The Effects of Cadmium and Lead on *Phaseolus vulgaris*

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KEYWORDS

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ABSTRACT

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The demand for better quality produce by consumers is on the increase, as higher heavy metal concentrations pose a problem in agriculture. They result in decreased yield and unsuitable food for human consumption. This brings about a negative economic effect as such products become unprofitable on the domestic or export markets thus affecting productivity of farms.

Four heavy metals (Cd, Cu, Pb and Zn) have been shown to be a problem in the farming areas in Cape Town. Pot and field studies were carried out on the effects and concentrations of cadmium and lead on *Phaseolus vulgaris*.

Field studies included collecting plant samples from the Joostenbergvlakte/ Kraaifontein farming areas and measuring the heavy metal concentrations within the different organs of the plants. Pot experiments were carried out, where *Phaseolus vulgaris* var. Contender were grown and then heavy metals were administered to these plants together with two heavy metal mitigation techniques, precipitation with phosphate and mobilisation with EDTA to see if they were successful in combating heavy metal pollution.

Samples taken from farms in the Joostenbergvlakte/ Kraaifontein area revealed that cadmium, lead and zinc concentrations were higher than the legal standard in the edible fruits. In the pot experiment, results revealed that cadmium reduced the chlorophyll index as well as the shoot fresh mass and changes in mineral uptake were seen. Lead did not affect growth or the chlorophyll index. The high cadmium treatment resulted in a marked increase in sodium concentration in the shoots. The phosphate treatments and EDTA treatments both resulted in increased cadmium concentrations in the roots and shoots. The higher phosphate and lead treatments also reduced lead concentrations in the roots. Low phosphate and the EDTA treatments increased the shoot sodium concentrations.

DECLARATION

I declare that "The Effects of Cadmium and Lead on *Phaseolus vulgaris*" is my own work, that it has not been submitted for any degree or examination at any other university and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Clarissa Brandt Date:

Signed ………………………

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"…He which soweth sparingly shall reap also sparingly; and he which soweth bountifully shall reap also bountifully."

2 Corinthians 9:6

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CHAPTER 1

Literature Review

1.1 Introduction

The term “heavy metals” has been used to describe a group of metals which have been linked to contamination and toxicity (Duffus 2002). Chemically, heavy metals are defined as metallic elements with a density of ≥ 5 g/ cm^3 (Schulze *et al.* 2005). Elements which fall into this category include Ag, Ar, Au, Bi, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, No, Pb, Pt, Sb, Ti, Tl, U, V, Zn and Zr (Schulze *et al.* 2005). Some heavy metals are essential in plant nutrition as micronutrients (Helal *et al.* 1990) but plants growing in a polluted environment can accumulate heavy metals at high concentrations (Voutsa *et al.* 1996, Schulze *et al.* 2005). Accumulation of heavy metals in agricultural soils has become a major concern for food crop production (Cieslinski *et al.* 1996) as they may cause a serious health hazard as they travel through the food chain unaltered (Bharti *et al.* 2001) as they are not biodegradable and have long biological half-lives (Jarup 2003).

1.2 Heavy metals

1.2.1 Sources of heavy metals

Soil, air and both organic and inorganic fertilizers are the main sources of heavy metals to plants (Martensson 1992) from which heavy metals are taken up by the root or leaves (Voutsa *et al.* 1996). Uptake of heavy metals by roots depends on soil and plant factors such as soil pH, organic matter, plant species, plant age (Voutsa *et al.* 1996). The concentration ranges of deficiency, optimal supply and toxicity for essential heavy metals as Zn and Cu are very close together (Schulze *et al.* 2005). Soil metal contamination has increased significantly in the previous century due to rapid industrialization including mining (Dudka *et al.* 1997). The surplus of heavy metals in soils is caused by the use of fertilizers, pesticides and sewage sludges, or by industrial activities (Gimeno-Gracia *et al.*1996). Elevated levels of metals in soil may lead to increased uptake by plants (Voutsa *et al.* 1996). The heavy metal distribution in soils and sediments can indicate the potential harm to the environment through the chemical associations (Mulligan *et al.* 2001) as a build up of heavy metals at high concentrations can cause serious risk to human health when food plants are consumed (Voutsa *et al.* 1996).

1.2.2 Toxicology of heavy metals

Heavy metals are of great environmental concern due to their toxicity and cumulative behaviour (Yusuf *et al.* 2002). Heavy metals such as copper and zinc are necessary for plant and human nutrition, and small amounts are required whereas other metals such as cadmium and lead are not needed. However, excessive amounts of any of the heavy metals can generate toxic effects (Voutsa *et al.* 1996).

Lead and cadmium are among the most abundant heavy metals and are particularly toxic (Yusuf *et al.* 2002). The extreme content of these metals in food is associated with aetiology of a range of diseases, especially with cardiovascular, kidney, nervous and bone diseases (Jarup 2003). Heavy metals are also implicated in causing carcinogenesis, mutagenesis and teratogenesis (Yusuf *et al.* 2002).

