, 2005) such as ours. In these countries, often high-quality irrigation water is not available for application to crops and brackish waters must be used.

Most studies on chelate-induced phytoextraction have focused on EDTA (ethylene diamine tetracetic acid)-mediated lead (Pb) phytoextraction. In this respect, the amount of Pb taken up by plants has been reported to be much smaller than the amount of Pb mobilized from the soil during EDTA-induced Pb phytoextraction. EDDS (ethylene diamine disuccinate) was proposed as an alternative for chelate-induced metal phytoextraction. EDDS has been shown to be easily biodegradable, to

form strong complexes with transition metals and radionuclides, to cause a much smaller leaching of Pb down the soil profile than EDTA, and to be less toxic to soil microorganisms (Wahla and Kirkham, 2007).

Contradictory results can be found in the literature regarding the capacity of EDTA and EDDS to induce plant Pb accumulation. Grčman *et al.,* (2001) found that EDDS and EDTA were equally efficient for the induction of Pb accumulation in Chinese cabbage shoots. By contrast, under similar experimental conditions, Kos and Leštan (2003) reported that EDTA was almost twice as efficient as EDDS for the induction of Pb accumulation in Chinese cabbage shoots. Likewise, conflicting data can be found in the literature regarding the phytotoxicity of these chelates and the formed metal-chelate complexes. Grčman *et al.,* (2001) observed a significant phytotoxic effect with EDTA but not with EDDS on *Brassica rapa*, while being equally effective at increasing metal uptake. Meers *et al*., (2005) did not observe any visual toxicity symptoms or growth inhibition in plants treated with EDDS or EDTA. The ultimate goal of any soil (phyto)remediation process must be not only to remove the contaminant(s) from the polluted site but to restore soil quality, i.e. the continued capacity of soil to perform or function according to its potential (Hernández-Allica *et al.,* 2008).

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1.3.2 Precipitation: alternative solution for farms in Cape Town

Of the available remediation techniques, chemical stabilization seems a good alternative due its cost-effectiveness and environmental sustainability (Cao *et al.,* 2009; Thawornchaisit and Polprasert, 2009). Stabilization is based on the

modification of pollutant characteristics (speciation, valence) and soil properties (solution capacity and buffering potential) by the addition of immobilization agents (Diels *et al.,* 2002; Raicevic *et al.,* 2009). This aims to reduce the bioavailability fraction of the metal by increased metal sorption, precipitation, or the formation of distinct minerals (Cao *et al.,* 2009; Thawornchaisit and Polprasert, 2009). Phosphate treatments have been shown to stabilize a number of metals especially Pb^{2+} from contaminated soils (Cao *et al.,* 2009). Sources of P include phosphate containing minerals, and soluble P in the form of salts or phosphoric acid. Phosphorus occurs in the soil solution as either a negatively charged phosphate ion $H_2PO_4^-$ in acidic soil or HPO_4^{2-} in alkaline soils, where they readily react with positively charged soil compounds and heavy metals (Rufyikiri *et al.*, 2006).

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When leachable metals come into contact with phosphate they form metal phosphates with less solubility and greater geochemical stability. This mechanism has been observed in Pb-contaminated soils where addition of phosphate leads to the formation of lead-phosphate minerals. The efficiency of stabilization however varies according to the type of contaminant. Brown *et al.* (2004) found that the addition of triple superphosphate with a high Fe by-product reduced the in-vitro extractible Pb in the soil, but the Cd content of the soils was raised. Mineral apatite however, was found to reduce the solubility and bioavailability of Pb, Cd and Zn in contaminated soils (Chen *et al.,* 1997). Conflicting findings on the stabilization of Cd using P exist, for example Brown *et al.,* (2004) found the addition of phosphate may reduce the bioavailability of Pb but increased Cd concentrations. Further research is therefore needed, as the presence of competing ions can reduce the specific treatments efficiency (Rufyikiri *et al.,* 2006; Raicevic, 2009). The efficiency of phosphate fertilizers like triple

superphosphate, which cost significantly more than minerals, also begs investigation (Thawornchaisit and Polprasert, 2009).

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Chapter 2: Is the water the source of the heavy metals to the Cape Town vegetable farms?

2.1 Introduction

Groundwater has played an important role in the economic and social development in South Africa. The history of water resources development in the country has resulted in disparate emphases on the present day use of groundwater. Located at the southern tip of Africa, Cape Town finds itself surrounded by ocean and prominent mountain ranges. The topographic low, located between Table Mountain and the Drakenstein and Hottentotsholland mountains, is known as the Cape Flats. The rainfall over the area of the Cape Flats is much less than in the surrounding mountains, and averages about 600 mm per annum, with most rainfall occurring during winter. Large parts of the Cape Flats are covered by urban development. Today most of the city's population live within the area of the Cape Flats. Based on 1996 census Fig.ures (Statistics South Africa, 1996) the 2001 population of the larger Cape Town Metropolitan area was estimated at just over 3 million (Saayman and Adams, 2002).

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Cape Town‟s two agricultural areas, the Joostenbergvlakte/Kraaifontein and Philippi areas‟ soils are of the Cenozoic Sandveld group deposit, which lies on top of the meta-sedimentary Malmesbury Shales and Cape Granite bedrock. The Cape Flats aquifer, a primary aquifer, lies in the Sandveld group deposit (Cole and Roberts, 1996, Fraser and Weaver, 2000; Harris *et al.*, 1999; Rose, 1996; Wright and Conrad, 1995). The majority of Cape Town‟s farming communities' use water from both primary and secondary aquifers for irrigation , some sourcing boreholes and well points fed by these aquifers (Saayman and Adams, 2002).

The Philipi and Kraaifontein-Joostenbergvlakte areas located on the Cape Flats supply markets throughout the country with vegetables. Both these areas are under constant threat from urban residential and industrial expansion, and researchers over the past decade (Sogayise, 2003 and Meerkotter, 2003 and 2012) have found that on occasion the soil, water and vegetables heavy metal concentrations exceeded the South African water affairs departmental guidelines (DWAF guidelines for Irrigation, 1996).

Philippi's soils are sandy and rich in silica which combined with the high water table allows cross contamination into irrigation water resources (Rice and Rice, 1997; Brown 1996) from fertilizers and other agricultural amendments, which may contain a number of contaminants among them heavy metals. The Philippi agricultural area is also one of the many on the Cape Flats aquifer prone to Phosphate contamination which is direct result of various agricultural practices such as fertilizer use (Bertram, 1989). Leaching of wastewater from treatment plants in the neighbouring Mitchells Plain area and the mineralisation of groundwater and has also been recorded (Chittenden Nicks Partnership, 1997; Fraser and Weaver, 2000; Harris *et al.*, 1999; Rose, 1996; Wright and Conrad, 1995).

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At several sites in the Philippi farming area, the water table is approximately 1,5m below the surface for the majority of the year facilitating the transfer of surface pollutants to subterranean water bodies. Due to farmers in many of Cape Town‟s agricultural areas making use of a combination of both surface and subterranean water for irrigation, this

cycling of pollutants and heavy metals may lead to the contamination of subterranean water resources (Alam *et al.*, 2003).

Soils from the Joostenbergvlakte and Kraaifontein areas are only slightly different in that they are sandy, but rich clay layers exist which in winter often leads to much water logging (Brown, 1996; Cole and Roberts, 1996; Rice and Rice, 1997). Nutrients and contaminants such as heavy metals may be leached from surface waters to soils and vice versa but, contaminants may remain in top soil layers longer since it cannot easily be leached past deeper clay rich soil layers (Brown, 1996; Eigenhuis, 1997; Rice and Rice, 1997).

Due to the seasonal cycling of elements through the agricultural system during different seasons of the year, it seems that Philippi's groundwater is a target for accumulation of heavy metal and other contaminants, while in the Kraaifontein area, the groundwater resources seem to be protected by clay layers leaving the topsoil a target for accumulation of heavy metal and other contaminants. The mere presence of contaminants in soils do not necessarily imply that crops will be contaminated (Brown, 1996; Eigenhuis, 1997; Li *et al.*, 2008; Rice and Rice, 1997).

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In both Phil and KJ, sprinkler irrigation is most often used during summer while especially in the Philippi area, during the rainy winter season, little sprinkler irrigation is needed as land is often waterlogged due to a high water table. In these two areas the flow of water is often regulated where run-off from land is collected in canals and in the case of winter drained away or, as in the case of dry summers, recycled. In the Philippi area, where farmers often face a lack of water in summers due to water leaching speedily into the deeper levels of very sandy soils, many farmers have lined their irrigation water

holding dams with water impermeable layers and have even done so under some cropped fields so that water may not be lost to readily during dry summer months (Kane, 2002; Kinchen and King, 2003; Personal communication with A Terreblanche, 2007; Rice and Rice, 1997; Summerfield, 1994). This poses a problem as pollutants may become more concentrated in cropped soils and irrigation water holding dams lined with impermeable layers to keep water from leaching to deeper soil levels (Kane, 2002; Kinchen and King, 2003).

2.2 Water quality

Water resource studies have shown that groundwater is fresh in most parts of the Cape Flats Aquifer (Fraser and Weaver, 2000). Water samples taken from the University of the Western Cape borehole site show a fresh groundwater character in the primary aquifer and a slightly brackish water in the Secondary Malmesbury aquifer at depth (Table 2.1). Table 2.1 illustrates the excellent quality of groundwater found in the Cape Flats Aquifer. The exception to this is the elevated iron content observed in places. Preliminary saturation state calculations indicate that the groundwater is supersaturated with respect to the iron oxide mineral phases. When used for irrigation the elevated iron content may result in the staining on walls and pavements. Biofouling of the screens by iron bacteria may become a problem with time. However, with low abstraction rates the impact of this should be insignificant (Saayman and Adams, 2002).

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Table 2.1 Water quality of the two aquifers tapped at the University of the Western Cape borehole site (Saayman and Adams, 2002).

	Primary aquifer	Secondary aquifer	water quality general*
Na	31.9	166.6	57
Mg	4.1	13.3	11
K	1.6	1.8	1.5
Ca	49.4	37.3	95
Si	2.3	2.9	-
Fe	0.1	0.56	-
Cl	43.7	262	99
HCO_3	192.8	200.2	-
NO_3-N	0.0	0.2	<0.1
pH	7.1	7.5	7.7
EC(mS/m)	41	96	78

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* Fraser and Weaver, 2000 (Cape Flats water quality)

**all values in ppm unless otherwise indicated

Irrigated areas in arid and semi arid areas are often underlain by aquifers of poor quality due to natural topography, groundwater recharge and geochemistry of the area, normally resulting from anthropogenic activities like inefficient irrigation and canal seepage, leading to increased soil and groundwater salinity (Kamra *et al.*, 2002).

Fresh water is a finite resource with an ever increasing demand that requires careful management. Agriculture, as a source of contamination, exerts direct and indirect

effects on the rates and compositions of groundwater recharge and aquifer biochemistry, as it uses about 67-70% of all freshwater supplies worldwide (Gallardo and Tase, 2007; Banedjschafie *et al.,* 2008). Hydrological efficiency is only about 37% in agriculture. Hydrological efficiency is the percentage of water used by evapotranspiration from the total of water irrigated, and differs from water use efficiency which relates to crop yield (Banedjschafie *et al.,* 2008).

2.3 Materials and Methods

2.3.1 Site selection

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The Philippi (Phil) and Kraaifontein-Joostenbergvlakte (K-J) vegetable farming areas were chosen for this survey as they occur in the Greater Cape Town Area and have been the subject of various studies (Meerkotter 2003; Sogayiso 2003; Feng 2005; Ma 2005). These two areas are jointly the major suppliers to the Cape Town vegetable markets, major chain stores, as well as some export markets (Personal communication, A Terblanche).

After the project was briefly explained to them, farmers were asked for permission to collect water samples from the sites they use for irrigation purposes. They were also asked to provide information relating to the depth of the boreholes, their aquifer material and the origins of the dam water, which in many cases they were unable to provide conclusively. The survey was conducted between March and June of 2007.

2.3.2 Water collection, preparation and analysis

A total of 50 samples were collected over the course of the survey, 35 from Phil (12 dams 23 boreholes) and 15 from K-J (14 dams and 1 borehole). The farms differed in the number and size of collection points for surface or groundwater, which fell into two main categories; dams and boreholes. All sites were sampled using a 300 ml plastic jar, which was lowered into the dam away from the edge or used to take up a sample from the borehole pump/pipeline. A GPS reading was taken at each site and recorded. The pH of the water was taken in the laboratory, with a Orion model 210A pH meter immediately after collection, and then the water was preserved with the addition of 2 mL of HCl (32%). Then the samples were then stored at 2°C in a cold room until all were collected.

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BemLab Ltd in Somerset West, South Africa, a certified commercial laboratory analysed the water samples following parameters: EC, OP, Na, K, Ca, Mg, Fe, Mn, HCO_3, SO_4, B, Adj. SAR, Langelier index Cu, Zn, P, Cd, Pb, which were tested against certified standards.

2.4 Results and Discussion

The average pH for the water samples in the study ranged in value from 7.0 to 7.5, with the borehole values tending lower than those of the dams at both study sites. These values do however meet the target range for South African irrigation water set by the Department of Water Affairs and Forestry (DWAF 1996) which is 6.5-8.4 (Table 2.2).

Table 2.2 A summary of the results of water analyses (not significantly different between sites) from both sites including the limits for various variables for irrigation and stock farming as set by the Department of Water affairs and forestry (DWAF 1996).

Parameter	Units	Phil Dam	Phil Borehole	K-J Dam	K-J borehole	Limit for irrigation	Limit for stock farming
pH		7.35 ± 0.46	7.08±0.39	7.39±0.86	7.23	**6.5-8.4 (target range)**	**n/a**
EC	(mS/m)	2137±473	2135±582	2176±290	2070	**n/a**	**n/a**
OP	(kPa)	769.3±170	768.7±210	783.4±104	745.2	**n/a**	**n/a**
Fe	$mg.l^{-1}$	0.6±0.9	0.8±1.3	0.3±0.3	0.1	**20**	**n/a**
Mn	$mg.l^{-1}$	0.020±0.02	0.025±0.02	0.029±0.03	0.030	**10**	**10**
Cu	$mg.l^{-1}$	0.013±0.02	0.003±0.01	0.008±0.01	0.010	**5**	**1**
.Cd	$mg.l^{-1}$	0.000	0.000	0.000	0.000	**0.05**	**0.01**
Pb	$mg.l^{-1}$	0.005±0.01	0.007±0.01	0.004±0.01	0.000	**2**	**0.1**

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The quality of groundwater from the Cape Flats aquifer is generally good, with the overall salinity being quite low. There are natural peaks in salinity where the water table is at or just below surface, and high evaporation results. High salinity and poorer

groundwater quality is however generally associated with the Philippi (Phil) agricultural area (Fraser and Weaver, 2000), attributed to the irrigation and fertilization practices of the area (Aza-Gnandji, 2011), but not withstanding this was found to be suitable for irrigation purposes for certain crops.

Electrical conductivity (EC) or specific conductivity is a measure of a material's ability to conduct an electric current. The conductivity of a solution of water is highly dependent on its concentration of dissolved salts and sometimes other chemical species which tend to ionize in the solution. Soil EC is influenced by a number of physiochemical properties in combination. These are soil water content, bulk density, organic matter, and even soil temperature (Corwin and Lesch, 2005). Salinity in water, which is a useful indicator in agriculture, is measured in electrical conductivity (EC). The EC in both Phil and K-J was in the same range; 2050-2200 $mS.m^{-1}$, with no difference in the dam and borehole values (Table 2.2).

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During its passage into the xylem, the composition of the water and minerals taken up into the plant may change due to electrochemical gradients or transporter systems (Marschner, 1995). Sodium occurs as a cation that is generally excluded by plants, if it is present at elevated levels in the soil solution, to avoid toxic effects. Several carrier systems have been identified that interact with sodium and the existance of a sodium/hydrogen pump in the root membrane was postulated. The function and localisation of amongst others, the Na+/H+ antiporter and the other ion channels in plant cell membranes play key roles in salt tolerance mechanisms (Trapp *et al.*, 2008).

Sodium (Table 2.3) is significantly higher in Phil than K-J, with dam water being significantly higher than borehole. The Phil boreholes and K-J dams are statistically

identical (Table 2.2). The borehole values are all over 100 mg/l and sodium toxicity is therefore expected in some plant species (BemLab Ltd, Somerset West, South Africa). These are all above the target range set by DWAF for irrigation waters which is $70mg/l^{-1}$.

The dam values at both sites were only significantly different for sodium, calcium, SO_4 , zinc and phosphate, with values at Philippi being the highest (Table 2.3).

Table 2.3 A comparison of significant dam values between Phillipi (Phil) and Kraaifontein Joostenbergvlakte (KJ). Values with the same subscript letter do not differ significantly

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Parameter	Phil dam mean value	KJ dam mean value	lsd	DWAF standard for irrigation
Na	171.59	105.49	51.619	70 $mg.l^{-1}$
Ca	180.92	66.27	54.14	n/a
SO_4	284.25	130.07	115.08	n/a
Zn	0.03	0.005	0.0222	5 $mg.l^{-1}$
P	2.4879	0.5150	1.2697	n/a

There were only seven significant ($p < 0.05$) values (t-test data) for the water survey. Ca and P were found to be significantly different when comparing the two areas (K-J

and Phil) to each other (Table 2.4), and when comparing the dams in the two areas there were significant differences found in Na, Ca, SO_4, Zn and P values (Table 2.6).

Table 2.4 A comparison of significant parameters when looking at the areas as a whole (including both water bodies). Values with the same subscript letter do not differ significantly

Parameter	Phil mean value	KJ value	lsd	DWAF standard for irrigation
Ca	165.65	62.47	43.715	n/a
P	2.3220	0.6089	1.0473	n/a

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Only calcium and phosphorus are significantly different between both areas when comparing results for both water bodies combined, with values at Philippi being the highest.

Table 2.5 Significant parameters in comparing water bodies at Kraaifontein Joostenberg vlakte (KJ). Values with the same subscript letter do not differ significantly.

Parameter	KJ Borehole mean value	KJ Dam value	lsd	DWAF standard for irrigation
K	28.292	48.481	17.784	n/a
B	0.06708	0.11731	0.0361	0.5 $mg.l^{-1}$

In the Kraaifontein Joostenber vlakte area potassium and boron were the only significantly different elements between water bodies, with damn values being higher as can be expected as dams concentrate these elements (Table 2.5).

Table 2.6 Significant parameters in comparing water bodies at Philippi (Phil) and the dam values at KJ. Values with the same subscript letter do not differ significantly

Parameter	Phil Dam	Phil borehole	KJ dam	lsd	DWAF standard for irrigation
Na	171.59 $_{a}$	115.78 $_{b}$	105.49 $_{b}$	54.87	70 $mg.l^{-1}$
K	40.05 $_{ab}$	29.47 $_{b}$	55.71 $_{a}$	22.84	n/a
Ca	180.92 $_{a}$	157.69 $_{a}$	66.27 $_{b}$	51.496	n/a
SO_4	284.25 $_{a}$	183.26 $_{ab}$	130.07 $_{b}$	115.89	n/a
B	0.11 $_{ab}$	0.06783 $_{b}$	0.12357 $_{a}$	0.0463	0.5 $mg.l^{-1}$
P	0.5150 $_{b}$	0.6578 $_{b}$	2.4879 $_{a}$	1.2337	n/a

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The sodium values are highest in Phil Dam, the potassium KJ dam, calcium Phil dam, sulphate Phil dam, boron KJ dam and phosphorus KJ dam. All the highest values are from dams which can well be expected as they accumulate due to evaporation.

Table 2.7 Parameters that displayed significantly differences between both water bodies in both the Philipi and Kraaifontein-Joostenbergvlakte areas. Values with the same subscript letter do not differ significantly (DWAF standards not available for any of these parameters)

Parameter	Phil Dam	Phil borehole	KJ dam	KJ borehole	lsd
K	40.05 $_{ab}$	29.47 $_{ab}$	55.71 $_{a}$	1.3 $_{b}$	48.63
Ca	180.92 $_{a}$	157.69 $_{ab}$	66.27 $_{bc}$	9.3 $_{c}$	109.64
Mg	30.68 $_{a}$	20.88 $_{ab}$	24.26 $_{ab}$	4.6 $_{b}$	24.899
SO_4	284.3 $_{a}$	183.3 $_{ab}$	130.1 $_{ab}$	2.0 $_{b}$	246.74

The element found to be most commonly significantly different between the two research area is calcium. This is followed by sulphate, potassium and phosphorus. Sodium and boron occur more than once, whereas zinc and magnesium only make single appearances.

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2.5 Summary

No cadmium was found in any of the water samples taken during the survey (Table 2.2). Minimal Pb was detected, but in a range far below the target range set by DWAF for irrigation waters. The pH of the waters in both Phil and K-J are of reasonably good quality in the range of 7. The EC of the samples were all above what is suitable for irrigation (Bemlab), which is in agreement with Aza-Gnandji (2011). The Na content of the samples is above the target range of 100mg/L and found to show variation among water bodies and areas (Tables 2.3 and 2.6).

Mn levels all equal to or above the target range and B, Zn, Cu were all far below target range. Aza-Gnandji (2011) found that chloride, nitrate, potassium and sodium ion concentrations in Phil exceeded the target ranges set up by DWAF. All heavy metals were either not present or present in concentrations far below target range,

therefore it can be concluded that the water is not the main source of heavy metals in both areas. This conclusion is further supported by Meerkotter (2012) that found cattle and chicken manure to be the greatest sources of heavy metals Pb, Cd and Zn, in both research areas.

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Chapter 3: An assessment of the effect of Cadmium & Lead, and two heavy metal mitigation techniques using a pot experiment: Growth and development.

3.1 Introduction

Two approaches are most commonly taken to phytoextraction of heavy metals: the first being the use of natural hyperaccumulator plants and high biomass plants with chemically enhanced methods (Chen *et al.*, 2004). Alternative to in situ phytoextraction, synthetic chelates are also used to increase the removal of metal in the ex situ methods. Cheators enhance leaching of heavy metals and while many batch and column-leaching experiments have been conducted (Chen *et al.*, 2004), there is still very little information available on the long term effectiveness of soil additives in regulation of trace elements under field conditions (Madejón *et al.*, 2009). Anthropogenic activities often pollute soils with multiple metals, which then results in antagonistic, additive or synergetic effects due to their interactions. Murakami and Ae (2009) found that Zn and Cu removal by plants was most effective when a single metal was present.

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A greenhouse experiment was conducted in order to test the effectiveness of two additives; Triple Super Phosphate and EDTA, in reducing the uptake of the heavy metals Cd and Pb of two crop species; namely spinach and turnip. Pb and Cd are non-essential elements for plants that can cause adverse effects on the plant's

photosynthesis and chlorophyll synthesis which lead to symptoms such as chlorosis, reduction of biomass, inhibition of root elongation and finally death (Milone et al., 2003).

It was designed in conjunction with a trained biostatistician, Mr F. Calitz of the Agricultural Research Councils' Biometry Unit. A randomised complete block experimental design (Huang *et al.,* 2003; Luo *et al.,* 2006) was chosen (Table 3.1), and the effectiveness of the two agents was tested at three concentration levels of the selected heavy metals, prepared using stock solutions. This work was specifically carried out using pure silica sand without ion exchange possibilities; as opposed to normal soil. The experiment was conducted in a glasshouse of the Environmental Education Resource Unit (EERU) nursery at The University of the Western Cape (UWC)'s Bellville campus.

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Table 3.1 The random block split plot design, where 25 treatments were replicated three times, and the two species were the split factor.

Block		
1	**2**	**3**
17	18	14
1 2	2 1	2 1
16	2	6
2 1	1 2	1 2
25	6	9
2 1	2 1	2 1
14	3	11
1 2	2 1	1 2
4	15	21
1 2	2 1	1 2
19	4	4
1 2	1 2	2 1
24	16	12
2 1	1 2	1 2
8	25	23
1 2	2 1	2 1
6	24	24

1 2	2 1	1 2
15 1 2	21 1 2	25 2 1
7 1 2	10 1 2	7 2 1
20 2 1	22 2 1	1 2 1
11 1 2	17 2 1	3 2 1
18 2 1	8 2 1	5 1 2
22 1 2	9 1 2	17 2 1
12 1 2	11 2 1	8 2 1
23 1 2	12 2 1	20 2 1
10 2 1	13 1 2	10 1 2
5 1 2	5 1 2	18 1 2
13 2 1	23 2 1	16 2 1
21 1 2	1 1 2	13 2 1
1 1 2	20 2 1	15 2 1
9 2 1	14 2 1	22 1 2
3 2 1	19 1 2	2 2 1
2 1 2	7 1 2	19 1 2

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Chapter three focuses on the effects of the additives on crop growth and development, and chapter four on the chemical composition after the treatment and mitigation combinations.

3.1.1 Cadmium (Cd) and its effect on growth and development

Cadmium is a non essential element which causes damage to plant metabolism at even very low concentrations and can easily be taken up by crops (Benavides *et al.,* 2005). Contamination of soils with Cd may happen through the disposal of municipal and industrial wastes, irrigation with sewage effluent and application of phosphate fertilizers. It then enters the food chain directly via contaminated soils (Mench 1998; Sanita di Toppi and Gabbrielli, 1999; Yang *et al.,* 2009). Cd can cause essential nutrient deficiencies and changes in N and P concentrations in plant tissue (Jalloha *et al.,* 2009) which could then directly impact growth and development as these are essential nutrients. In tomato plants, Hédiji *et al.* (2010) reported Cd treatments to disturb physiological and metabolic processes, leading to growth inhibition and alterations of anatomical structures.

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Farmlands around cities, like those in this study, Phillipi (Phil) and Kraaifontein-Joostenbergvlakte (K-J) are highly vulnerable to metal contamination of the soils and waters used for growing vegetables. The solution would be to assess the risk of metals exceeding the national limits in various metals, and advise farmers accordingly. This would require knowledge of Cd accumulation in various species as well as the soil factors that control its bioavailability (Yang *et al.,* 2009).

In a study conducted by Yang *et al.* (2009), it was found that leafy vegetables and carrots had a large Cd accumulation in their edible parts compared with fruit vegetables, which is in agreement with previous studies that found leafy vegetables

and root crops to have higher Cd uptake than fruits or seeds. In general plants have been found to tend to sequester metals in their roots, with only small amounts being translocated to the above ground parts (Yang *et al.,* 2009), but this has not been the experience of our research group (Meerkotter 2003, Sogayiso 2003).

3.1.2 Lead (Pb) and its effect on growth and development

Lead is a heavy metal well-known to be extremely toxic to humans. A number of studies have also concluded that these toxic effects of lead also affect many plant metabolic processes, e.g. photosynthesis, transpiration, DNA synthesis, mitotic activity and cell division and seed germination (Wierzbicka and Obidzińska, 1998; Singh *et al.*, 1997; Van Assche and Clijsters, 1990).

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Metallic lead does occur in nature, but it is rare. Lead is usually found in ore with zinc, silver and (most abundantly) copper, and is extracted together with these metals. In uncontaminated soils the Pb concentration is generally less than 50 $mg.kg^{-1}$ soils and 10 $mg.kg^{-1}$ in plant material. Mining, industrial and agricultural activities increase Pb availability to the environment. Lead is one of the most commonly found metals in the environment as it has a high adsorption affinity on soils (Zhang *et al.*, 2008, Saifullah Khan *et al.,* 2009), and is largely concentrated at the soil surface with just a little in the soil solution.

Ruley *et al.* (2006) found that EDTA greatly increased the translocation of Pb into the shoots while causing little harm to the plant. It is suggested that EDTA chelates Pb outside the plant and the soluble EDTA-Pb complex is transported through the plant and accumulated in the leaves (Vassil *et al.*, 1998, Saifullah Khan *et al.*, 2009). Huang

et al. (1997) also showed EDTA to greatly increase the concentration of soluble Pb in contaminated soils, and subsequently Pb uptake in plants with high biomass production such as Indian mustard or maize, though there are no Pb hyperaccumulator species known to date (Saiffulah Khan *et al.,* 2009).

This study aimed to investigate the use of different concentrations of a triple super phosphate fertiliser and EDTA solutions to mitigating the uptake of cadmium and lead by spinach and turnip plants, with a particular focuse on the growth and development effects in this chapter.

3.2 Materials and Methods - Greenhouse experiment - March 2008

3.2.1 Experimental design and statistical analysis

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The experimental design was a split plot with heavy metal and mitigation treatment combinations as main plot treatments and crop species (spinach and turnip) as split plot factor. The main plot design was a randomized complete block with 25 heavy metal and mitigation treatment combinations replicated at random in 3 blocks (Tables 3.1 & 3.2). The treatment design of the main plot factors was a 5x5 factorial with 5 heavy metal treatments (Control (0 mg/kg), Limit (maximum permissible limit) Cd (2 mg/kg), 10x Limit Cd (20 mg/kg), Limit Pb (6.6 mg/kg), 10x Limit Pb - 66 mg/kg) and 5 mitigation treatments (Control, low EDTA, high EDTA, low Phosphate, high Phosphate). Each experimental unit consisted of one plant. Fresh mass, dry mass and chlorophyll index were assessed for each plant.

Analysis of variance was performed according to the experimental design, using the GLM (General Linear Models) procedure of SAS statistical software version 9.2 (SAS Institute Inc., 2000, Cary, NC, USA)). The Shapiro-Wilk test was performed to test for normality (Shapiro and Wilk, 1965). The Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

Table 3.2 Detail of the twenty five treatments applied, and the ANOVA

Where

Trt	Heavy Metal	Mitigation
1	Contr (0)	Contr (0)
2	Contr (0)	Low EDTA
3	Contr (0)	High EDTA
4	Contr (0)	Low phosphate
5	Contr (0)	High phosphate
6	Limit Pb	Contr (0)
7	Limit Pb	Low EDTA
8	Limit Pb	High EDTA
9	Limit Pb	Low phosphate
10	Limit Pb	High phosphate
11	10x Limit Pb	Contr (0)
12	10x Limit Pb	Low EDTA
13	10x Limit Pb	High EDTA
14	10x Limit Pb	Low phosphate
15	10x Limit Pb	High phosphate
16	Limit Cd	Contr (0)
17	Limit Cd	Low EDTA
18	Limit Cd	High EDTA
19	Limit Cd	Low phosphate
20	Limit Cd	High phosphate
21	10x Limit Cd	Contr (0)
22	10x Limit Cd	Low EDTA
23	10x Limit Cd	High EDTA
24	10x Limit Cd	Low phosphate
25	10x Limit Cd	High phosphate

and

	Species	**Crop**
	1	leaf crop
	2	root crop

ANOVA

Source	**d.f (2 blocks)**	**d.f (3 blocks)**
Block	2-1=1	3-1=2
Heavy Metals	5-1=4	5-1=4
Mitigation	5-1=4	5-1=4
MetalxMitigation	(5-1)(5-1)=16	(5-1)(5-1)=16
Error (a)	(2-1)(5x5-1)=24	(3-1)(5x5-1)=48
Species	2-1=1	2-1=1
MetalxSp	(5-1)(2-1)=4	(5-1)(2-1)=4
MitigxSp	(5-1)(2-1)=4	(5-1)(2-1)=4
MetalxMitigxSp	(5-1)(5-1)(2-1)=16	(5-1)(5-1)(2-1)=16
Error	(2-1)[(2-1)+ (5x5-1)(2-1)]=25	(3-1)[(2-1)+ (5x5-1)(2-1)]=50
Total	2x2x5x5 - 1=99	2x3x5x5 - 1=149

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3.2.2 Preparation of growth medium and choice of crop

Pure silica sand, obtained from a glass manufacturer was used as a growth medium, in order to avoid contamination. Pots with a top diameter of 18 cm were used, each weighing approx 2.5 kg when filled. The crops chosen were spinach (*Spinacea oleracea* cv. 'ford hook giant') and turnip (*Brassica campestris* cv. 'early purple top globe'), a leaf and root crop respectively. Both a root and shoot crop were used in order to test the common idea in present literature on the matter, that all heavy metals are accumulated in greater amounts in the roots than the shoots (Greger 1999; Yang *et al.*, 2009). Marschner (1995) reported monocotyledon species to be more tolerant to metals than dicotyledonous species. Species used in this study were, however, both dicotyledonous species.

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3.2.3 Seedling germination

Performance tested seed, chemically treated and packaged by Starke Ayres was obtained from a local nursery. The seeds were sown in non-contaminated (99% pure) silica sand sourced from Consol Glass, in a seedling tray. These were then watered every alternate day for two weeks with tap water. After two weeks they were then treated with Chemicult (Chemicult – Kompel : 65 g/kg N, 27 g/kg P, 130 g/kg K, 70 g/kg Ca, 22 g/kg Mg, 75 g/kg S, 1.5 g/kg Fe, 0.24% Mn, 0.24% B, 0.05% Zn, 0.02% Cu and 0.01% Mn before dilution), a commercial plant nutrient supplement (made up with water per on pack instructions) once a week, while still continuing to be watered every alternate day with tap water. When the seedlings were established they were

transplanted in pairs into one hundred and fifty 18 cm top diameter pots and after an initial acclimatization period were introduced to their particular treatment.

3.2.4 Increasing the sand heavy metal content

The effectiveness of the two additives was tested at three concentration levels of the chosen heavy metals, Cd and Pb. The concentration levels of the selected heavy metals were chosen using the guidelines for the maximum permissible total concentration limit of each metal in South African soils as proposed by the Department of Water Affairs and Forestry (DWAF, 1996). These were (1) the control (0 mg/kg), (2) the maximum permissible concentration for each in the soil and (3) ten times the maximum. These were as follows:

Control Cd 0 mg/kg	Limit Cd 2 mg/kg	10 x Limit Cd 20 mg/kg soil
Control Pb 0 mg/kg	Limit Pb 6.6 mg/kg	10 x Limit Pb 66 mg/kg soil

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Aqueous stock solutions were prepared for each heavy metal at the required concentrations and used to spike the soil. These were prepared by dissolving the specific metal nitrate; Cadmium nitrate tetra hydrate (99%) and Lead III nitrate (99%) in tap water.

Recommended P/Pb molar ratios for effective remediation of Pb-contaminated soils range from 3/5 to 4/1 depending on the presence of other metals that may compete with Pb for dissolved P, for example Cd and Cu. In this experiment 4/1 molar ratios were used.

The amount of these heavy metals supplied in the tap water at the nursery was negligible so it was used both to water the plants and prepare the stock solutions. Each week the plants were spiked with 100 mL stock solution (made up of 50 mL heavy metal treatment and 50 mL mitigation agent). This was 100% of each pots holding capacity, which meant each solution was replaced afresh each week. In the case of control treatments tap water was used. Metal treatment stock solutions and mitigation agents were applied in 25 combinations to each species once a week for a period of seven weeks which included controls for both treatment and mitigation agent (Geebelen *et al,.* 2002; Luo *et al.,* 2006).

3.2.5 Soil additives

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A granular triple super phosphate (TSP) fertilizer was used for the phosphate additive treatment. The appropriate amount of P needed for each treatment was calculated based on the percentage of P in the TSP fertilizer (19.8%). Appropriate amounts were weighed out, crushed and dissolved in the aqueous solution.

EDTA on the other hand is the most commonly used additive in bioremediation studies to enhance the uptake of heavy metals (Epelde *et al.,* 2008), and found to be the most efficient at increasing water-soluble metal concentrations (Wu *et al.,* 2003). Synthetic chelators have been showed to significantly increase Pb and Cd uptake by roots and translocation to the shoots (Prasad, 1999). This study investigated the effect that an EDTA solution would have in enhancing the concentration of metals available for uptake by the roots of the plants, by making them more soluble and the contrasting effect of a phosphate addition which would reduce the amount of heavy metals bio available to the root zone (Lai and Chen, 2004; Lai and Chen, 2005; Liphadzi and

Kirkham, 2007). It is also to be borne in mind that metal speciation and ionic activity, and not just total amount of dissolved metal that determine plant metal uptake (Wu *et al.,* 2003).

For the EDTA additive treatment, three different concentration levels were investigated and EDTA solutions were prepared using ethylenediaminetetetraacetic acid disodium salt dihydrate (Na_2-EDTA.$2H_2O$) (99%). The heavy metal concentrations used in this study were considerably lower then similar remediation studies in other literature, and therefore it was decided to use EDTA concentrations based on equimolar concentrations of EDTA and Pb as a starting point to select suitable EDTA concentration levels for this experiment.

3.2.6 Preparation of stock solutions

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The DWAF guidelines for the maximum permissible total concentrations for each metal in agricultural soil were used to calculate the stock solutions based on each pot weighing approx 2.5 kg (DWAF, 1996). The limit and ten times the limit were used for each metal solution and the Phosphate and EDTA concentrations worked out from their molar ratios with Pb where EDTA has a 1:1 and Phosphate a 1: 4 molar ratio, gathered from prior students work (Meerkotter 2003, 2012) and a number of other sources (Geebelen *et al.,* 2002, Wu *et al.,* 2004 and Zhu *et al.,* 2004). Several investigations into the immobilization of Pb and Cd by phosphate indicated that this ratio was effective in immobilizing Pb and some other metals to a lesser extent. The effectiveness of each of the additives was tested at the following concentrations,

EDTA at 4 and 12 mg/kg soil and Phosphate at 1 and 8 mg/kg soil (Thayalakumaran *et al.*, 2003; Cui *et al.*, 2004; Lai and Chen, 2005).