1.2.3 Effects of heavy metals on plants

Most heavy metals are not necessary for plant growth and metabolism, yet they are often readily taken up and accumulated by plants (Bharti *et al.* 2001). Heavy metals have shown to reduce the "qualitative and quantitative productivity" of plants (Bharti *et al.* 2001). These metals have been reported to affect seed germination (Mukherji and Maitra 1976), seedling growth (Kumar *et al.* 1992), photosynthesis (Muthuchelian *et al.* 1988), nitrate assimilation (Chugh *et al.* 1992) and other metabolic processes (Stiborova *et al.* 1988) of plants. Other effects include chlorosis of leaves of plants and restricted yields of dry matter (Xian 1989). Roots proved more sensitive than the aerial parts, because of direct exposure to metals while translocation to the aerial parts is steady (Bharti *et al.* 2001). Cadmium, lead and copper have been thought to inhibit growth by affecting uptake of water, activity of hydrolytic enzymes and hydrolysis of storage macromolecules in endosperms or cotyledons, transfer of hydrolytic products to the embryonic axis, root and shoot, and resynthesis of essential macromolecules such as nucleic acids and proteins (Bharti *et al.* 2001). The occurrence of heavy metals in certain chemical forms may have more effect on plant growth, than their concentration in soil (Xian 1989) yet too high metal concentrations in contaminated soils can end in less soil microbial activity and soil fertility and yield losses (McGrath 1998).

1.3 Agriculture and Heavy Metals

Metals pose a problem in agricultural soils and crops as metals are elements and as such they do not breakdown but rather persist in the environment essentially forever (McGrath 1998, Jarup 2003). Even if only a small amount of a metal is added each year to a soil, its concentration will slowly increase over time. The only natural way that the total concentrations of a metal can decrease over time is physical removal from a site by processes such as erosion and leaching (Anderson 1993). Plants accumulate many metals and nutrients from the soil (Kachenko and Singh 2006). As concentrations of metals in soils increases, the uptake of metals into plants also increases (Muchuweti *et al*. 2006).

Although metals naturally occur in soils, heavy metals found in agricultural soils and crops are introduced from a number of different sources, some natural and some man-made (Kachenko and Singh 2006). Natural concentrations of metals in soils depend on the parent rock from which the soil originated and these are highly variable. In terms of metals in rain and irrigation water, very low concentrations are found in this medium. However, if sources are from sewage treatment plants, irrigation water should be monitored as it may be contaminated with metals (Moeletsi *et al.* 2004). Fertilizers are generally deliberately added in order to improve plant growth and yields, and these may contain high concentrations of cadmium and other non-essential metals (Martensson 1992). Cadmium occurs naturally with deposits of zinc and also phosphorus depending on the source of rock phosphate used in producing fertilizer.

1.4 Heavy metals in vegetables

Commercial and residential vegetable growing areas are usually located in urban areas, and are subject to man-made contamination (Kachenko and Singh 2006). Both commercial and residential growing areas are subject to atmospheric pollution, in the form of metal containing aerosols (Kachenko and Singh 2006). These aerosols can enter the soil and be absorbed by vegetables, or otherwise be deposited on leaves and adsorbed (Kachenko and Singh 2006). Excessive accumulation of heavy metals in agricultural soils may not only result in environmental contamination, but lead to elevated heavy metal uptake by crops, which may affect food quality safety (Muchuweti *et al.* 2006, Sharma and Chettri 2005, Sharma *et al.* 2007). Studies of vegetables grown in locations in close proximity to industrial areas have reported high levels of heavy metals (Kachenko and Singh 2006). Most plant species have a tendency to sequester metals in their roots, with only small amounts of metals being translocated to the aboveground parts (Yang *et al.* 2009). Fruit and root vegetables appear to be low accumulators of cadmium in their edible parts, whereas leafy vegetables tend to accumulate more cadmium in leaves (Yang *et al.* 2009). A number of factors influence the concentration of heavy metals on and within plants (Muchuweti *et al.* 2006). These factors include climate, atmospheric deposition, the nature of the soil on which the plant is grown and the degree of maturity of the plant at the time of harvesting (Scott *et al.* 1996; Voutsa *et al.* 1996; Lake 1984). The soil is one of the most important factors in determining the heavy metal content of food plants (Itanna 2002, Madyiwa *et al.* 2002). Heavy metal contamination of agricultural soils can pose long-term environmental problems and is not without health implications (Sauve *et al.* 1996; Ferguson 1990; Chumbley 1982).

1.5 Tolerance to heavy metals

There are two basic strategies by which plants react to high concentrations of heavy metals in the environment: exclusion mechanisms, whereby plants avoid too much uptake and transport of metals and the other - accumulation and sequestration mechanisms, whereby large amounts of metals are taken up and transported to the plant shoots (Baker 1981). There are two groups of plants evolved to tolerate heavy metals, namely chemoecotypes and metallophytes. These plants may serve as indicator plants for heavy metals (Larcher 2001) as concentrations of heavy metals in plants have been used to measure pollution (Leavitt *et al.* 1979). Chemoecotypes display characteristic patterns of isoenzymes (Larcher 2001). These plants show element-specific increases in the ability of the protoplasm to resist the high concentrations of heavy metals in tissues when growing on soils rich in these elements (Larcher 2001). The greater the exposure to a certain element, the more tolerance is adopted toward the element (Larcher 2001). These are taxa with a high degree of genetic plasticity from which a number of specialized ecotypes evolved that are resistant to a number of heavy metals (Larcher 2001). Metallophytes take up large amounts of heavy ions and store them (Larcher 2001). Non-resistant plants may also collect certain elements (Larcher 2001). There are also hyperaccumulators i.e. plants that have very high concentrations of heavy metals (Larcher 2001). These plants are capable of accumulating more than 100 times greater concentrations of metals than normal plants (Brooks *et al.* 1997). Heavy metal ions can be extracted from the harvested biomass of these plants (Larcher 2001). In some species this tolerance is limited to a particular heavy metal, in other species co-tolerance to numerous heavy metals exists (Cox and Hutchinson 1979). These plants have also developed a range of avoidance mechanisms by which the surplus of heavy metals can be rendered harmless, these mechanisms include:

1) immobilization of toxic ions in the cell walls
2) slowing down of permeation across boundary layers of the protoplasm
3) chelation in the cytoplasm
4) compartmentalization and complex formation with organic and inorganic acids, phenol derivatives and glycosides in the vacuole (Marschner 1986)

Plants may also produce peptides called phytochelatins which attach naturally and detoxify toxic metals such as lead, mercury and cadmium (Spiro 2003).

1.6 Monitoring of Heavy Metals

Heavy metals are naturally present at low concentrations in agricultural soils (Korkmaz *et al.* 2010). A number of factors contribute to heavy metal contamination of agricultural soils including industrial and traffic emission, atmospheric deposition from town wastes, using metal-containing agricultural expedients and metal production (Alloway and Jackson 1991, Korkmaz *et al.* 2010). Heavy metal ions in contaminated soils may easily enter the human food chain through crop plants (Korkmaz *et al.* 2010).

Once heavy metals are accumulated as contaminants, they can neither be destroyed nor can they be altered by chemically or physically, and are spread in the ecosystems (Sharma and Chettri 2005). Plants tolerate heavy metals either by detoxification mechanism or accumulation in different plant parts or cell organelles (Sharma and Chettri 2005). Some plants have the ability to absorb and accumulate heavy metals, which makes them valuable as indicators of environmental pollution (Buszewski *et al.* 2000) and therefore provide an understanding of the bioavailability and mobilization of heavy metals (Murphy *et al.* 2000). However, few studies have been done to investigate plants and their role as biomarkers to identify metal mobility for continuous monitoring purposes (Murphy *et al.* 2000).

Most common methods of assessing metal toxicity to plants from soil:

(1) monitoring the presence or absence of specific plant ecotypes and/or plant species (indicator plants)

(2) measurements of metal concentration in tissues of selected species (accumulative bioindicators)

(3) recording of physiological and biochemical responses (biomarkers) in sensitive bioindicators (Buszewski *et al.* 2000).

1.7 Cadmium

Cadmium is one of the most toxic pollutants found in air, water and soil (Dixit *et al.* 2000) and is not essential for humans, animals and plants. It enters the environment mainly from industrial processes and phosphate fertilizers and then is transferred to the food chain (Sandalio *et al.* 2001). It is known to be easily taken up by plants and translocated within the plant (John *et al.* 1972). Once entered, cadmium accumulates immediately in roots, later in the stem and leaves (Dixit *et al*. 2000). About 75 % of cadmium entering the plant is in one way or another bound in the root system (Jastrow and Koeppe 1980, Krupa 1999). Up to 11 % of the total cadmium content in plants can be founding stems and about 15 %, depending on plant species and the ability to detoxicate this heavy metal, enters the leaves (Jastrow and Koeppe 1980). In terms of cadmium accumulation within mesophyll cells, 48 % is in the cell wall, 39 % in the cytoplasm and vacuole and 13 % in chloroplasts and mitochondria (Ernst 1980). Cadmium produces alterations in the functionality of membranes by inducing changes in lipid composition and by affecting the enzymatic activities associated with membranes (Sandalio *et al.* 2001).

Cadmium toxicity is also correlated with disturbances in the uptake and distribution of macro and micronutrients in plants (Sandalio *et al.* 2001). It interacts with the uptake of essential nutritional elements for example, iron, calcium, potassium, magnesium and manganese (Krupa 1999). Cadmium is strongly phytotoxic and causes growth inhibition and even plant death (Sandalio *et al.* 2001). Cadmium also initiates senescence in plants (Krupa 1999). Leaf chlorosis is the most visible symptom of cadmium toxicity, as cadmium inhibits chlorophyll synthesis and interacts with iron (Krupa 1999). Photosynthesis is also sensitive to cadmium (Sandalio *et al.* 2001). Cadmium interacts with photosynthetic, respiratory and nitrogen metabolism in plants and produces oxidative stress by producing free radicals and active oxygen species, resulting in poor growth and low biomass accumulation (Dixit *et al.* 2000). Increasing the concentration of cadmium produced a significant growth inhibition of pea plants which was accompanied by a significant decrease in the photosynthesis rate (Sandalio *et al.* 2001) and causes a decrease in biomass (Dixit *et al.* 2000). Once plants are exposed to cadmium, a variety of detoxification processes are triggered in the cells, including complexing of the metal by phytochelatins, compartmentalization in vacuoles, immobilization at the level of cell wall, exclusion through the action of plasma membrane, and production of stress proteins play very major roles (Dixit *et al.* 2000).