3.2.7 Chlorophyll index measurements

In this study, shortly before harvesting, the chlorophyll index readings were taken from selected plants using an OPTI-Sciences CCM-200 chlorophyll index meter (CCI). This meter is useful in that it is non-destructive. It measures the ration of radiation transmitted through the leaf at 940nm and 660 nm. An area of 0.71 cm^2 is measured (N Knighton and B Biegbee, date unknown, www.optisci.com/datasheet/ccmosspad.pdf). The treatments selected for measurement are given in Table 3.3 and are a fair representation of all the treatment and mitigation factor combinations for both species (raw data appendix A).

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Table 3.3 - Plants selected for CCI measurements

Plants selected for CCI measurements					
Block 1		Block 2		Block 3	
Spinach	Control	Turnip	Control	Spinach	Control
	Control		Control		Control
Spinach	10 x Pb	Turnip	Control	Turnip	Control
	High Phos		Limit Cd		Control
Spinach	10 x Pb	Turnip	Limit Cd	Turnip	Limit Cd
	Low Phos		Low Phos		Control
Spinach	10 x Pb	Turnip	Limit Cd	Spinach	Limit Pb
	Low EDTA		High Phos		Control
Spinach	10 x Pb	Turnip	Limit Cd	Spinach	Limit Cd
	High EDTA		Low EDTA		High Phos
				Turnip	Limit Pb

					High Phos
				Spinach	Limit Pb
					High EDTA
				Turnip	Limit Cd
					High EDTA

3.3 Results

The fresh mass of both the spinach and the turnip shoots (Fig. 3.1) has been reduced significantly by the cadmium treatment, but not by lead treatments.

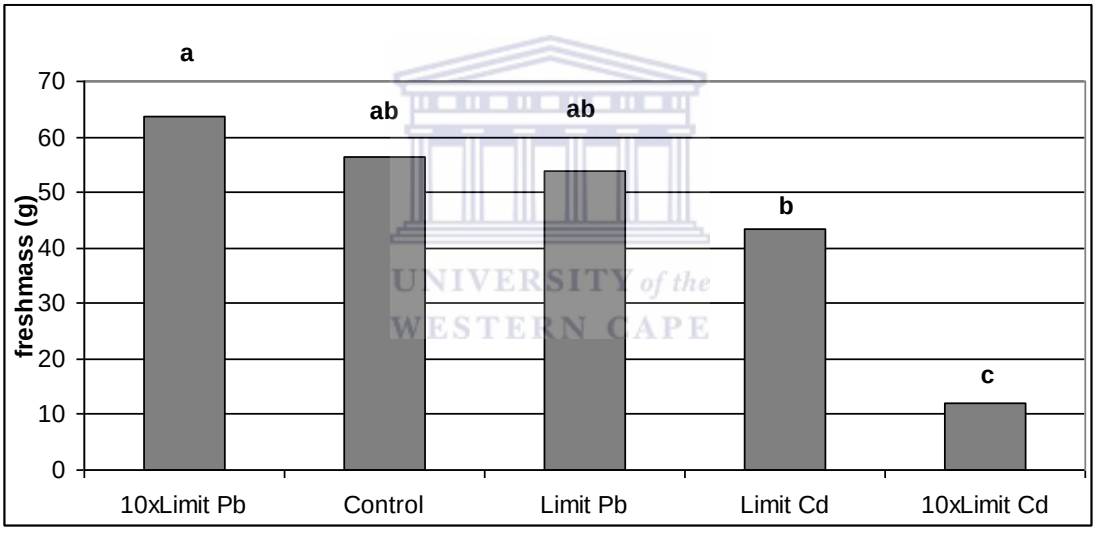

Fig. 3.1 The effect of lead and cadmium treatments on the fresh mass of both spinach and turnip shoots; the lsd at p = 0.05 is 17.022, bars with the same letter do not differ significantly.

In dry mass (Fig. 3.2) The lowest mean dry mass values are from 10 x Limit Cd, at almost ten times lower than that of the controls. Both the roots and shoots (Fig. 3.2 and 3.3) show the strongest inhibitor of growth to be cadmium treatments, though Pb also reduced the dry mass.

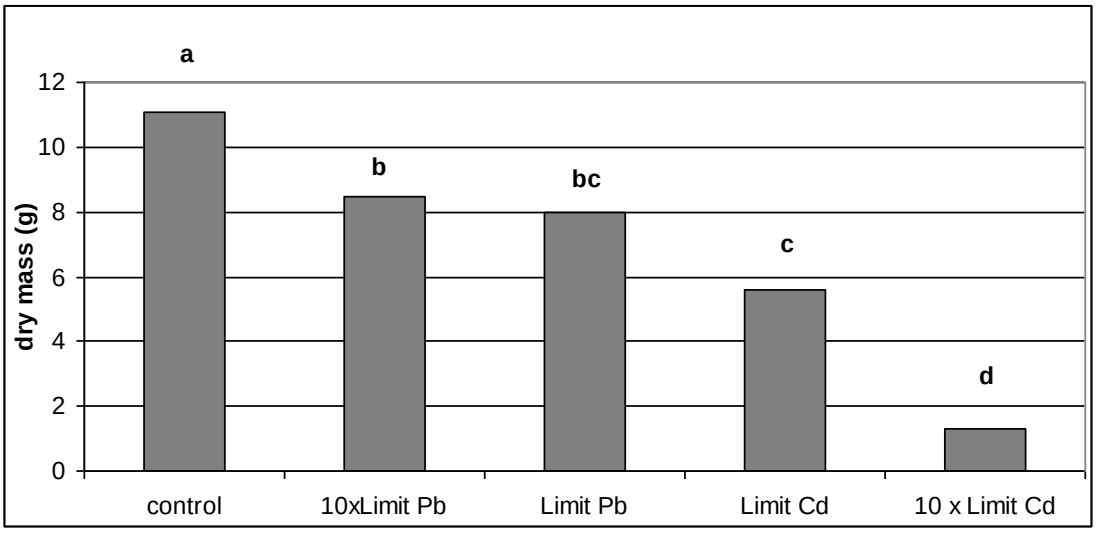

Fig. 3.2 The effect of lead and cadmium on the dry mass of both spinach and turnip shoots; the lsd at p = 0.05 is 2.6755, bars with the same letter do not differ significantly.

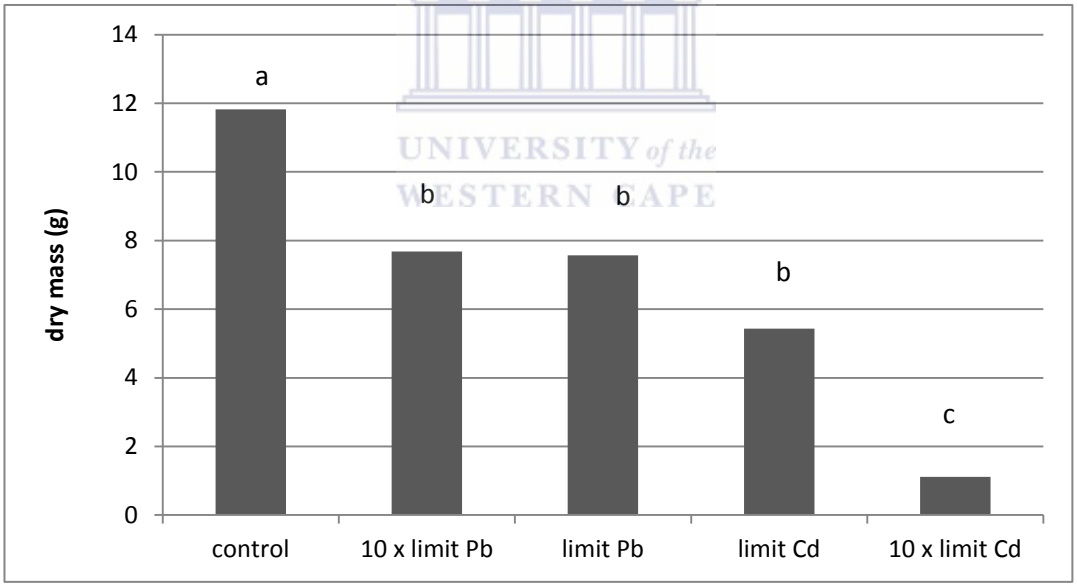

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Fig. 3.3 The effect of lead and cadmium on the dry mass of both spinach and turnip roots; the lsd at p = 0.05is 2.4495, bars with the same letter do not differ significantly.

The chlorophyll readings per treatment show an interesting trend (Fig. 3.4). Crops at the lower dose of Pb and Cd show statistically identical data, which indicate elevated chlorophyll content when compared to the higher treatments. The 10 x limit dose for Pb showed minimal effects, with chlorophyll content equal to that of the control.

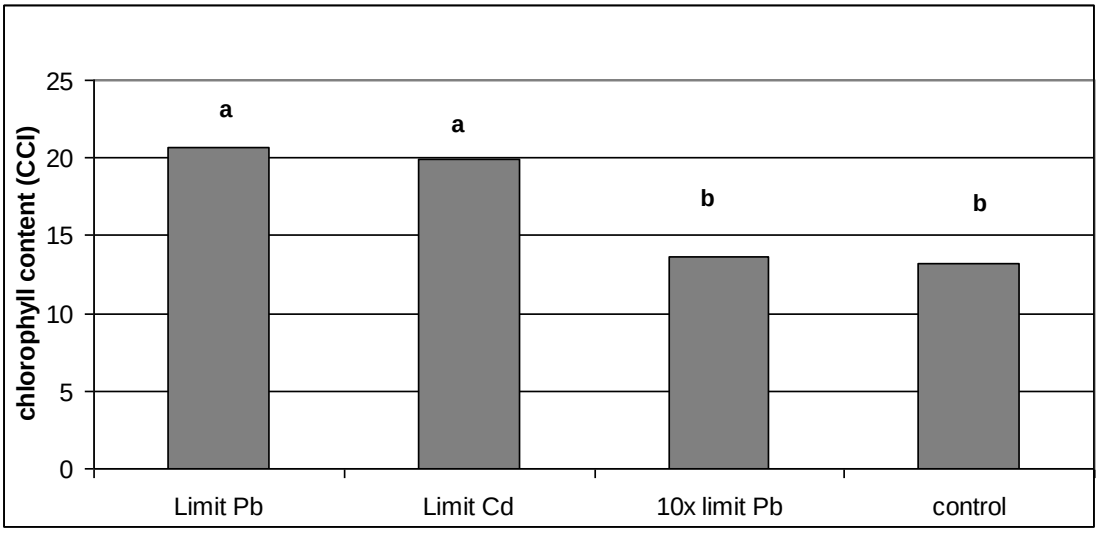

Fig. 3.4 The effect of lead and cadmium on the Chlorophyll index (CCI) for spinach and turnip plants; the lsd at p = 0.05 is 1.8377, bars with the same letter do not differ significantly.

When comparing the effects of mitigation treatments, EDTA at the low dose produced plants with the highest chlorophyll content (Fig. 3.5), and at high dose, did not differ from the control. P also increased the chlorophyll content.

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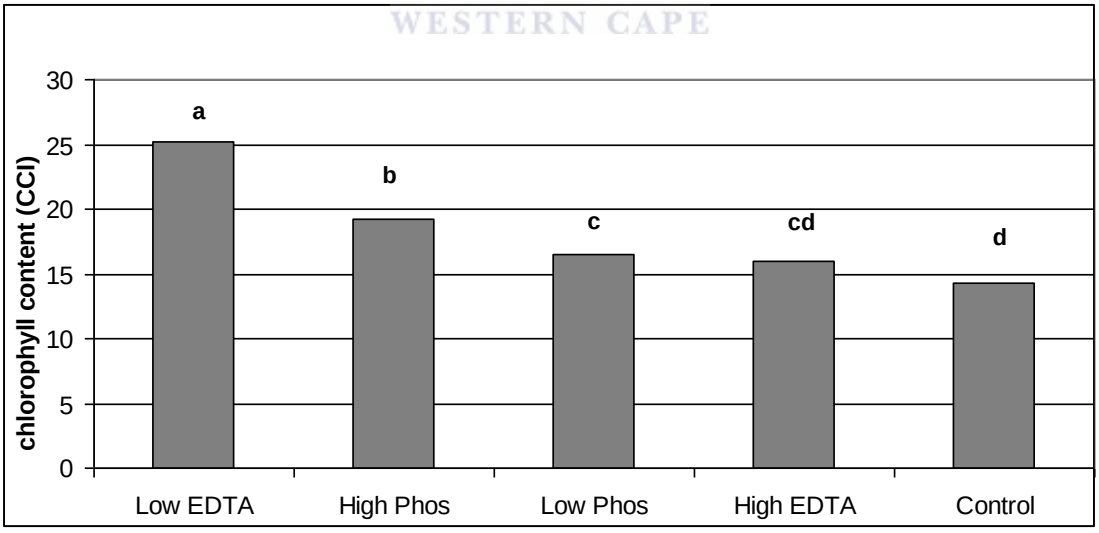

Fig. 3.5 The effect of mitigation treatments on the Chlorophyll index readings spinach and turnip plant; lsd at p = 0.05 is 2.1858, bars with the same letter do not differ significantly.

Analysis of both heavy metal treatment and mitigation factor together(Fig. 3.6) shows Limit Cd x Low EDTA and Limit Pb x High Phosphate had statistically similar values. Limit Cd whether combined with high EDTA, high Phosphate, or indeed the control, reveals similar values, which effectively means that the heavy metal treatment and not the mitigation factor dictated the increased chlorophyll content. The last two bars further illustrate this point with a eightfold difference in the mitigation factor, revealing insignificant differences in chlorophyll content.

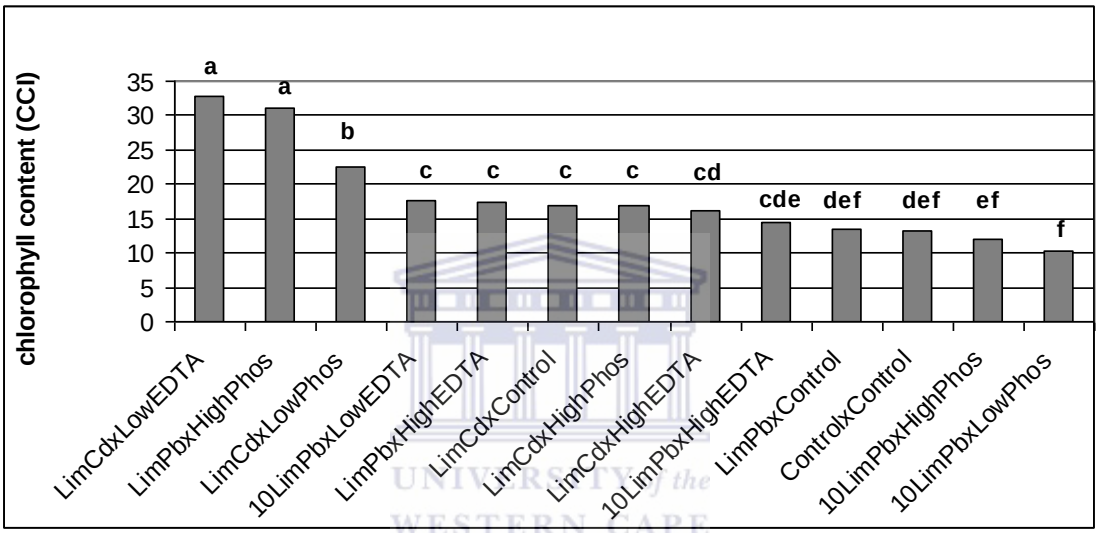

Fig. 3.6 The effect of the combination of heavy metals and mitigation factor on the Chlorophyll index values for both spinach and turnip plants; the lsd at p = 0.05 is 3.4606, bars with the same letter do not differ significantly.

There were no significant differences found in the fresh and dry mass of the shoots when tested by mitigation treatment.

The effect of the mitigation treatments on the dry mass of the roots is in agreement with the other findings of the research group (Meerkotter 2012) in that phosphate (Fig. 3.7) in fact promoted plant growth, while there were no significant differences between the other mitigation treatments.

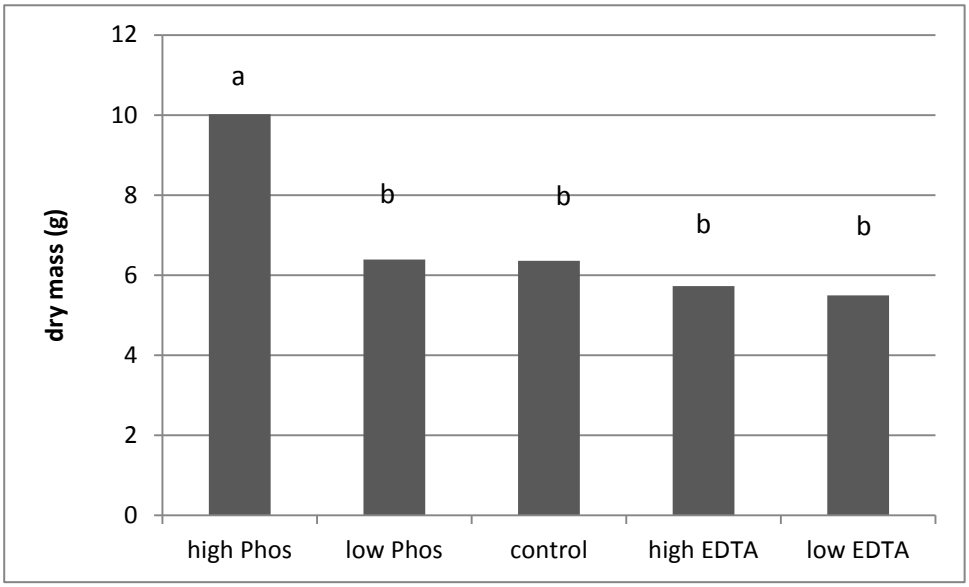

Fig. 3.7 The effect of mitigation treatment on the dry mass of both spinach and turnip roots; the lsd at p = 0.05 is 2.4495, bars with the same letter do not differ significantly.

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As expected, in spinach treated with heavy metals, the highest root dry mass was expressed in the control plants (Fig. 3.8). The 10x limit Pb and the limit Cd and Pb plants have similar values, and the lowest values are in the 10x limit Cd. The greatest effect on spinach root growth was at the high concentration of cadmium.

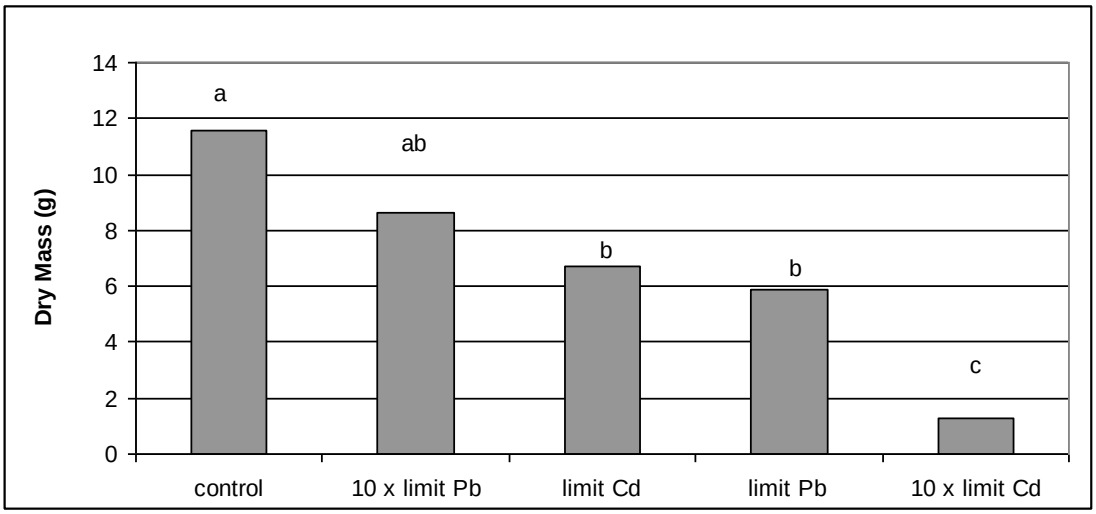

Fig. 3.8 The effects of the heavy metal treatments on the dry mass of spinach roots; the lsd at p = 0.05 is 3.3659, bars with the same letter do not differ significantly.

The fresh mass readings of the above ground parts of the spinach plants showed (Fig. 3.9) minimal variation amongst heavy metal treatments, except for the 10 x limit Cd which showed a major growth inhibition compared to the other treatments.

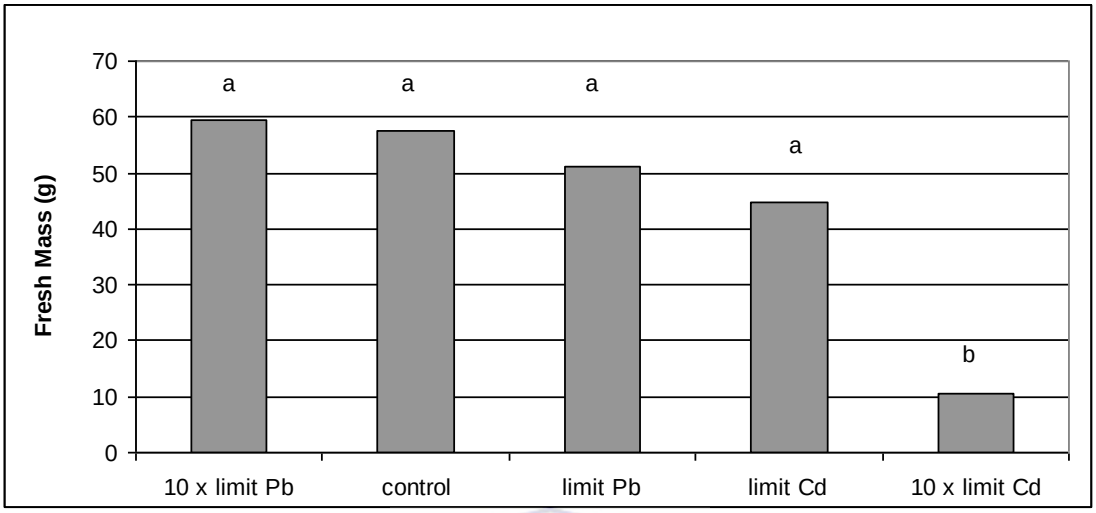

Fig. 3.9 The effect of heavy metal treatment on spinach shoot fresh mass; the lsd at p = 0.05 is 20.839, bars with the same letter do not differ significantly.

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The dry mass of the spinach roots (Fig. 3.10) fitted into three categories when treated with heavy metals. The first is the control where the values are highest, then the 10 x limit Pb, limit Pb and Cd that are all similair, then finally the last category, with the lowest values, is the 10 x limit Cd treatment, as is the case with the previous Fig.ures.

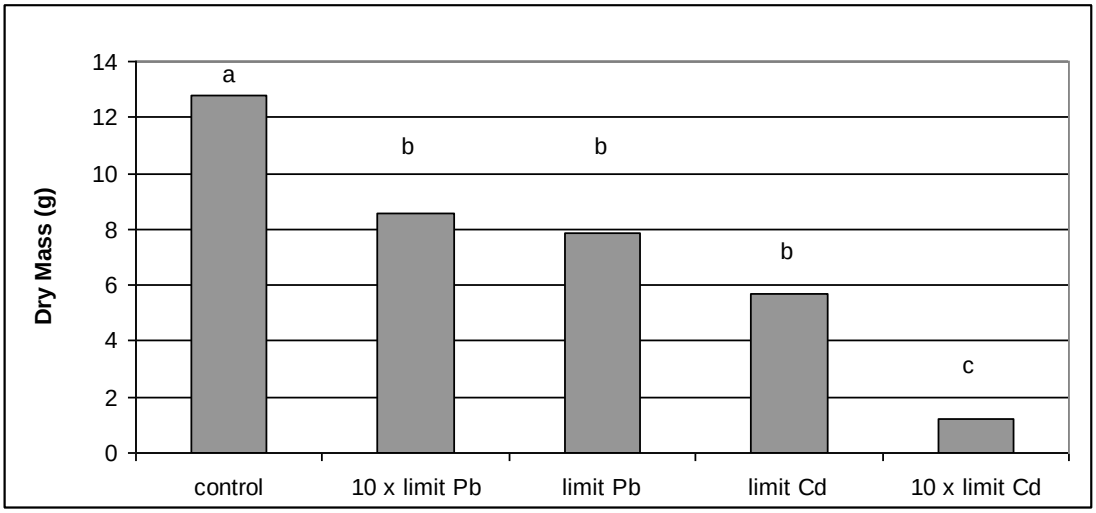

Fig. 3.10 The dry mass of spinach shoots arranged by heavy metal treatment; the lsd at p = 0.05 is 3.4146, bars with the same letter do not differ significantly.

The effect of the heavy metal mitigation treatment on the dry mass of the turnip roots (Fig. 3.11) showed decreases from the control to the 10 x limit Cadmium treatment, the general trend among most organs. The limit Pb, 10 x limit Pb and limit Cd values overlap with each other, leaving four categories of measurements, of which 10 x limit Cd is the lowest. This shows that cadmium has the greatest limiting effect on turnip root growth.

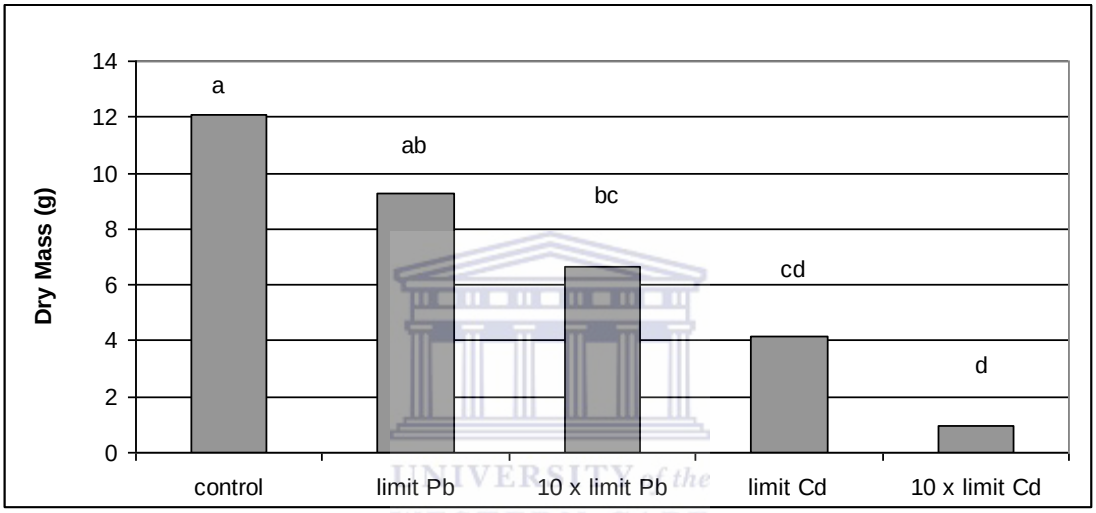

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Fig. 3.11 The dry mass of turnip roots arranged by treatment; the lsd at p = 0.05 is 3.2069, bars with the same letter do not differ significantly.

The fresh and dry mass of turnip shoots (Fig. 3.12 and Fig. 3.13) show very little variation among heavy metal treatments and do not differ from the control, except for the lowest value which is 10 x limit Cd, as is the case with many other parameters.

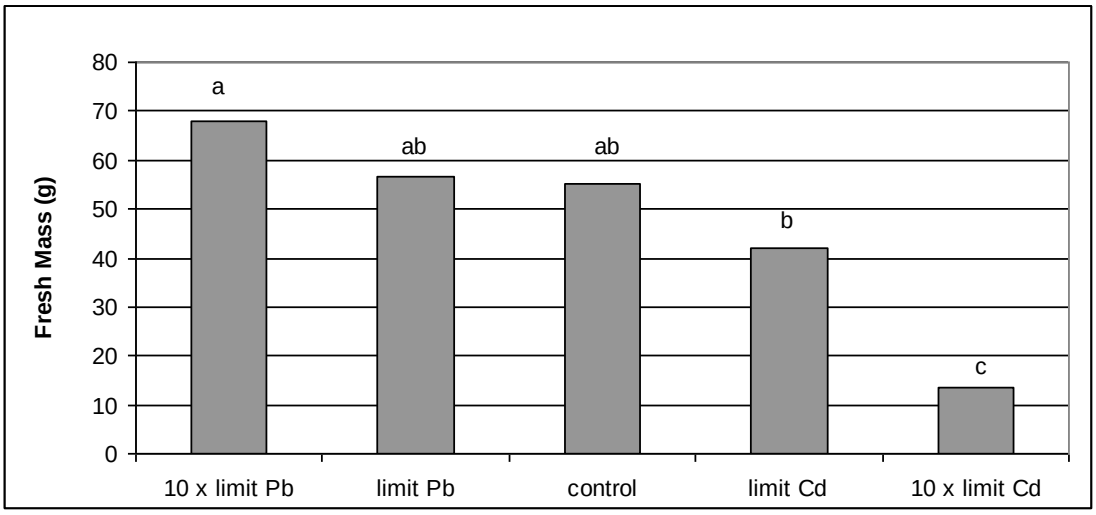

Fig. 3.12 The fresh mass of turnip shoots arranged by heavy metal treatment; the lsd at p = 0.05 is 21.164, bars with the same letter do not differ significantly.

The pattern in the dry mass values of the turnip shoots (Fig. 3.13) is similar to that of the fresh mass, with the Cadmium treatments having the most severe effect, of growth reduction.

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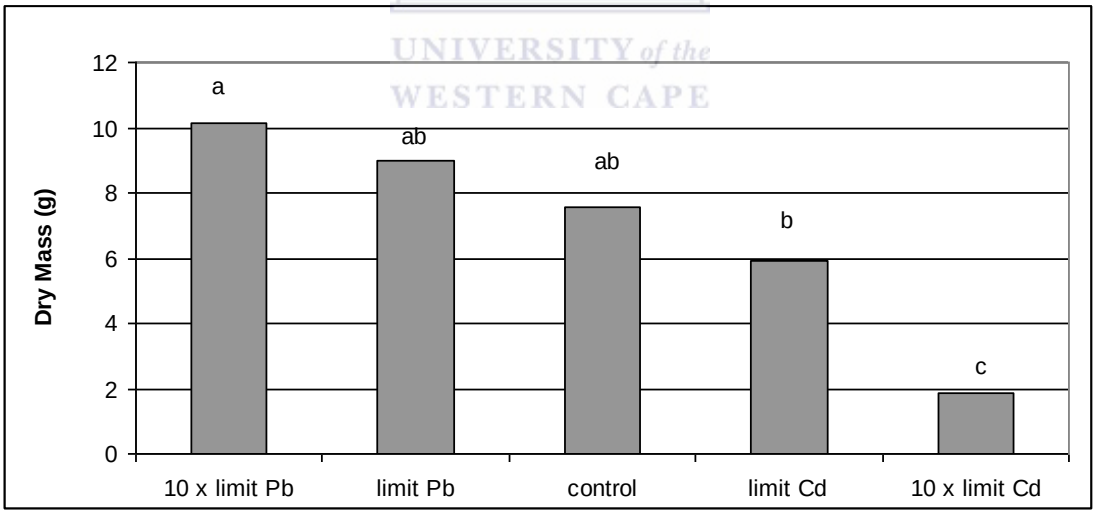

Fig. 3.13 The dry mass of turnip shoots arranged by heavy metal treatment; the lsd at p = 0.05 is3.1763, bars with the same letter do not differ significantly.

3.4 Discussion

The results described here show the growth and development of the spinach and turnip crops grown with Cd and Pb, mitigated by two methods, EDTA (chelating) and phosphate (precipitation).

A number of heavy metals are available to crops through the intensive use of agrichemicals (pesticides, fertilizers) and the photosynthetic apparatus appears to be quite sensitive to the toxicity of these heavy metals (Zhang and Shan, 2008). Cd, Cu, Hg, Pb, Zn ions affect photosynthetic functions both direct and indirectly. The scale and character of these effects is dose dependant and also species specific, depending on the crops tolerance levels. Reactions occur at various levels of organisation and architecture: in the leaves, leaf tissues, interactions with cytosolic enzymes and organics, and may even alter the chloroplast membrane. Cadmium has proven to be the most effective inhibitor of photosynthetic activity. Research has shown that cabbage production yields could be reduced by as much as 20% to 25% in the presence of a solution of only 1 mg.l-1 Cd (DWAF, 1996; Meerkotter, 2003; WRC, 1997). Effects on net photosynthesis have been shown in tomato, rice and maize, and even very small amounts entering the chloroplast can have large scale effects, due to the phenomena known as the “effect of multiplication” (Mysliwa-Kurdziel *et al.*, 2004).

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As was the case when both species were taken together (Fig. 3.3) Cd resulted in a greater reduction of root dry mass than Pb in both spinach (Fig. 3.8), and turnip (Fig. 3.11). Pb did not affect shoot fresh mass in spinach (Fig. 3.9) or turnip (Fig. 3.12), however in both cases 10 x Cd resulted in major depression of growth, as similarly

found by Meerkotter (2012). Considering the shoot dry mass, both Pb and Cd caused a reduction in spinach (Fig. 3.10) with 10xCd accounting for an even greater response. However Pb did not reduce turnip dry mass whereas the high Cd treatment did.

Lead at the levels supplied had little effect on fresh mass but decreased dry mass production**,** cadmium had the greatest effects on both fresh and dry mass. Lead did not significantly reduce root growth (Fig. 3.3 /3.8/ 3.11), in this experiment, as it is well documented to (Li *et al.,* 2010; Marschner, 1995; Prasad and De Oliveira, 2003; Salisbury and Ross, 1992; Zhu *et al.,* 2004)

The fresh and dry mass results revealed that cadmium caused a severe depressive effect on the biomass production of both crops (Fig. 3.1-3.3). The control plants fresh mass (Fig. 3.1) were about six times larger than those treated with the high dose of cadmium. Dry mass values of the control plants showed ten times the growth of those treated with high-dose Cd.

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Chen *et al.,* (2004) found that both the direct adverse action of EDTA and the increased bioavailability of soil metals could reduce plant growth. Lai and Chen (2004) reported that plants grown in highly concentrated Pb-contaminated soil reduced in biomass, growth and total chlorophyll content. Monocot species showed a reduced response to the addition of EDTA, exhibiting little chlorosis, and minor reductions of shoot mass. Dicot species on the other hand (as in the case of spinach and turnip in this study) were highly sensitive to EDTA application with leaves exhibiting visual symptoms such as curling, chlorosis, necrosis and stunting (Chen *et al.,* 2004).

Mitigation measures had no effect on shoot fresh and dry mass. In the roots however (Fig. 3.7) high phosphate actually increased growth, which is in agreement with similar research conducted by Meerkotter (2012).

The chlorophyll content readings showed crops treated with the lower doses of Cd and Pb to be statistically identical and higher than with the higher doses of Pb and the control (Fig.. 3.4) which agrees with literature that heavy metals inhibit the photosynthetic process (Lai and Chen, 2004). Using the mitigation factors as basis for comparison, low EDTA and both Phosphate treatments resulted in increased chlorophyll (Fig.. 3.5)

The compound effects of both the treatments and mitigation factor on chlorophyll production revealed little or no effect of the mitigation factor on the chlorophyll content. In the case of Pb for instance and eightfold difference in phosphorus, showed insignificant effects on chlorophyll content (Fig.. 3.6). Fig.. 3.4 shows that moderate concentrations of Pb and Cd in fact increased chlorophyll content, as did some of the mitigation measures (Fig.. 3.5).

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Reduced chlorophyll content due to the toxicity of Cd^{2+} and Pb^{2+} as well as many other ions, is well documented, with Cadmium-induced inhibition of chlorophyll biosynthesis results in a direct lowering of pigment content. Retardation of plant growth and chlorosis of leaves are often observed, and a lower photosynthetic pigment content induces changes in plastid development, photosynthetic efficiency as well as general metabolism (Mysliwa-Kurdziel *et al.*, 2004).

In summary, Cd reduced growth more than Pb in terms of the heavy metal treatments. As far as the mitigation treatments are concerned high Phosphate even resulted in increased growth. Several treatments, both metal and mitigation enhanced the chlorophyll content. Our experiments did not use the high concentrations reported elsewhere, current thought is that high doses of heavy metal depress chlorophyll production in plants (Mysliwa-Kurdziel et al., 2004, Amrate and Akretche, 2005; Brown *et al.*, 2005; Clemente *et al.*, 2005; Madejón *et al.*, 2006; Meer *et al.*, 2005). It can then be concluded from the large variation in results that Cd and Pb at the concentrations administered, and the two mitigation factors at theirs, did not to significantly retard the growth or development of spinach (*Spinacea oleracea* cv. 'ford hook giant') and turnip (*Brassica campestris* cv. 'early purple top globe') grown on silica soil.