1.8 Lead

Soil lead contamination is a major environmental problem facing the modern world (Li Li *et al.* 2008). Sources of lead contamination in soils can be classified into three broad categories: industrial (mining and smelting processes), agricultural (application of insecticides and municipal sewage sludges) and urban activities (use of lead in gasoline, paints and other materials) (Shen *et al.* 2002). Lead is one of the most persistent metals (Li Li *et al.* 2008) and most frequently encountered heavy metals in polluted environments (Seaward and Richardson 1990). Severe lead contamination in soils and in ground and surface waters may cause a variety of environmental problems, including loss of vegetation, groundwater contamination, and lead toxicity in plants, animals and humans (Li Li *et al*. 2008). Lead is accumulated in the roots with some transportation to the leaves (Robb and Pierpoint 1983, Li Li *et al*. 2008) and the majority of lead within the plant may be retained in the root system (Schulze *et al.* 2005). If lead is bioavailable in the plant growth media, only a small proportion of absorbed lead is translocated to shoots (Li Li *et al.* 2008).The mobility of lead from roots to shoots of plants is usually low (Begonia *et al.* 1998) and the lead concentration in the underground parts is two to eight times higher than it in the leaves (Li Li *et al.* 2008). Toxicity of this heavy metal is due to the leakage of some lead into the cytoplasm, as lead is bound irreversibly to the cell wall (Schulze *et al.* 2005). When lead enters the plant root, it encounters the neutral pH, high phosphate and high carbonate environment of the intercellular spaces (Li Li *et al.* 2008) which would result in it being immobilized.

1.9 Heavy Metal Pollution in South Africa

South Africa has a high concentration of industrial and mining activity. Studies of vegetables grown in locations close to industrial and mining industries have reported elevated levels of heavy metals (Kachenko and Singh 2006). Poor monitoring and control of safe disposal of waste means that industrial waste is illegally dumped in urban areas, causing health risks to communities and environments (Whyte 1995). The deposition of processed and unprocessed waste materials has led to continuous, persistent leaching of dissolved metals into soil and water systems (Naicker *et al.* 2003; Roychoudury and Starke 2006). Numerous reports have indicated heavy metal contamination of South African rivers and soils (Abbu *et al.* 2000; Binning and Baird 2001; Okonkwo and Mothiba 2005). There is also clear evidence of heavy metal contamination in some vegetable farming areas (Meerkotter 2003, 2012; Sogayiso 2003).

1.10 Aims and Objectives

The aim of this study is to investigate the heavy metals namely cadmium and lead which have shown to have a negative effect on vegetable farming in Cape Town (Meerkotter 2003, 2012; Sogayiso 2003).

1.10.1 Research Objectives

The main objective of the study is to examine the effect these metals have on the growth and chemical composition of *Phaseolus vulgaris*.

1.10.2 Research Questions

Do heavy metals accumulate in *Phaseolus vulgaris* in the Kraaifontein/ Joostenbergvlakte farming area? If so, in which organs? Are the edible fruits affected?

Can phosphate and EDTA mitigate the effects of Pb and Cd on the growth and nutrient content of *Phaseolus vulgaris*?

1.11 Conclusion

Because of their hazardous and toxic nature, effort should be expanded to alleviate heavy metal pollution. Excessive levels of cadmium and lead may be detrimental to the well-being of plants as well as humans. Heavy metals can have serious implications for farming practices, as they decrease crop yield and food quality.

1.12 References

Abbu R, Pillay AE, Moodley KG (2000) The use of ICP-AES and anodic stripping voltammetry (ASV) to determine the levels of cadmium and lead in river water samples from KwaZulu-Natal (KZN), South Africa. *Journal of Trace and Microprobe Techniques* **18**: 83-97.

Alloway BJ, Jackson AP (1991) The behavior of heavy metals in sewage sludge-amended soils. *Sci. Total Environ.* **100**: 151-155.

Anderson WC (1993) Soil washing/soil flushing. *Amer. Acad. Environmental Engineers*. Annapolis, MD *In*: Sun B, Zhao FJ, Lombi E, McGrath SP (2001) Leaching of heavy metals from contaminated soils using EDTA. *Environmental Pollution* **113**: 111-120.

Baker AJM (1981) Accumulation and excluders-strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**: 643-654.

Begonia GB, Davis MFT and Gray BCN (1998) Growth responses of Indian mustard and its phytoextraction of lead from a contaminated soil. *Bulletin of Environment Contamination Toxicology* **61**: 38-43.

Bharti N, Srivastava S, Srivastava PS (2001) Alleviation of Cadmium-Induced Decrease in Biomass of *Pisum* and *Sesamum* by Inorganic Nutrients. *Bulletin of Environmental Contamination and Toxicity* **67** (2001): 496-502.

Binning K and Baird D (2001) Survey of heavy metals in the sediments of the Swartkops River Estuary, Port Elizabeth South Africa. *Water SA* **27**: 461-466.

Brooks RR, Lee J, Reeves RD and Jaffe T (1997) Detection of nickeliferous rocks by analysis of herbarium species of indicator plants. *Journal of Geochemical Exploration* **7**: 49-57 *In:* McGrath SP, Shen ZG, Zhao FJ (1997) Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. *Plant and Soil* **188** (1997): 153-159.