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3.6 Appendix A: Chlorophyll index measurements CCI : raw data

Block 1				**Block 2**				
Spinach				**Turnip**				
Treatment	**Mitigation**	**CCI**				**CCI**		
Contol	Control	7.5		Control	control	14.2		
10 x Pb	High Phos	12.1		Control	Limit Cd	16.9		
	Low Phos	10.4		Limit Cd	Low Phos	22.6		
10 x Pb	High EDTA	14.4			High Phos	16		
	Low EDTA	17.7		Limit Cd	High EDTA			
					Low EDTA	32.8		
Block 3			**CCI**					
Control	Control	Spinach	15.7					
Control	Control	Turnip	15.3					
Limit Cd	Control	Turnip	17.1					
Limit Pb	Control	Spinach	13.4					
Limit Cd	High Phos	Spinach	17.9					
Limit Pb	High Phos	Turnip	31.1					
Limit Pb	High EDTA	Spinach	17.4					
Limit Cd	High EDTA	Turnip	16.2					

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Chapter 4: An assessment of the effect of two heavy metal and two mitigation techniques on the chemical composition of spinach and turnip using a pot experiment: Chemical analysis

4.1 Introduction

4.1.1 Remediation: Chelation and Precipitation

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Remediation of trace element-contaminated soils can be improved by in situ inactivation of contaminants through the use of soil additives. Forming insoluble chemical species reduces the amount leached through the soil profile, and that which is available for biological interaction. The use of soil additives is seen as a low input, cost effective remediation alternative for metal-contaminated soils (Salt *et al.*, 1998), and while it is well documented that additives such as lime, phosphates (like the triple super phosphate used in this experiment) and some organic waste products are effective in reducing the mobility and availability of trace elements in soils, no long term information is available about the stability and longevity of additives as soil parameters change. There is also little data on their effectiveness under field/real world conditions (Madejon *et al.*, 2009). A second type of soil additive is chelating (solubilising) agents that make the metal more available, to be taken up more easily the plant, and removed from the soil matrix, or leached to the ground water (Lestan *et al.*, 2008)

A number of conditions are important to achieve successful phytoextraction of a particular metal, the most import of these being bioavailability to the plant root (Marques *et al.,* 2008). Chelating agents have been found to be most effective extractents due to their high metal extraction efficiency, high thermodynamic stabilities and solubility of the metal complexes they form, and low adsorption to soils. They also have a very minor impact on the physical and chemical properties of the soil matrix (Lee and Marshall 2002, Lim *et al.,* 2004).

Numerous studies have reported that EDTA is able to extract large percentages of lead and cadmium from contaminated soils and have found a linear relationship between EDTA concentration and metal removal from soils (Steele and Pichtel, 1998; Papassiopi *et al.,* 1999; Garrabrants and Kosson, 2000; Kim and Ong, 2000; Wassay *et al.,* 2001).

There have however been few studies conducted on the effect of multiple heavy metals that are more bioavailable. A danger in chemically assisted phytoextraction is leaching to groundwater (Sun *et al.,* 2001). Chen *et al,* (2004) found that both the direct adverse action of EDTA and the increased bioavailability of soil metals could reduce plant growth.

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4.1.2 Lead and Cadmium

High levels of metal accumulation in the soil, ground and surface water affects the normal functioning of both plants and animals. In animals both the alimentary canal and respiratory system are affected, with lead and cadmium being listed among the most dangerous of the metals. Plants can accumulate quite considerable amounts of both cadmium and lead without any significant changes to their habit or yield, so these metals are easily introduced into the food chain (Piechalak *et al.,* 2003).

Plants, and even some microorganisms have a number of mechanisms to counter the effects of heavy metals, which include active translocation of metals, synthesis of peptides that bind metals (phytochelatins and metallothioneins), and storage in the vacuole (Piechalak *et al.,* 2003).

Cadmium can cause essential nutrient deficiencies and changes in N and P concentrations in plant tissue (Jalloh *et al.,* 2009). cadmium is strongly phytotoxic, and causes growth inhibition and even plant death due to its interference in photosynthesis, respiration and nitrogen assimilation in plants (Sanita *et al.,* 1999). The fact that cadmium influences N metabolism, has lead to the train of thought that N application may alleviate the toxic effect of cadmium in real soil conditions, by increasing the stromal proteins and photosynthetic capacity of the leaves and plant growth.

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Metals like lead are quite immobile in soils and the low solubility and bioavailability of some toxic metals is a major limiting factor in phytoextraction (Chen *et al* 2004). The exchangeable fraction of lead is very low in soils and the role of synthetic chelation agents is then to remobilise these metals and form strong soluble complexes (Neugschwandtner *et a.l.,* 2008). EDTA was also found to be the most effective at releasing soil-bound lead. The mobilizable and bioavailable fraction of lead (water soluble and exchangeable) is usually very low due to the strong association of lead with organic matter (Chen *et al.,* 2004). Chen *et al.* (2004) found the application of EDTA to the soil increased significantly the amount of lead extracted by the shoots of the plants tested. Reeves and Baker (2000) reported that the normal range of lead in shoots to be 0.1-5 $mg.kg^{-1}$ dry mass with most crops being unable to protect cells from excessive lead levels. The allowed maximum concentrations of heavy metals

allowed in vegetables by regulation of the Foodstuffs, Cosmetics and Disinfectants Act (Act no. 54 of1972)(Government gazette, 1994) for cadmium is 0.1 $mg.kg^{-1}$ and for lead is 0.3 $mg.kg^{-1}$ for spinach and 0.5 $mg.kg^{-1}$ for beetroot (which is the most similar listed vegetable). Larcher (2003) lists the range of lead in phytomass as up to 20 $mg.kg^{-1}$.

This study aimed to investigate the use of different concentrations of a triple super phosphate fertiliser and EDTA solutions to mitigating the uptake of cadmium and lead by spinach and turnip plants, with a particular focus on chemical composition in this chapter.

4.2 Materials and Methods

The experimental design, growth, harvesting and statistical analysis for this chapter are described in chapter three, as this chapter is analysis of the data focused on chelation and precipitation, while chapter three focused on growth and development. This chapter aims to focus on the effect of EDTA and phosphate on the chemical composition of the plant after the cadmium and lead additions. For the chemical analysis, the samples were dry-ashed and the ash was taken up in hydrochloric acid. The concentrations of the various elements were then determined with a Varian Radial ICP. The cadmium and lead determinations were carried out with a Perkin Elmer Optimia ICP after digestion with nitric acid and hydrogen peroxide.

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4.3 Results : Chelation and Precipitation

4.3.1 The relative species elemental content as affected by heavy metals and or mitigation treatments

The results found for this suite of elements follow. Turnip plants contained more N, P, K, Ca and B, while spinach had more Na, Mn and Zn, as shown in Table 4.1. Na shows the largest difference with approximately a 35% difference in accumulation values between turnip and spinach. cadmium and lead uptake have statistically similar uptake in both plants. The trend among most elements is that uptake was greater in the root crop, turnip, which is in agreement with current trends in the literature that roots take up and hold onto heavy metals and other elements (Greene 1993, Marschner, 1995; Prasad and De Oliveira, 2003; Salisbury and Ross, 1992).

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Table 4.1 – A comparison of the mean (n=3) elemental content of both crops. Significant differences are highlighted in bold ($p \leq 0.05$).

	Turnip	Spinach	Units
N	**1.764**	**1.518**	%
P	**0.458**	**0.410**	%
K	**3.058**	**2.753**	%
Ca	**1.092**	**0.566**	%

Na	**2720**	**4225.5**	$mg.kg^{-1}$
Mn	**51.939**	**72.136**	$mg.kg^{-1}$
Zn	**79.660**	**110.830**	$mg.kg^{-1}$
B	**31.748**	**27.184**	$mg.kg^{-1}$
cadmium	310.31	292.43	$mg.kg^{-1}$
lead	735.4	908.3	$mg.kg^{-1}$

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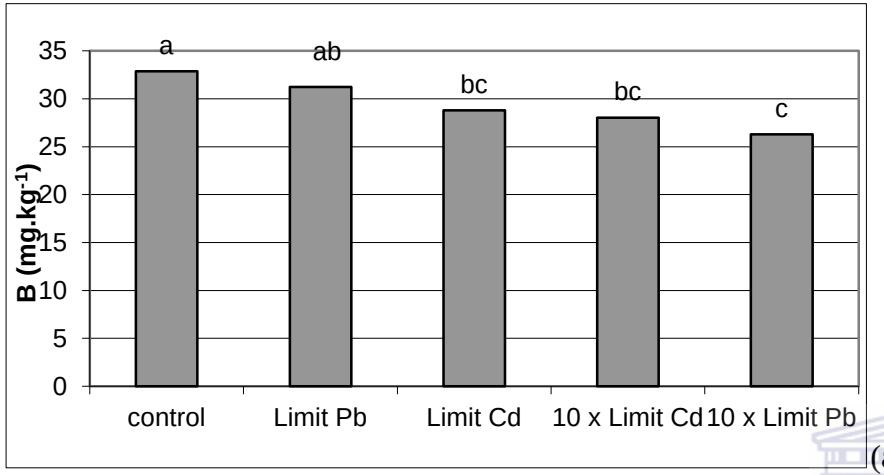
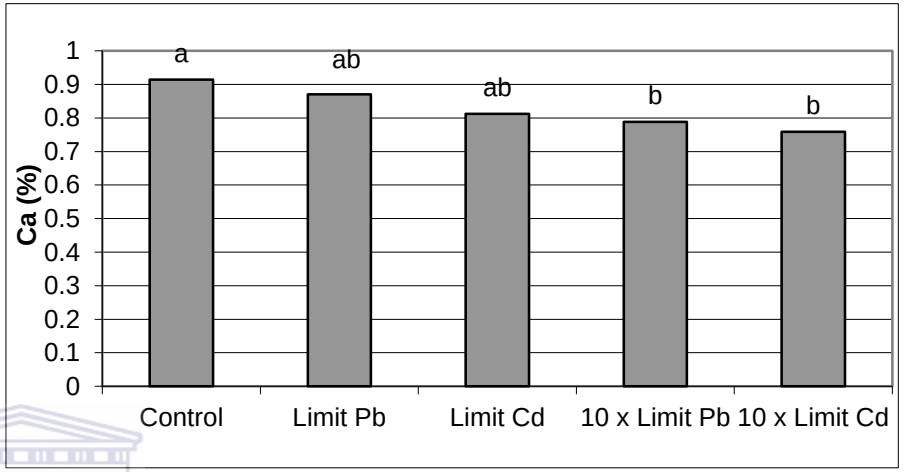
When comparing heavy metal treatments (Fig. 4.1 a-d), the greatest uptake of the elements B, Ca, Cu and K was in the control plants, as they had no inhibiting factors, with increasing inhibition as cadmium increased in the growth medium.

B uptake was highest in the control and lowest among the two highest heavy metal treatments (Fig. 4.1a). The calcium content was significantly lower in the high metal treatments as compared with the control, a sure sign of inhibition (Fig.ure 4.1b).

There was a decreasing trend in copper content, as the heavy metal treatment concentration increases (Fig. 4.1c). cadmium, as is the case with other elements (Fig. 4.1b+d), appeared to have the greatest inhibitory effect on copper content. Potassium content (Fig. 4.1d) was found to be highest in the control and the Limit lead treatments – showing lead had no effect on K uptake at low concentrations.



(a)



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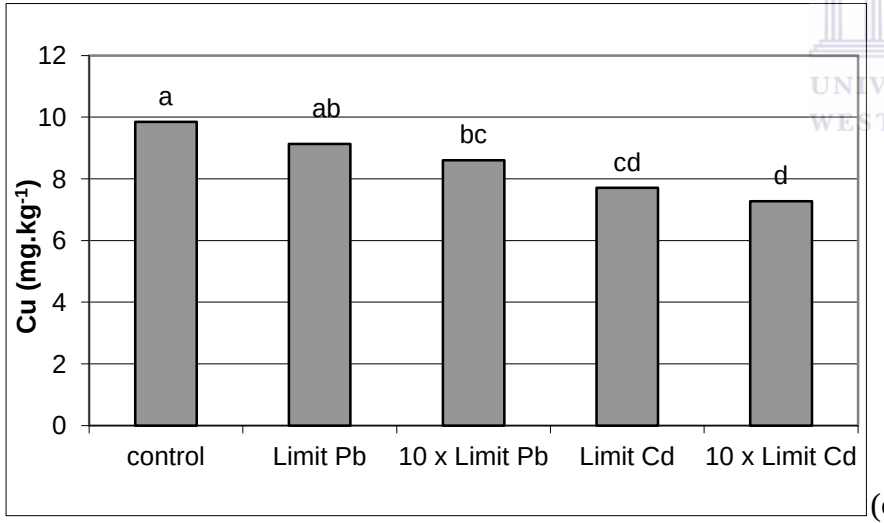
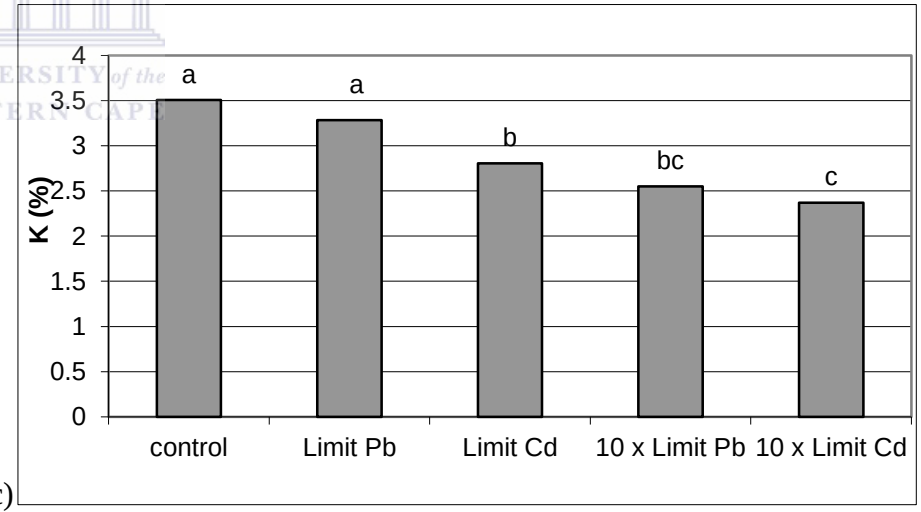


(c)



(d)

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Fig. 4.1 – The effect of heavy metal treatments on boron, calcium, copper and potassium concentrations in the spinach and turnip plants;
(a) Boron ($mg.kg^{-1}$), the lsd at p = 0.05 was 3.3729; bars with the same letter do not differ significantly.
(b) Calcium (%), the lsd at p = 0.05 was 0.1182; bars with the same letter do not differ significantly.
(c) Copper ($mg.kg^{-1}$), the lsd at p = 0.05 was 0.8974; bars with the same letter do not differ significantly.
(d) Potassium (%), the lsd at p = 0.05 was 0.331; bars with the same letter do not differ significantly.

The elemental concentrations in Fig. 4.2a, b and c (Mg, Mn and Na) follow the same trend. The highest concentrations are in the control plants, and the lowest in the plants treated with the highest cadmium concentration. They are all significantly different from the control, and the crops therefore show significant inhibition of these elements by high doses of cadmium. In addition it is clear that cadmium has a more severe effect than lead, as the lowest value is found with the highest cadmium additions. With sodium content there is a significant difference in uptake between species, with the spinach crop being highest (Table 4.1). There is however no difference in the mean values of the control and lead and cadmium (Fig. 4.2c) at their respective legal limits for agriculture (DWAF, 1999). The ten times limit values for cadmium and lead however show some inhibitory effects, the most severe of these being cadmium at ten times the limit. In the case of nitrogen, all the heavy metal treatments appear to have the same effect, with only the control being significant different from the low cadmium treatment (Fig. 4.2d)

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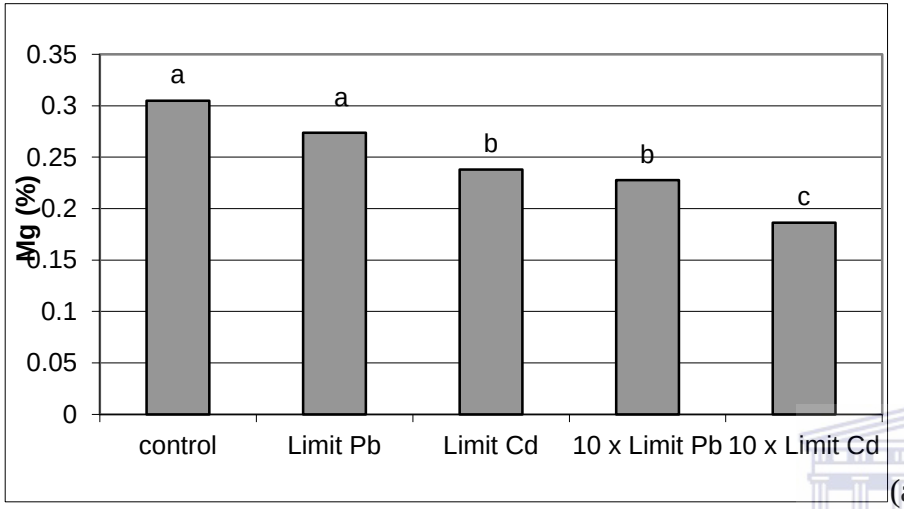
The Phosphorus content, Fig. 4.2e was found to be highest in the control plants, and lowest in the two highest metal contents, which would indicate an inhibition of phosphorus uptake by heavy metals at higher concentrations, or bound phosphate being unavailable to plants.