Buszewski B, Jastrzebska A, Kowalkowski T, Gorna-Binkul A (2000) Monitoring of selected heavy metals uptake by plants and soils in the area of Torun, Poland. *Polish Journal of Environmental Studies* **9** (6): 511-515.

Chugh LK, Gupta VK and Sawhney SK (1992) Effect of Cadmium on enzymes of nitrogen metabolism in pea seedlings. *Phytochemistry* **31**: 395-400.

Chumbley CG (1982) Cadmium and lead content of vegetable crop grown on land with a history of sewage sludge application. *Environmental Pollution* **4**: 231-237.

Cieslinski G, Neilsen GH and Hogue EJ (1996) Effect of soil cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruit of strawberry plants (*Fragaria manassa* Duch.). *Plant and Soil* **180**: 267-276.

Cox R and Hutchinson TC (1979) Metal co-tolerances in the grass *Deschampsia cespitosa*. *Nature* **279**: 231-233 *In* Marschner H (1986) Mineral Nutrition of Higher Plants. Academic Press Publishers, London, UK.

Dixit V, Pandey V and Shyam R (2000) Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *Journal of Experimental Botany* **52** (358): 1101-1109.

Dudka S, Piotrowska M and Terelak H (1997) Transfer of Cadmium, Lead and Zinc from Industrially Contaminated Soil to Crop Plants: A Field study. *Environmental Pollution* **94** (2): 181-188.

Duffus JH (2002) “Heavy metals”- a meaningless term? IUPAC Technical report. *Pure and Applied Chemistry* **74**: 793-807.

Ernst WHO (1980) Biochemical aspects of Cadmium in plants *In*: Krupa Z (1999) Cadmium against Higher Plant Photosynthesis- a Variety of Effects and where do they possibly come from? *Z. Natur forsch*. **54**: 723-729.

Ferguson EJ (1990) Heavy metals in plants. *In*: Muchuweti M, Birkett JW, Chinyanga E, Zvauya R, Scrimshaw MD and Lester JN (2006) Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agriculture, Ecosystems and Environment* **112**: 41-48.

Gimeno-Garcia E, Andreu V and Boluda R (1996) Heavy metals incidence in the Application of Inorganic Fertilizers and Pesticides to Rice Farming soils. *Environmental Pollution* **92** (1): 19-25.

Helal HM, Arisha H and Rietz E (1990) The uptake of trace elements by spinach and bean varieties of different root parameters. *Plant and Soil* **123**: 229-232.

Itanna F (2002) Metals in leafy vegetables grown in Addis Ababa and toxicological implications. *Ethiopian J. Health Dev*. **6**: 295-302.

Jarup L (2003) Hazards of heavy metal contamination. *British Medical Bulletin*. **68**: 167-182.

Jastrow J and Koeppe DE (1980) Uptake and effects of Cadmium in higher plants *In* Krupa Z (1999) Cadmium against Higher Plant Photosynthesis- a Variety of Effects and where do they possibly come from? *Z. Natur forsch*. **54**: 723-729.

John MK, Van Laerhoven C and Chuah HH (1972) Factors affecting plant uptake and phytotoxicity of Cadmium added to soil *In* Cieslinski G, Neilsen GH, Hogue EJ (1996) Effect of soil cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruit of strawberry plants (*Fragaria manassa* Duch.). *Plant and Soil* **180**: 267-276.

Kachenko AG and Singh B (2006) Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water, Air and Soil Pollution* **169**:101-123.
Korkmaz K, Metin Kara S, Ozkutlu F and Gul V (2010) Monitoring of heavy metals and selected micronutrients in hempseeds from North-Western Turkey. *African Journal of Agricultural Research*. Vol **5** (6) 463-467.

Krupa Z (1999) Cadmium against Higher Plant Photosynthesis- a Variety of Effects and where do they possibly come from? *Z. Natur forsch*. **54**: 723-729.

Kumar G, Singh RP and Dabas S (1992) Nitrate assimilation and biomass production in *S. indicum* seedlings in a lead enriched environment. *Water Air Soil Po*llution **66**: 163-177.

Lake DL (1984) Chemical speciation of heavy metals in sewage sludge and related matrices *In*: Muchuweti M, Birkett JW, Chinyanga E, Zvauya R, Scrimshaw MD and Lester JN (2006) Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agriculture, Ecosystems and Environment* **112**: 41-48.

Larcher W (2001) Physiological Plant Ecology, 4th Edition. Springer-Verlag, Germany.

Leavitt SW, Dueser RD and Goodell HG (1979) Plant Regulation of Essential and Non-Essential Heavy Metals. *Journal of Applied Ecology* **16** (1979): 203-212.

Li Li Y, Liu YG, Liu JL, Zeng GM and Li X (2008) Effects of EDTA on Lead uptake by *Typha orientalis* Presl. : A new lead-accumulating species in southern China. *Bulletin of Environmental Contamination and Toxicity* **81**: 36-41.

Madyiwa S, Chimbari M, Nyamangara J and Bangira C (2002) Cumulative effects of sewage sludge and effluent mixture application on soil properties of a sandy soil under a mixture of star and kikuyu grasses in Zimbabwe. *Phys. Chem. Earth parts* A/B/C **27**: 747-753.

Marschner H (1986) Mineral Nutrition of Higher Plants. Academic Press Publishers, London, UK.

Martensson AM (1992) Effects of Agrochemicals and Heavy Metals on Fast-growing rhizobia and their symbiosis with small-seeded legumes. *Soil Biol. Biochem* **24** (5): 435-445.

McGrath SP (1998) Phytoextraction for soil remediation. *In*: Sun B, Zhao FJ, Lombi E, McGrath SP (2001) Leaching of heavy metals from contaminated soils using EDTA. *Environmental Pollution* **113**: 111-120.

Meerkotter M (2003) Heavy metals and vegetable farming in Cape Town. Unpublished MSc thesis. University of the Western Cape, Cape Town.

Meerkotter M (2012) Sources of heavy metals in vegetables in Cape Town and possible methods of remediation. Unpublished PhD thesis. University of the Western Cape, Cape Town.

Moeletsi M, Mazema H and Halday I (2004) Strategy for mitigating the impacts of heavy metals in wastewater and the economic and environmental implications for the city of Cape Town. Proceedings of the 2004 Water Institute of Southern Africa (WISA) Biennial Conference.

Muchuweti M, Birkett JW, Chinyanga E, Zvauya R, Scrimshaw MD and Lester JN (2006) Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agriculture, Ecosystems and Environment*. **112**: 41–48

Mukherji S and Maitra P (1976) Toxic effects of lead on growth and metabolism of germinating rice (*Oryza sativa*) seed and on mitosis of onion (*Allium cepa*) root tip cells. *Indian Experimental Biology* **14**: 519-521.

Mulligan CN, Yong RN and Gibbs BF (2001) An evaluation of technologies for the heavy metal remediation of dredged sediments. *Journal of Hazardous Materials* **85** (2001) 145-163.

Murphy AP, Coubert M, Barker J (2000) Plants as biomarkers for monitoring heavy metal contaminants on landfill sites using sequential extraction and inductively coupled plasma atomic emission spectrophotometry (ICP-AES). *J. Environ. Monit.* **2** (6): 621-627.

Muthuchelian NS, Rani MV and Paliwal K (1988) Differential action of copper and cadmium on chlorophyll biosynthesis and nitrate reductase activity in *Vigna sinensis* L. *Indian Plant Physiology* **31**: 169-173.

Naicker K, Cukrowski E and McCarthy TS (2003) Acid mine drainage arising from gold mining activity in Johannesburg, South Africa. *Environmental Pollution* **122**: 29-40.

Okonkwo JO and Mothiba M (2005) Physico-chemical characteristics and pollution levels of heavy metals in the rivers in Thohoyandou, South Africa. *Journal of Hydrology* **308**: 122-127.

Robb DA and Pierpoint WS (1983) Metals and Micronutrients: Uptake and Utilization by Plants. Academic Press Inc., London.

Roychoudhury AN, Starke MF (2006) Partitioning and mobility of trace metals in the Blesbokspruit: impact assessment of dewatering of mine waters in the East Rand, South Africa. *Applied Geochemistry* **21**: 1044-1063.

Sandalio LM, Dalurzo HC, Gomez M, Romero-Puertas MC and delRio LA (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany* **52** (364): 2215-2126.

Sauve S, Cook N, Hendershot HW and McBridge BM (1996) Linking tissue copper concentrations and soil copper pools in urban contaminated soils. *Environ. Pollut*. **94**: 153-157.

Schulze E-D, Beck E, Muller-Hohenstein K (2005) Plant Ecology. Springer-Verlag Berlin, Germany.

Scott D, Keoghan JM and Allan BE (1996) Native and low-input grasses – a New Zealand high country perspective. *N.Z.J Agric. Res* **39**: 499-512.

Seaward MRD and Richardson DHS (1990) Atmospheric sources of metal pollution and effects on vegetation. *In*: Piechalak A and Tomaszewska B (2002) Accumulation and detoxification of lead ions in legumes. *Phytochemistry* **60**: 153-167.

Sharma B and Chettri MK (2005) Monitoring of Heavy metals in vegetables and soil of agricultural fields of Kathmandu Valley. *Ecoprint* **12**:1-9

Sharma RK, Agrawal M and Marshall FM (2007) Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. *Environmental Pollution* (2007): 1-10.

Shen Z-G, Li X-D, Wang C-C, Chen H-M and Chua H (2002) Lead phytoextraction from contaminated soil with high biomass plant species. *Journal of Environmental Quality* **31**: 1893-1900.

Sogayiso M (2003) Contamination of vegetables with heavy metals. Unpublished BSc Honours project. University of the Western Cape, Cape Town.

Spiro B (2003) Assessment of the distribution of heavy metals in plants, waters and soils around a Cu smelter and mining town, Karabash, Urals, Russia. *Geophysical Research Abstracts* **5** (06775).

Stiborova M, Ditrichova M, Brezinova A (1988) Mechanism of action of Cu $^{2+}$, Co $^{2+}$ and Zn $^{2+}$ on ribulose-1-5-biphosphate carboxylase from barley (*Hordeum vulgare* L.). *Photosynthetica* **22**: 161-167.

Voutsa D, Grimanis A, Samara C (1996) Trace elements in vegetables grown in an industrial area in relation to soil and air particulate matter. *Environmental Polluion* **94**: 325-335.