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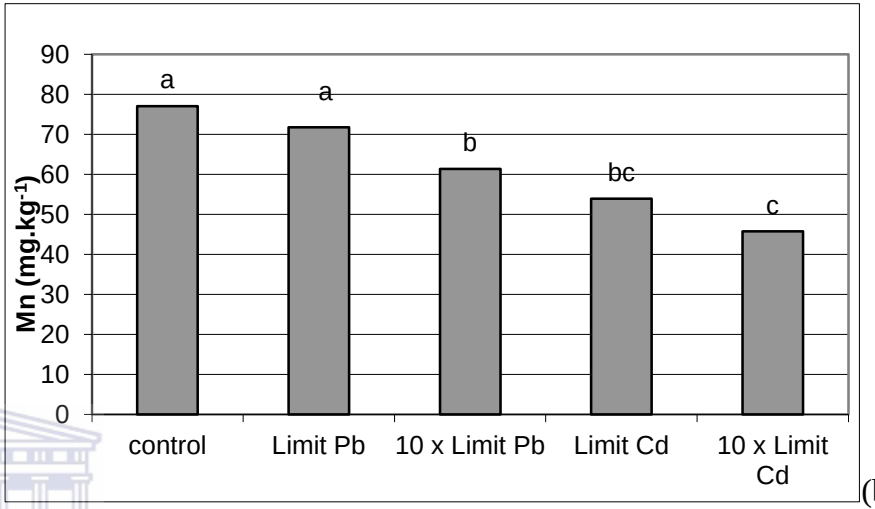

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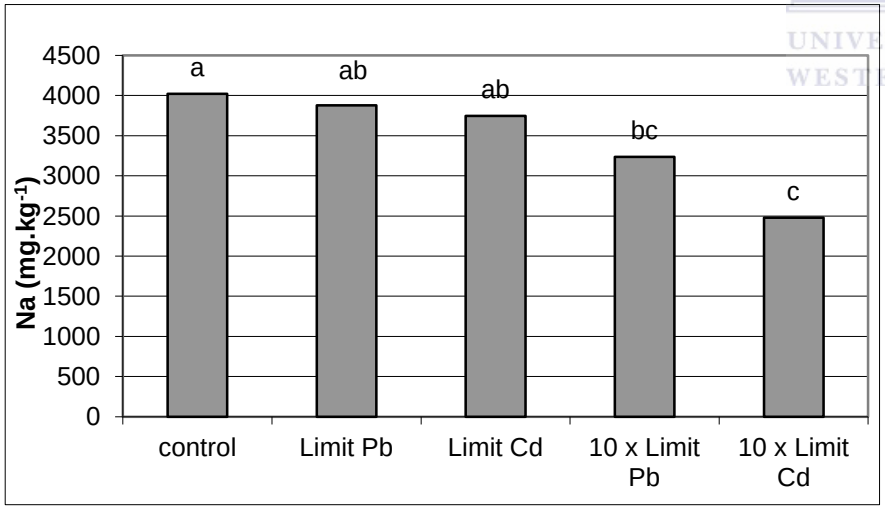

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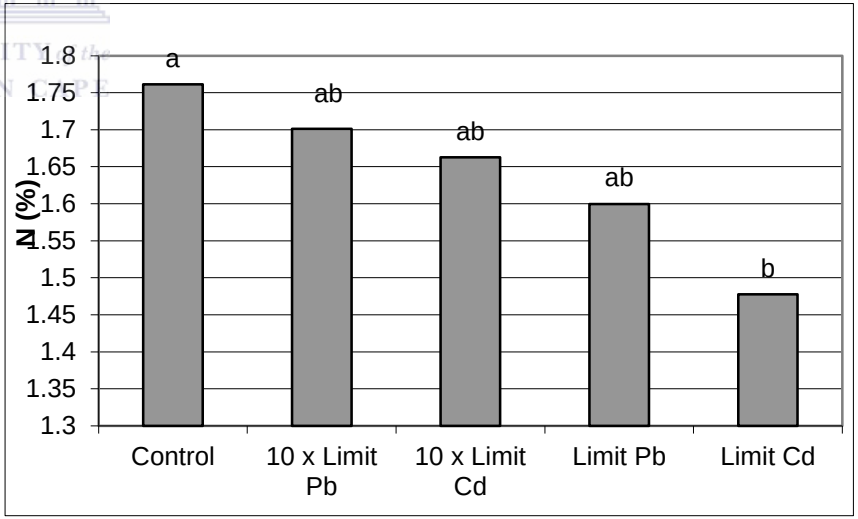

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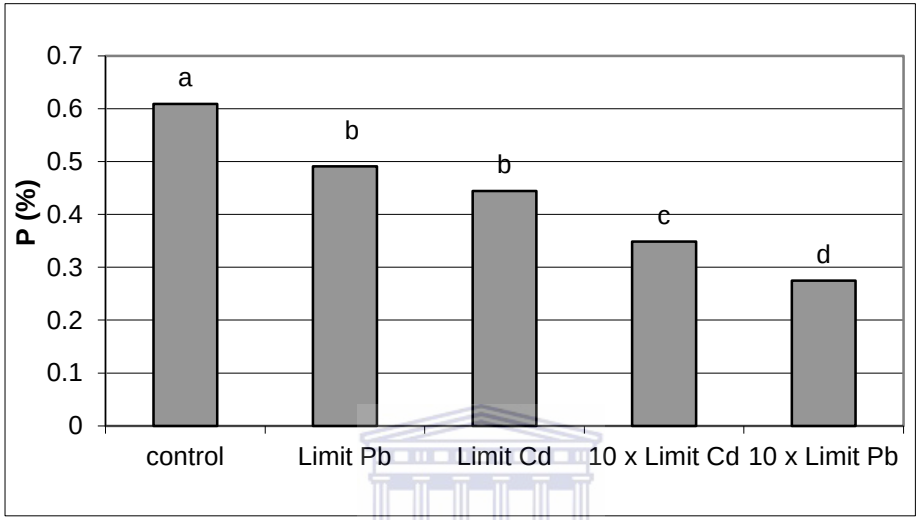


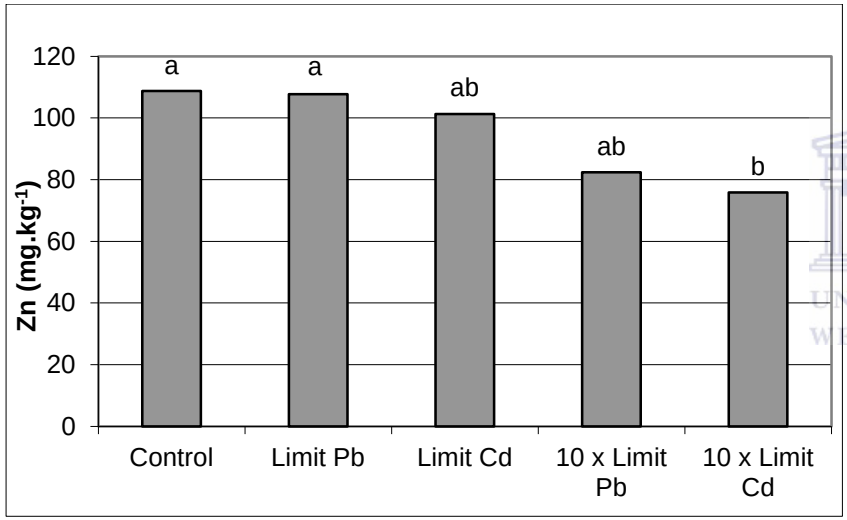
(e)

Fig. 4.2 – The effect of heavy metal treatments on elemental uptake:

(a) magnesium content (%) of both crops; the lsd at p = 0.05 was 0.0351; bars with the same letter do not differ significantly.
(b) manganese content ($mg.kg^{-1}$) of both crops; the lsd at p = 0.05 was 9.1397; bars with the same letter do not differ significantly.
(c) sodium content ($mg.kg^{-1}$) of both crops; the lsd at p = 0.05 was 779.79; bars with the same letter do not differ significantly.
(d) nitrogen content (%) of both crops; the lsd at p = 0.05 was 0.2329; bars with the same letter do not differ significantly.
(e) phosphorus content (%) of both crops; the lsd at p = 0.05 was 0.0626, bars with the same letter do not differ significantly.

Zinc accumulation (Fig. 4.3a) was inhibited the most by the high cadmium treatment, with no significant difference between the other treatments. There is a decrease in cadmium content from left to right in Fig. 4.3b, with the lowest being in the plants not supplied with cadmium. The highest cadmium content is predictably highest in the plants treated with 10 times the legal limit of cadmium.

There is a similar decrease in lead content from left to right (Fig. 4.3c), with the highest value being the 10 times limit lead and the lowest values recorded in the plants supplied with lead, as expected. While mitigation treatments did not affect cadmium content, the lead content was reduced by the high phosphorus mitigation treatment.



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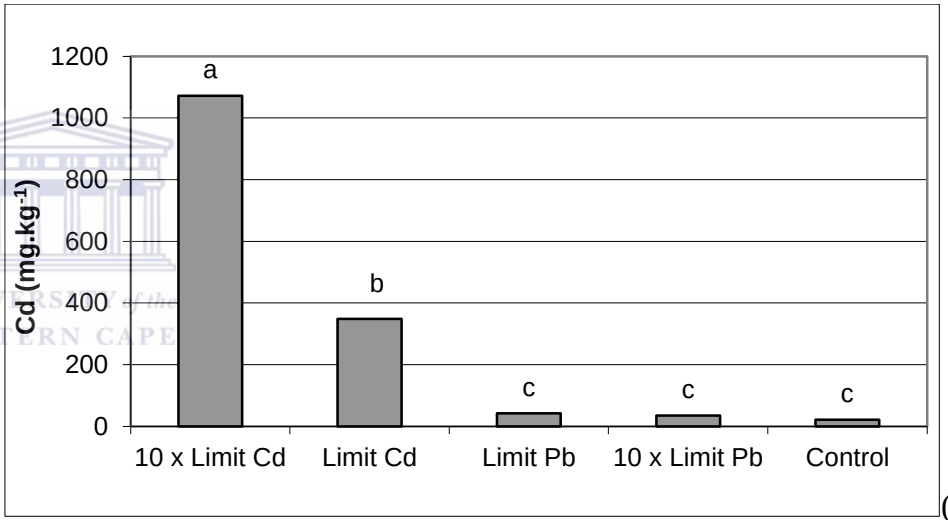

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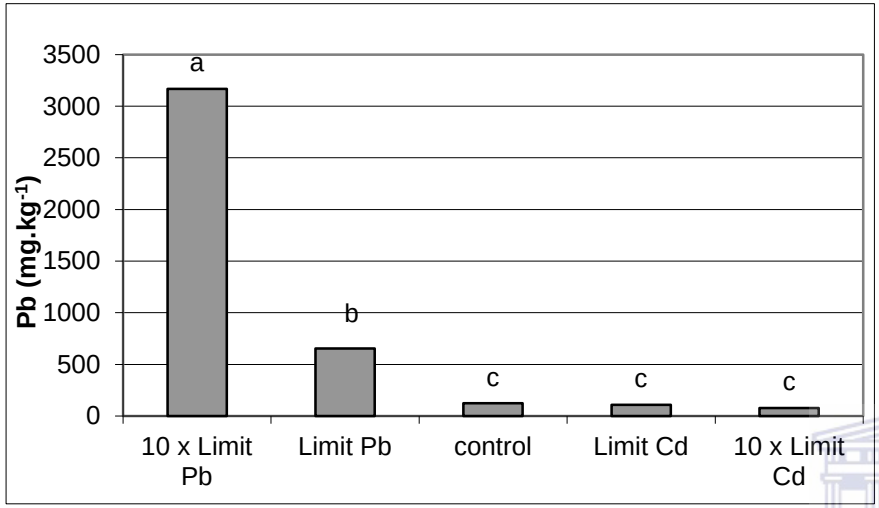

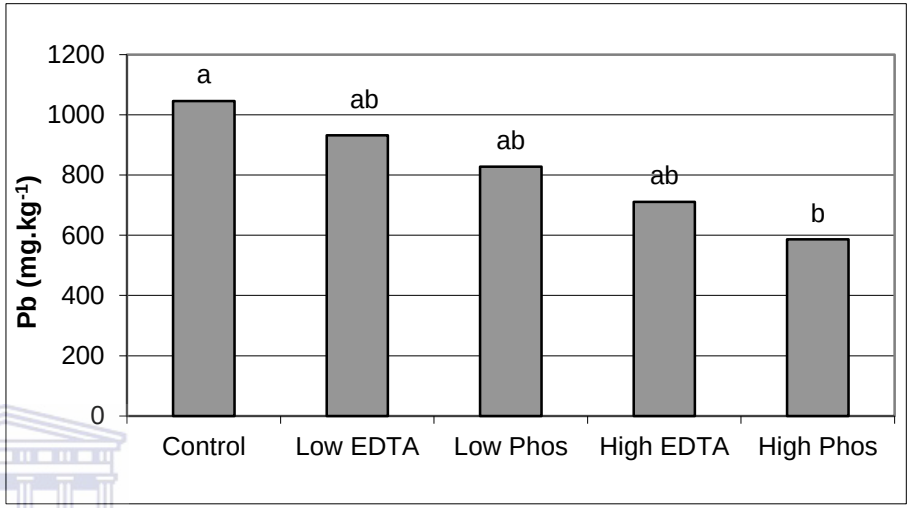

(c)

Fig. 4.3 – The effect of heavy metal treatments on the zinc, cadmium and lead content
(a) zinc content ($mg.kg^{-1}$) of both crops; the lsd at p = 0.05 was 27.281; bars with the same letter do not differ significantly.
(b) cadmium content ($mg.kg^{-1}$) of both crops; the lsd at p = 0.05 was 155.47; bars with the same letter do not differ significantly.
(c) lead content ($mg.kg^{-1}$) of both crops; the lsd at p = 0.05 was 377.49; bars with the same letter do not differ significantly.

Fig. 4.4 - The effect of mitigation treatments on lead content ($mg.kg^{-1}$) of both crops; the lsd at p = 0.05 was 377.49**; b**ars with the same letter do not differ significantly

While mitigation treatments did not affect cadmium content, the lead content was reduced by the high phosphorus mitigation treatment.

4.3.2 The combined effects of heavy metal and mitigation treatments on the concentrations of the elements studied.

The concentration of the heavy metals has proved to be more important than any mitigation treatment in determining the level of that heavy metal in the plant Fig.ures: 4.5-4.8). Irrespective of mitigation treatment, the high cadmium treatments resulted in significantly enhanced cadmium in spinach plants (Fig. 4.5). Low and High P and High EDTA resulted in even higher cadmium than no mitigation treatment with the high cadmium treatments. The fact that low cadmium treatments are equivalent to lead treatments as far as cadmium uptake, may mean that significant uptake of cadmium in spinach plants is only possible at high concentrations. All the lead treatments did not differ significantly from the control, so lead had no influence over cadmium uptake in spinach plants.

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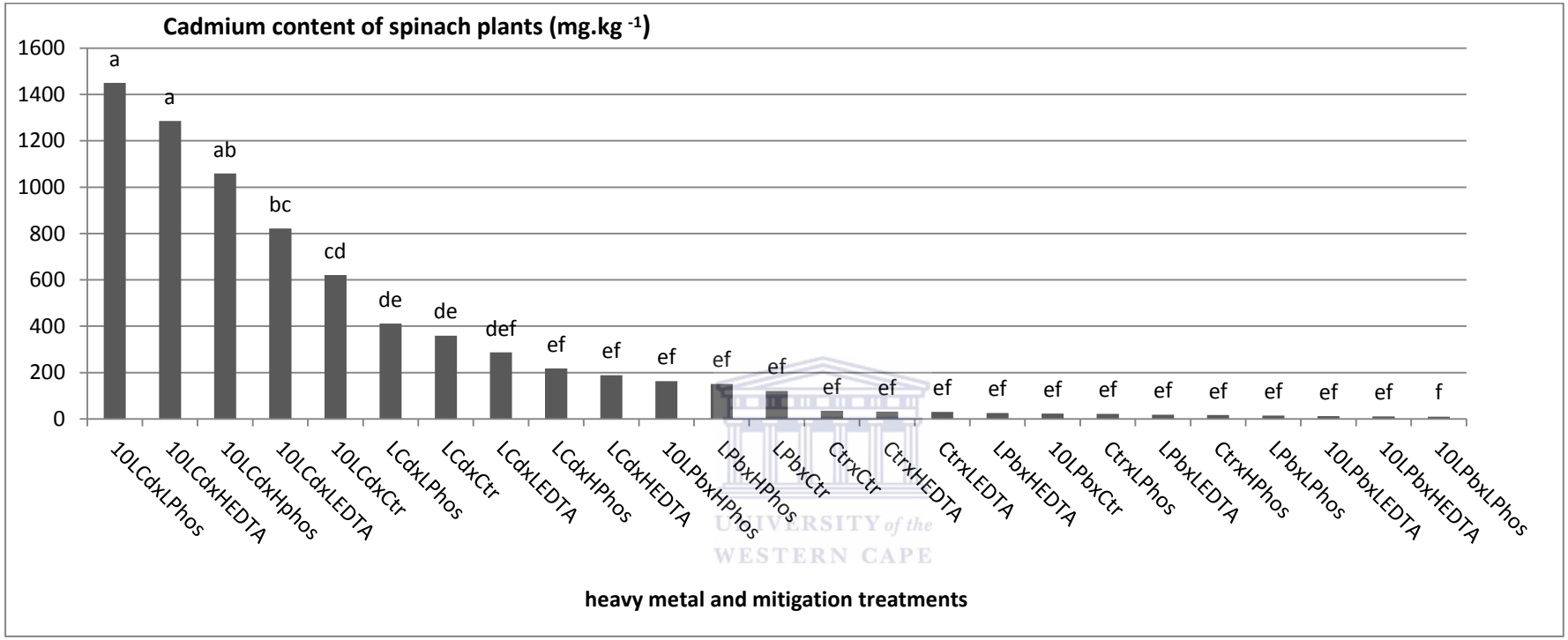

Fig. 4.5 The effect of heavy metal and mitigation treatments on cadmium content ($mg.kg^{-1}$) of spinach plants; the lsd at p = 0.05 was 401.29**;** bars with the same letter do not differ significantly.

High lead treatments, irrespective of mitigation treatment resulted in significantly higher lead in spinach plants than in the control plants (Fig. 4.6). High lead with low EDTA significantly enhanced lead uptake over the low lead with no mitigation treatment and the high lead high phosphorus treatment. All the cadmium treatments did not differ significantly from the control, so cadmium clearly had no influence on lead

uptake in spinach plants. At low lead concentrations, all mitigation treatments resulted in a reduced lead concentration when compared with the control.

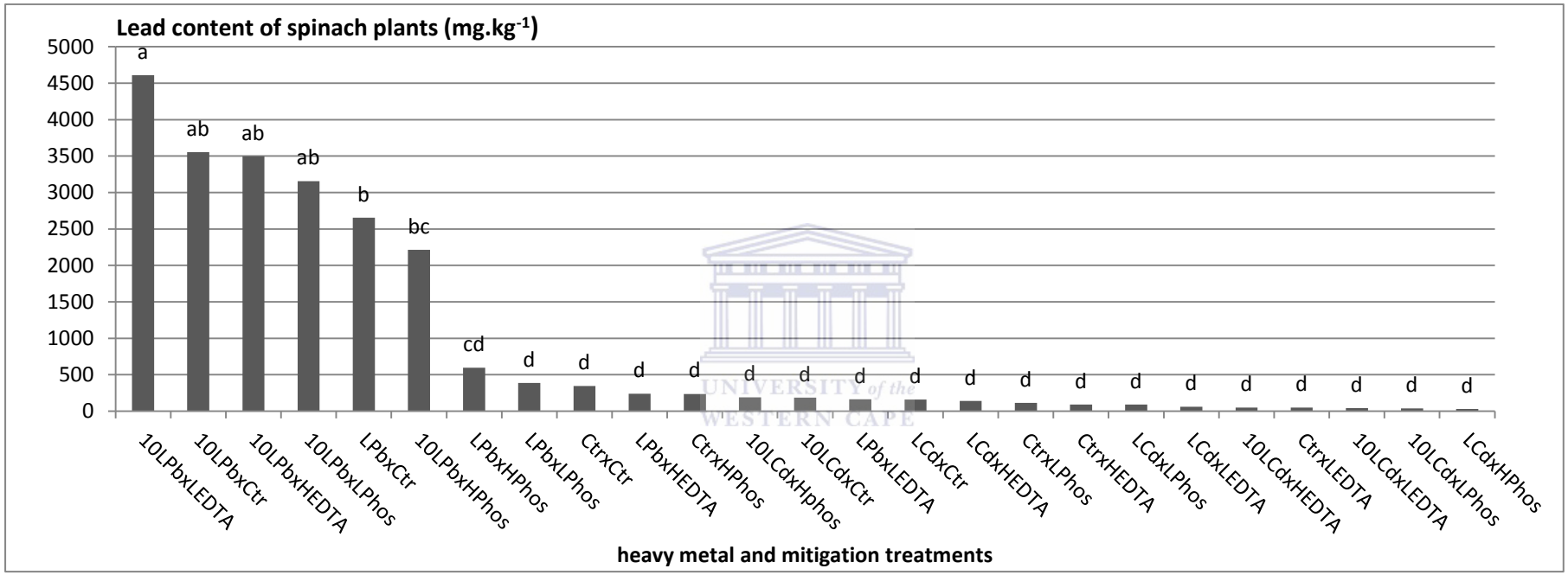

Fig. 4.6 The effect of heavy metal treatments on lead content of spinach (mg.kg^{-1}); the lsd at p = 0.05 was 1732.4; bars with the same letter do not differ significantly.

All high cadmium treatments and the low cadmium with the low phosphorus treatment resulted in significantly increased cadmium in turnip plants (Fig. 4.7). Both mitigation treatments of the high cadmium plants resulted in reduced cadmium concentrations. This differs from what was

found with spinach, where most mitigation treatments seemed to have insignificant effects. All the lead treatments did not differ significantly from the control, so lead clearly had no influence on cadmium uptake in turnip plants.

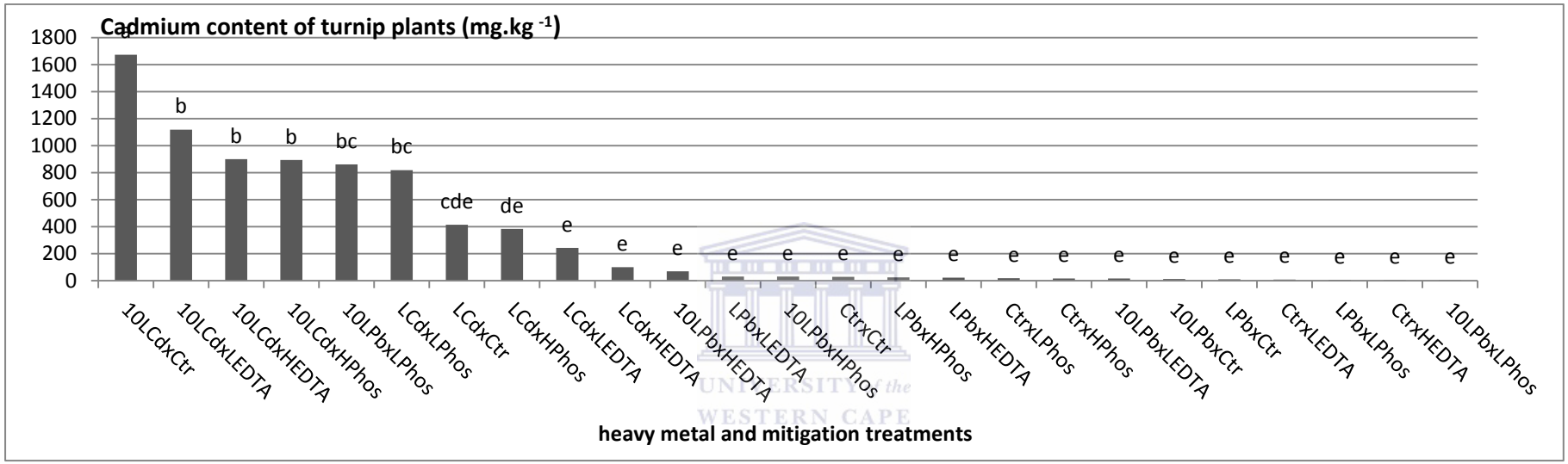

Fig. 4.7 The effect of heavy metal and mitigation treatments on cadmium content of turnip (mg.kg^{-1}); the lsd at p = 0.05 was 446.87**;** bars with the same letter do not differ significantly.

All high lead treatments resulted in significantly increased lead concentrations in turnip plants (Fig. 4.8). the low level mitigation treatments resulted in higher lead concentrations in the turnip plants than the high level mitigation treatments.. All the low lead and all the cadmium treatments did not differ significantly from the control, so cadmium treatments and low lead had no influence on lead uptake in the turnip plants.

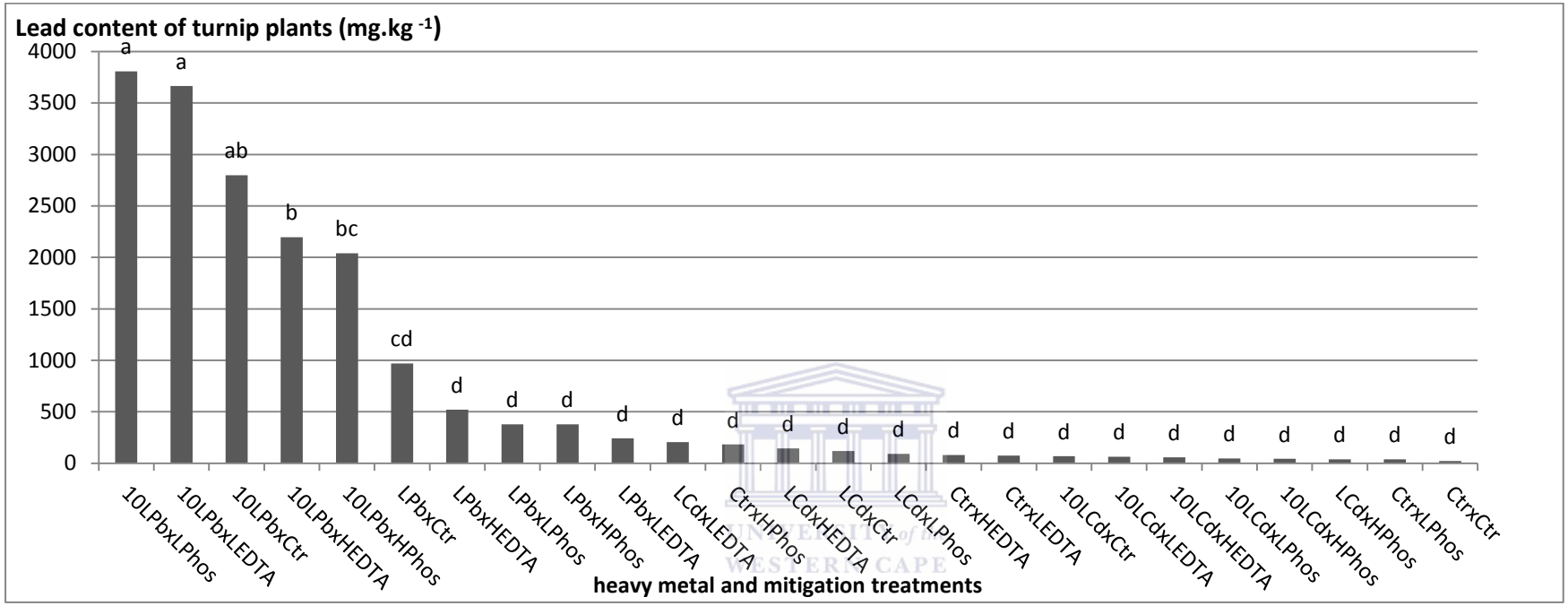

Fig. 4.8 The effect of heavy metal and mitigation treatments on the lead content of turnip plants (mg.kg^{-1}); the lsd at p = 0.05 was 1208.3**;** bars with the same letter do not differ significantly.

4.3.3 The relative shoot and root elemental content as affected by heavy metals and or mitigation treatments

The shoots took up significantly more Mg and Mn (Table 4.2), while the roots contained more Fe, Cu, lead and Zn. Roots have seven times as much lead as the shoots. cadmium uptake is the same for both organs. In the case of manganese and magnesium, both elements were taken up more in the shoots than the roots, which puts Mn in contrast to the popular thought currently that the roots retain most of the heavy metals taken up by crops (Table 4.2). The trend in most current literature is that roots are the major storage organs of heavy metals taken up from soils (Meighan *et al*., 2011).

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Table 4.2 – A comparison of the elemental content of the organs of both crop species in % and $mg.kg^{-1}$ respectively. All the elements with significant differences are displayed in bold ($p \leq 0.05$).

	Shoots	Roots	Units
N	2.507	0.788	%
P	0.641	0.233	%
K	4.810	1.053	%
Ca	1.352	0.320	%
Mg	**0.404**	**0.093**	%
Na	5065.700	1922.700	$mg.kg^{-1}$
Mn	**74.483**	**49.926**	$mg.kg^{-1}$
Fe	**521.430**	**2363.490**	$mg.kg^{-1}$
Cu	**6.090**	**10.879**	$mg.kg^{-1}$
Zn	**47.345**	**141.859**	$mg.kg^{-1}$

B	47.552	11.866	$mg.kg^{-1}$
cadmium	273.6	328.14	$mg.kg^{-1}$
lead	**171.1**	**1451.3**	$mg.kg^{-1}$

4.3.4 Elemental content of the different species shoots and roots

The boron concentration was greater in the shoots than in the roots, with higher levels in turnip shoots than spinach shoots (Fig. 4.9a) but an identical concentration of boron in the roots of both crops. Calcium content (Fig. 4.9b) shows great variety, with each being significantly different from the other, and turnip shoots concentration being by far the highest. Concentration of calcium was greater in the shoots than in the roots, with the turnip plants overall accumulating more calcium than the spinach. Copper concentration (Fig. 4.9c) fits into two groups – by organ, with the roots of both species higher than in the shoots, with no significant differences between species. This is agreement with popular thought on heavy metal uptake (Marshner 1995). The iron content (Fig. 4.9d) of both species was highest in the roots, with significant differences seen between both species and organs. Spinach roots had more, and shoot less iron than turnip roots and shoots respectively. Shoot potassium concentration (Fig. 4.9e) was identical between species and greater than root, with turnip roots accumulating more than spinach roots. Shoot Magnesium concentration (Fig. 4.9f) was greater than root, with no significant differences between species. Manganese content (Fig. 4.9g) was highest in the spinach shoots (edible parts), and lowest in the turnip roots (edible parts) with the turnip shoots and spinach roots not differing significantly from each other. Sodium concentration (Fig. 4.9h) shows a great variety and was greatest in the spinach shoots and lowest in the spinach roots, with turnip roots and shoots as significantly different intermediates. The uptake of nitrogen (Fig. 4.9i) was greater in the shoots than in the

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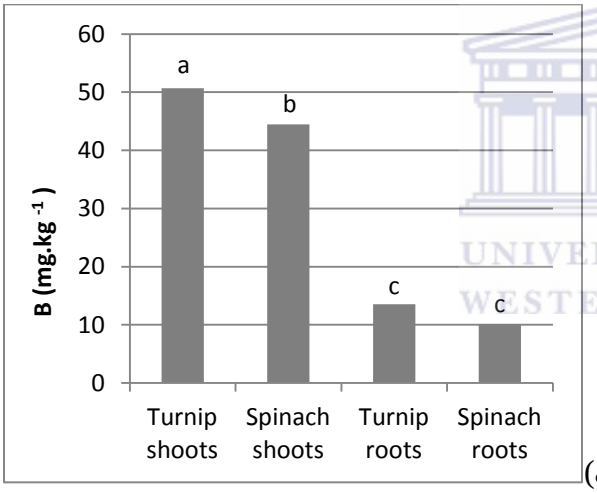
roots, with no significant difference between shoots of both species. The uptake of phosphorus (Fig. 4.9j) was greater in shoots than in roots, with spinach shoots and roots being the highest and lowest values, and turnip shoots and roots as intermediates. Zinc uptake (Fig. 4.9k) was greater in the roots than in the shoots, with no significant differences between the shoot concentrations of both species. Cadmium uptake (Fig. 4.9 l) was greater in the shoots than in the roots with no significant differences between species. Lead uptake (Fig. 4.9 m) was greater in the roots than in the shoots, with no significant differences between species. At species level roots and shoot crops show very similar results, so there is very little to suggest a difference in uptake of elements by root and shoot crops, as can be seen in the following Fig.ures.



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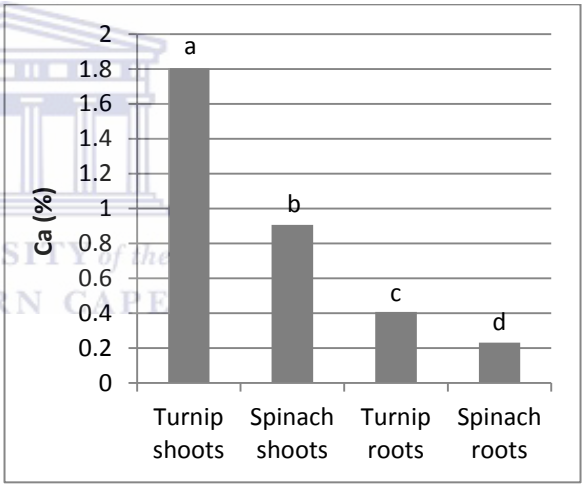

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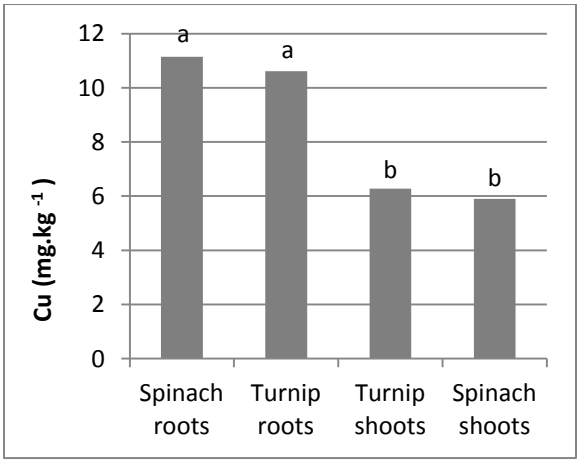

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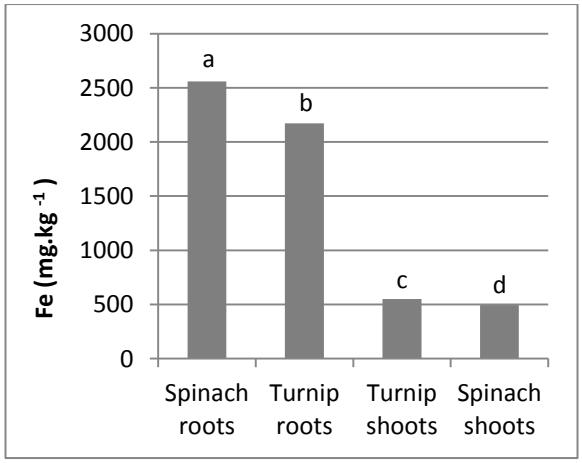

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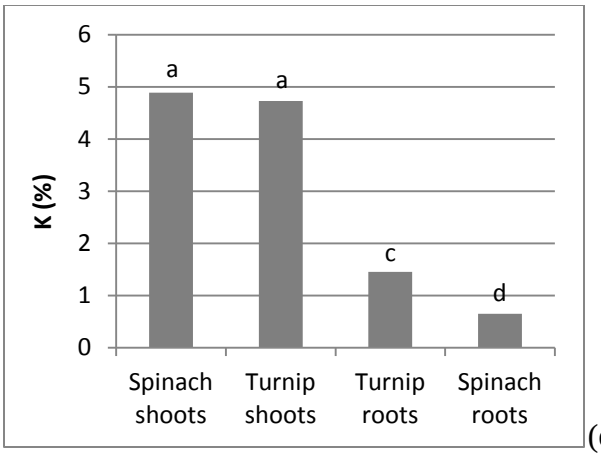

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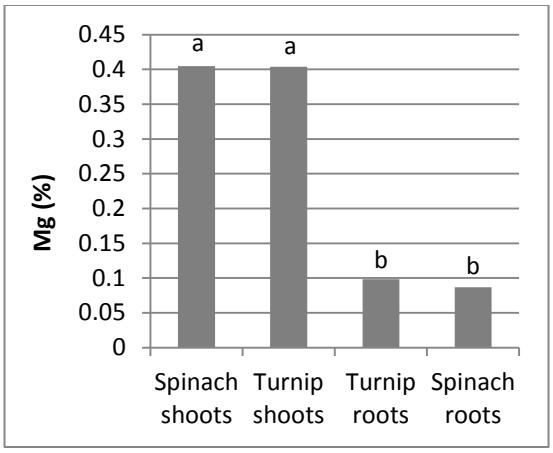

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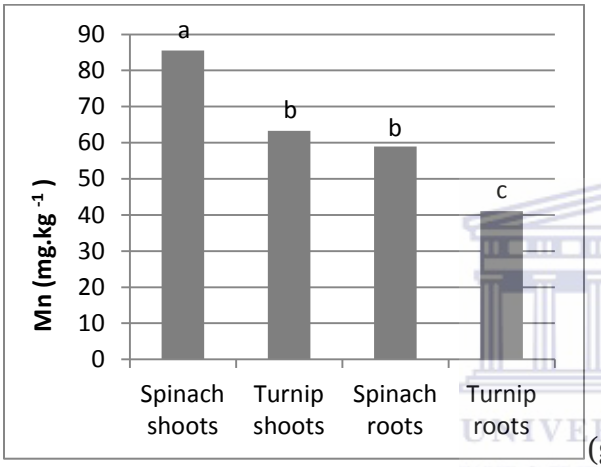