Whyte AV (1995) Building a new South Africa – environmental, reconstruction and development. *International Mission on Environmental Policy*. Canada, International Development Research Centre.

Xian X (1989) Effect of chemical forms of cadmium, zinc and lead in polluted soils on their uptake by cabbage plants. *Plants and Soil* **133** (1989): 257-264

Yang Y, Zhang F-S, Li H-F and Jiang R-F (2009) Accumulation of cadmium in the edible parts of six vegetable species grown in Cd contaminated soils. *Journal of Environmental Management* **90**: 1117-1122.

Yusuf AA, Arowolo TA and Bamgbose O (2002) Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria. *Food and Chemical Toxicology* **41** (2003): 375-378.

CHAPTER 2

Heavy metals in *Phaseolus vulgaris* on farms in Cape Town.

2.1 Introduction

Accumulation of heavy metals in agricultural soils has become a major concern for food crop production (Cieslinski *et al*. 1996) as contamination of vegetables with heavy metals poses a threat to their quality and safety (Sharma *et al*. 2007). Commercial and residential vegetable growing areas are often located in urban areas, and are subject to anthropogenic contamination (Kachenko and Singh 2006).

Heavy metals occur in many fertilizers and in some pesticides, for example, cadmium is predominantly found in phosphatic fertilizers, due to the presence of cadmium as an impurity in phosphate rocks (Alam *et al.* 2003) and although cadmium is not always present in food, certain vegetables and grains produced with heavy applications of commercial fertilizer have been shown to have high concentrations of cadmium in them (Schroeder and Balassa 1963, Kabata-Pendias and Pendias 1984, Alegria *et al*. 1990, Lugon-Moulin *et al*. 2006).

Lead is one of the most frequently encountered heavy metals in polluted environments (Seaward and Richardson 1990). Plants can accumulate large amounts of lead without any visible changes in their appearance or yield (Piechalak *et al.* 2002). The most difficult problem connected with lead contamination of soil is its persistence (Li Li *et al.* 2008), as lead can remain in soil for up to 300 years (Piechalak *et al.* 2002).

High levels of heavy metals had been found in roots and leaves of vegetables in the Cape Town farming area (Meerkotter 2003, Sogayiso 2003). This survey was to monitor the levels of cadmium, copper, lead and zinc in the roots, stems, leaves and fruits of *Phaseolus vulgaris* (dwarf bean).

The Kraaifontein farming area lies within the greater Cape Town area, adjacent to Kraaifontein and Brackenfell. The Bottelary River flows through the area (Fig 2.1) and is a source of irrigation water for the surrounding farms. A canal brings runoff which joins with effluent from the Scottsdene Waste Water Treatment Works and flows into the Bottelary River, west of the Botfontein and Bottelary Road junction. This is also used as water for irrigation.

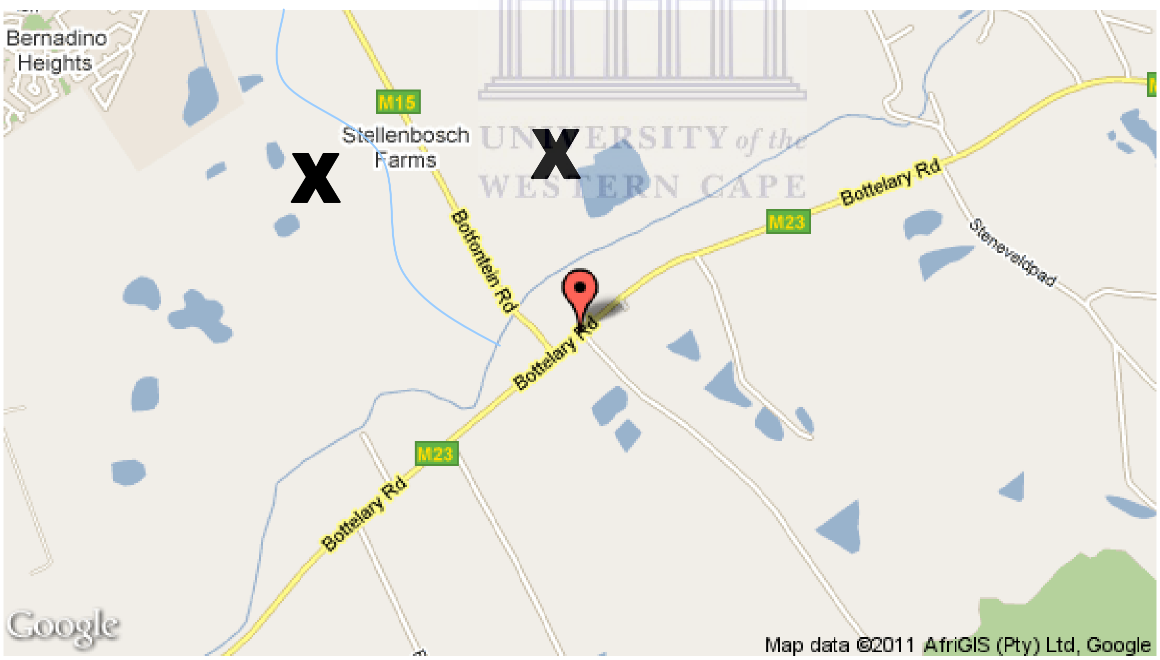

Figure 2.1 The Kraaifontein Farming Area (x – areas sampled) Adapted from Google map.