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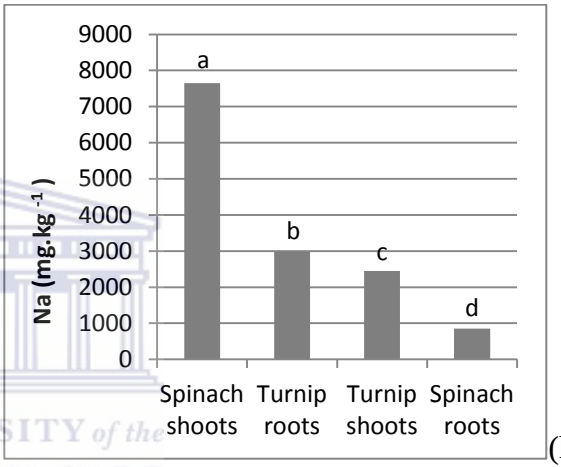

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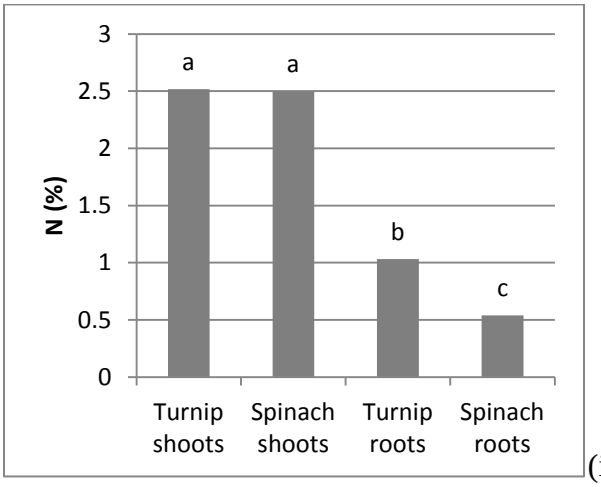

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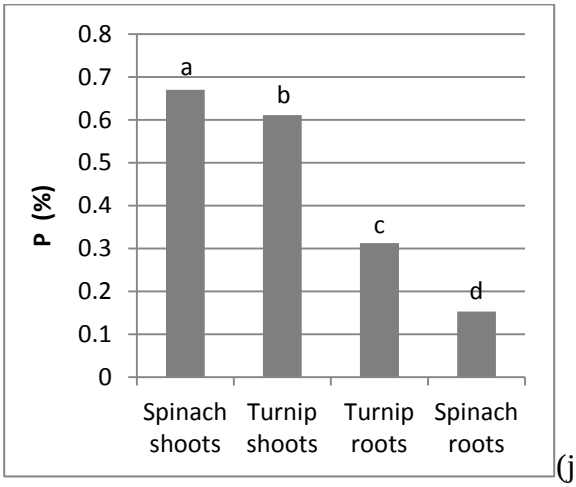

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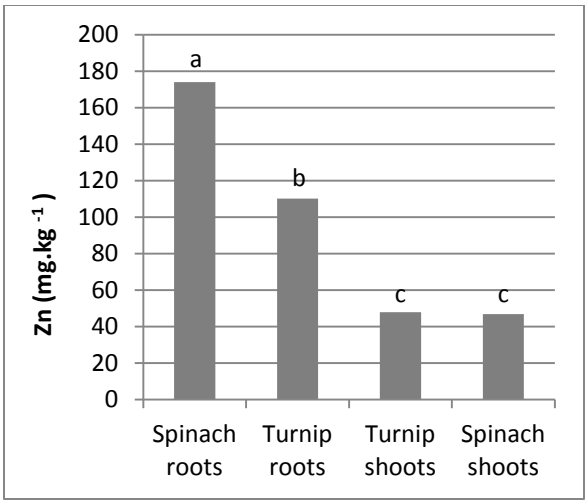

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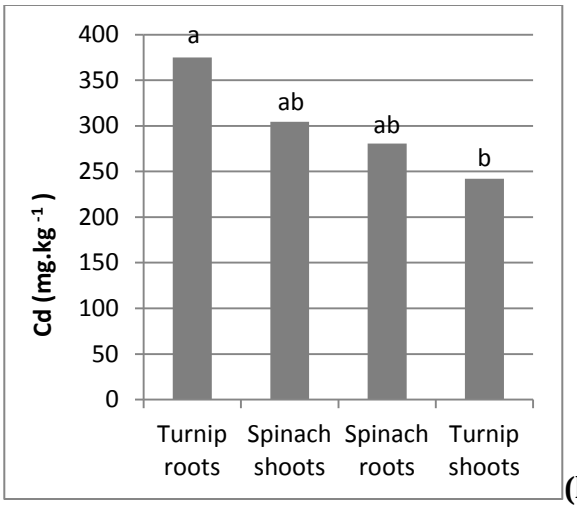

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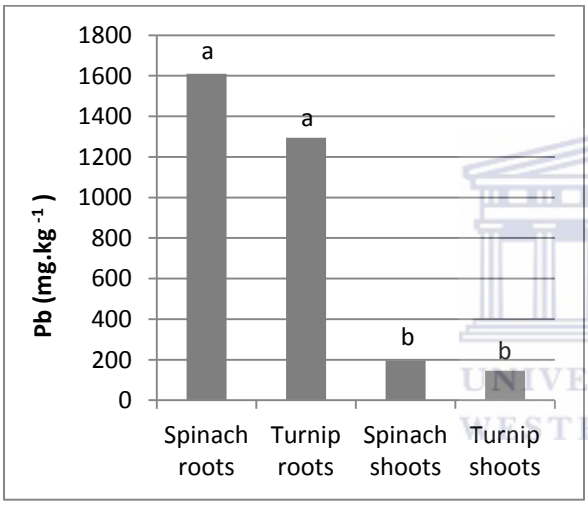

(m)

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Fig.ure 4.9 – The concentration of elements in shoots and roots of turnip and spinach plants:

(a) boron (mg.kg^{-1}); the lsd at p = 0.05 was 3.8196**; b**ars with the same letter do not differ significantly.

(b) calcium (%) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 0.1244**; b**ars with the same letter do not differ significantly.

(c) copper (mg.kg^{-1}) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 0.8877**; b**ars with the same letter do not differ significantly.

(d) iron (mg.kg^{-1}) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 0.244**; b**ars with the same letter do not differ significantly.

(e) Potassium (%) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 0.3396**; b**ars with the same letter do not differ significantly.

(f) magnesium (%) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 0.0368**; b**ars with the same letter do not differ significantly.

(g) manganese (mg.kg^{-1}) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 6.9709**; b**ars with the same letter do not differ

(h) sodium (mg.kg^{-1}) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 488.8**; b**ars with the same letter do not differ significantly.

(i) nitrogen (%) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 0.2185**; b**ars with the same letter do not differ significantly.

(j) phosphorus (%) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 0.0549**; b**ars with the same letter do not differ significantly

(k) Zinc (mg.kg^{-1}) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 22.836**; b**ars with the same letter do not differ significantly

(l) cadmium (mg.kg^{-1}) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 114.42**; b**ars with the same letter do not differ significantly

(m) lead (mg.kg^{-1}) in shoots and roots of turnip and spinach plants; the lsd at p = 0.05 was 372.68**; b**ars with the same letter do not differ significantly.

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4.4 Discussion

Comparison by species revealed that with the exception of manganese and sodium, all of the essential elements were taken up more in the turnip plants than the spinach (Table 4.1), which is in contrast to Cao *et al* 2010 and Nabulo *et al* 2011 that found leafy vegetables more having more of an affinity for accumulating heavy metals. In terms of the heavy metals being investigated, cadmium and lead, there was no significant difference in uptake between the two species.

When comparing heavy metal treatments, elemental uptake was highest in the control plants, suggesting that both heavy metals inhibited elemental accumulation. The greatest inhibitory effect on B, Ca, Cu and K (Fig.ures 4.1 b-d) and Mg, Mn and Na (Fig.4.2 a,b and c) is shown to be by cadmium. In the case of B both high cadmium and lead levels had a significant effect on levels of uptake of the element (Fig. 4.1a), as was the case with phosphorus (Fig. 4.2 e). Fig. 4.2 d+e are in agreement with the findings of Jalloh *et al* 2009 and Sanita *et al* 1999 that cadmium interferes with nitrogen and phosphorus concentration in plant tissue.

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Zinc was inhibited only by the high dose cadmium. In the case of cadmium and lead the uptake is directly proportional to the dose administered, but there is no evidence of a inhibitory effect on uptake by either on the other.

Fig.ure 4.4 is the influence of mitigation treatments on lead uptake, as there is no effect on cadmium uptake, showing that cadmium was not chelated or precipitated by the mitigation treatments given at those doses.

The high phosphate treatment showed the most significant inhibitory effect on reducing lead uptake.

When comparing elemental uptake by organ overall, cadmium content did not differ significantly between organs, while in the case of lead root uptake was much larger (Table 4.2). When considering the data at the level of organ Mg, Mn, Fe, Cu and Zn were found to be significantly different (Table 4.2), with the majority of elements were taken up in higher concentrations in the roots than the shoots, with the exception of magnesium and manganese

The interactions between heavy metal uptake and mitigation treatment (Fig. 4.5-4.8) showed no significant differences for cadmium concentrations, while lead concentrations displayed interesting groupings, with control plants having the highest lead content; low EDTA, low Phos and high EDTA grouped together and finally high Phos being the lowest (Fig. 4.5). As is to be expected the uptake of cadmium and lead in plants was greatest in their respective 10x treatments and lowest in control plants (Fig. 4.6, 4.7 and Fig. 4.8). The concentration of the heavy metals has proved to be more important than any mitigation treatment in determining the level of that heavy metal in the plant, which is in contrast to findings by Steele and Pichtel, 1998; Papassiopi *et al.*, 1999; Garrabrants and Kosson, 2000; Kim and Ong, 2000 and Wassay *et al.*, 2001 who found EDTA to extract large percentages of cadmium and lead.

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At the organ level, elements can be classed into two groups. Those that are taken up in greater concentrations in the roots, and secondly those that accumulate in the shoots. The first group

includes Cu, Fe, Zn and lead (Fig.ure 4.9 c,d, k and m). These are all heavy metals and this is agreement with literature (Meighan *et al*., 2011; Greene 1993, Marschner, 1995; Prasad and De Oliveira, 2003; Salisbury and Ross, 1992) that says they accumulate more in the roots and do not translocate to other organs.

The second group consists of B, Ca, K, Mg, Mn, Na, N, P and cadmium. The most harmful of these is cadmium, and the presence of lead seems to have also inhibited the absorption of Ca into the root (Fig. 4.9 b). Ca has been illustrated to allow for plants greater adaptation to environmental stresses (Huang *et al.,* 2008), so its inhibition by cadmium and lead would leave it vulnerable. cadmium translocated to the leaves (Fig. 4.9 l) and lead remained in the roots (Fig. 4.9 m). This renders cadmium more dangerous to whole plant health.

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In a study conducted by Yang et al (2009) leafy vegetables had a higher risk of cadmium accumulation to people than other vegetables, which is the case in this study (Fig. 4.9 l) which is in agreement with some literature that stated that leafy and root crops have higher cadmium concentrations than fruits and seeds (Jinadasa *et al.,* 1997) but not the view that heavy metals accumulate more in the roots than other organs.

There are no significant differences (Fig. 4.9 l and m) in cadmium and lead between spinach and turnip. This has some implications in agriculture when advising as to whether a root or shoot crop is best planted in a certain area. lead is accumulated more in the roots and cadmium is accumulated more in the shoots. Organ content of heavy metals seems to be very species specific, as found in Dahmani-Muller *et al.* (2000) where various hyperaccumulators had

differing results w.r.t translocation. All the elements tested showed inhibition by both cadmium and lead treatments.

Cadmium uptake (Fig. 4.9l) was greater in the shoots than in the roots with no significant differences between species. lead uptake (Fig. 4.9 m) was greater in the roots than in the shoots, with no significant differences between species.

Many contrasting results have been found to the effects of EDTA on lead uptake which could be explained by the many different plant species used, the experimental conditions, age of plants and the lead/EDTA ratio used (Huang *et al.*, 2008). Huang *et al* (2008) found in their results that lead treatments affected the concentrations of Mg, Ca and K in root, stem and leaves when compared with the control. The contradicting and large variation of results in this study, and indeed those of the available literature, indicate mainly that the remedial treatments investigated here are not necessarily the most effective and that other treatments should be investigated to control the uptake of either cadmium or lead should agricultural soil in future become contaminated with either or both of these heavy metals. These include the reduction of farmers' reliance on additives like fertilizers (organic farming methods); increasing the organic content or raising the pH to immobilize heavy metals.

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Vegetable farms in Cape Town: water quality and possible remediation techniques.

Conclusions and suggestions for further research

Overall aims

The overall aims of this project were firstly to find out if water was the source of the various heavy metals (Lead, Cadmium, Cu and Zn) previously found in vegetables (Meerkotter, 2003 and Sogayiso, 2003) and then to test possible remediation methods for tackling the problem. This project found that the water from the dams and the boreholes on all the farms in the areas surveyed was in fact relatively free of heavy metals, and it could therefore be concluded that this was not the source of the problem (Chapter 2). Minimal lead and no cadmium were found in all the sites surveyed in both Phillipi and Kraaifontein-Joostenbergvlakte. Cu and Zn were found in minimal quantities, far below the DWAF limit for irrigation waters. It is now fairly clear that agricultural additives (mainly various fertilizers) are the main source of the heavy metals (Meerkotter, 2012).

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The greenhouse experiment conducted concentrated on two specific heavy metals, namely cadmium and lead; and two mitigation techniques – chelation with EDTA and precipitation using phosphate treatments. This was carried out in sand culture rather than soil so that soil complexities would not mask the effects of the treatments. Two crop species; namely spinach and turnip, a leaf and root crop vegetable, to test both the methods of phytoisolation, making the element inaccessible to the plant and phytoextraction through the chelating of the element, making it more easily absorbed by the plant, to assess their suitability.

Lead at the levels supplied had little effect on fresh and dry mass production, whereas cadmium affected both fresh and dry mass, causing a severe depressive effect on the biomass production of both crops (Chapter 3). The control plants were about six times larger than those treated with the high dose of cadmium. Dry mass values of the control plants showed ten times the growth of those treated with high-dose Cadmium.

It was found that in general both mitigation treatments increased metal concentrations in plants, and that the concentration of metal treatment was more important than the mitigation in determining how much of it would be taken up into the plant. Cadmium accumulation did not differ across mitigation treatments and in Lead root accumulation was greater than in the shoots. The concentration of most elements was greater in the roots, with the exception of Mn and Mg. Cadmium uptake was greater in the shoots than in the roots with no significant differences between species.

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In this experiment we also found that moderate amounts of heavy metals and the mitigation treatments lead to an increase in chlorophyll content, which seems to suggest they have a reversal effect on the inhibition/hydrolysis caused by increasing heavy metal content (Mainos *et al.*, 2003). A more intensive study in this regard would need to be undertaken in order to be more conclusive.

Current thought is that the accumulation of most elements is greater in the root rather than the shoot, but this was not always the case here, there was minimal difference in the accumulation of elements between the spinach and turnip plants a leaf and root crop respectively. It was reported

by Meighan *et al.*, (2011) that EDTA increased translocation of Cadmium from root to shoots as well as significantly increasing Cadmium uptake in Sunflowers. Li *et al.,* (2010) reported a pot experiment carried out to evaluate the effects of heavy metals on biomass, chlorophyll, and antioxidative enzyme activities of eight vegetables grown in a saline soil. The biomass and chlorophyll content of crops decreased with the increase of heavy metal concentration, as was the case in this project.

Other researchers have found that chelate-assisted phytoextraction of heavy metals requires meticulous soil management and crop selection before it becomes practical in field conditions (Lestan *et al.,* 2008), and in the case of this project this is significant as pure silica sand was used, which isn't a representation of real world conditions in agriculture, but allows, due to the absence of exchange positions, for the easier uptake of elements. Another member of this research group (Meerkotter 2012) used soil samples taken from the same farming area, in order to make suggestions for what could be done in case of continued increase in heavy metal content of vegetables in Cape Town.

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Since there is no such thing as a ‘‘universal reference soil,’’ sand culture experiments, such as this one, are probably the most practical way of producing comparable, reproducible studies on plant performance under standardized conditions. The phytoextraction efficiency of a plant depends quite heavily on the soil conditions under which it was grown which directly affect metal mobility and phytoavailability because if metals are not available to plants, their phytoextraction potential cannot be tested (Herna´ndez-Allica *et al.,* 2008). In this experiment with pure silica sand, both mitigation agents increased the bioavailability of heavy metals.

Phytoremediation uses plants to completely remove or at least render harmless pollutants in the environment (Garbisu and Alkorta, 2001). Lead is often the target pollutant of remediation studies due to its widespread distribution, persistence, and toxicity to human health (Chen *et al.,*1997). Metals soil contamination is a widespread problem encountered at many contaminated sites of industrialised countries. Lead is one of the most prevalent contaminant metals in soil, posing a serious risk to public safety and groundwater supplies due to the fact that upon reaching the soil matrix, it can be strongly retained. Heavy metals can also be very dangerous to plant health, as was the case in this study where cadmium was found to significantly reduce chlorophyll content of both the spinach and turnip plants (Fig. 3.4). The extraction of metals by chelating agents has been the subject of numerous studies undertaken to explore the potential and further develop it (Peters, 1999, Hong and Jiang, 2005 and references cited therein).

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Cadmium is a toxic trace metal naturally present in soils that may be unintentionally added to soil as a contaminant in fertilizer, manure and sewage sludge and from aerial deposition (Grant *et al.,*1998). Increased cadmium concentrations threaten soil productivity, human and environmental health because of the ability if it to amass in the food chain. Therefore careful consideration must be taken in the management of cadmium in plant-soil systems and innovative strategies for reducing accumulation in crops (Grant *et al.,* 2008) are vital. The application of phosphate fertilizers (Grant and Sheppard 2008) is one of the major inputs of cadmium into agricultural soils. Meighan *et al.,* (2011) found EDTA to accelerate cadmium uptake and translocation, but in this project the results there was no significant difference in cadmium uptake between mitigation techniques, or between mitigation techniques and the control.

The high phosphate treatment proved to increase growth (Fig. 3.7) and cadmium was the greatest inhibitor of the two heavy metals, similarly Gao *et al.,* (2011) reported that plant biomass increased significantly with P application rates and decreased with increasing Cadmium concentration in the phosphate fertilizers. Application of phosphate fertilizer increased the concentration and accumulation of Cadmium in durum wheat.

The maximum heavy metal remediation potential of plants is yet to be realized and in order to achieve this we, as a scientific community, need to gain a better understanding of heavy metal tolerance in plants. A number of examples of heavy metal tolerant plants have showed enhanced metal accumulation, although this is not always the case (Chin *et al.*,2009).

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In conclusion, the results from the EDTA and phosphate treatments were not conclusive. So in general it was found that cadmium was more toxic to the plants than lead (chapter 3). Phosphate immobilized Lead better than Cadmium (Fig. 4.4) . More research on mitigation methods is required before they can be of use to the farmers.

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