Moeletsi *et al.* (2004) measured the heavy metal levels of the influent into the Scottsdene Waste Water Treatment Works and it was found that the concentrations of cadmium and lead were 1.0 mg. kg^{-1} and 8.1 mg. kg^{-1} respectively. These levels were higher than the general standards of waste discharge set by DWAF Guidelines (2008).

2.2 Materials and Methods

2.2.1 Sample collection and preparation

Samples were collected from several farms in the Kraaifontein farming area. Fields of dwarf beans were scattered throughout the area, and whole plants were dug up, one sample from each field, fourteen in total. The plants were then divided into different organs; roots, stems, leaves and fruits. They were dried in a forced draft oven at 70 °C and samples were then ground in a Wiley mill.

2.2.2 Chemical Analysis

The samples were dry ashed and digested in hydrochloric acid. The solution was then analysed on a Varian Radial ICP. For the heavy metals, the nitric acid-peroxide wet ashing method was used (Moore and Chapman 1987).

2.3 Statistical Analyses

A statistical programme (SAS 8.2; SAS 1999) was used to analyze the results of the chemical analyses. Analysis of variance was performed using GLM (General Linear Models) procedure of SAS. The Shapiro-Wilk test was used to check for normality. The student T test was used to calculate the least significant difference at the 5% level to compare organ means (Ott 1998). A probability level of 5% or less was considered to be significant.

2.4 Results

Figure 2.4.1 shows that the nitrogen content was higher in the leaves and fruits which in turn had a higher concentration than the stems which were in turn higher than the roots.

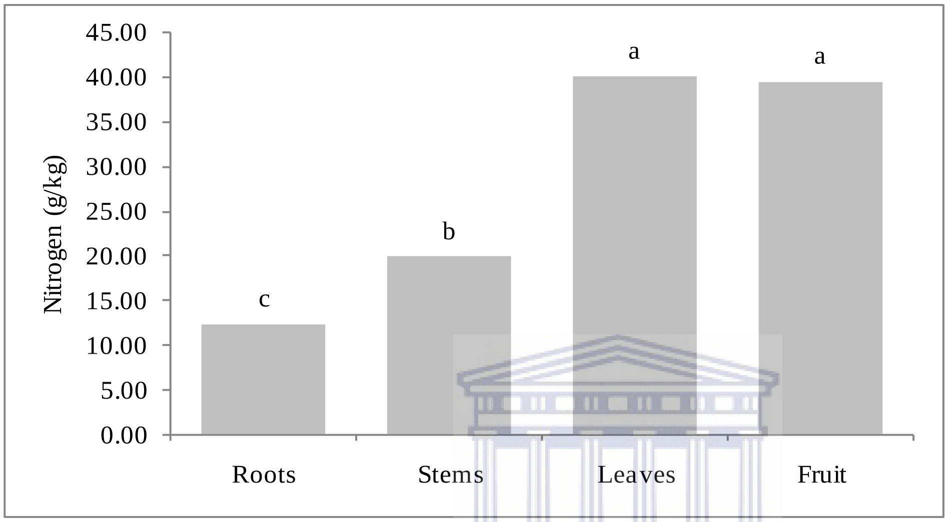

Figure 2.4.1: Nitrogen content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

Phosphorus concentrations were significantly higher in the fruits than any of the other plant organs (Fig. 2.4.2).

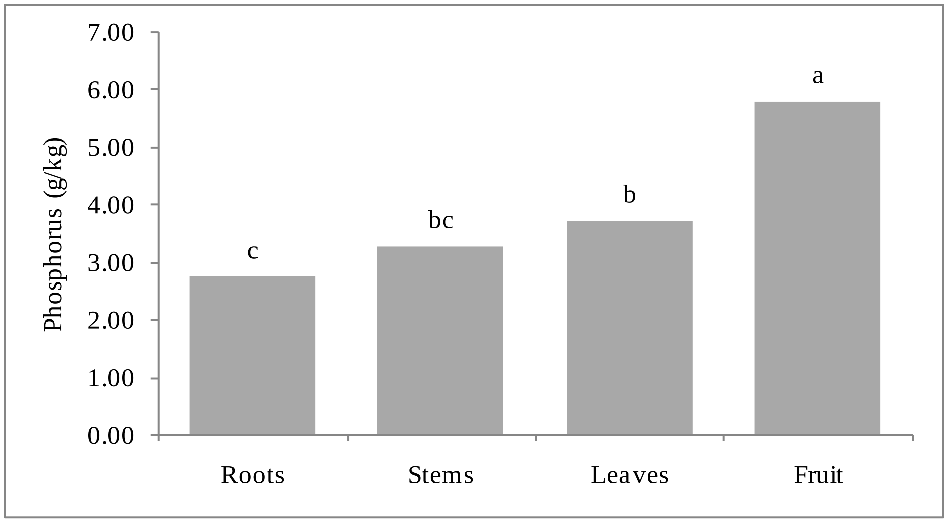

Figure 2.4.2: Phosphorus content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

Similar concentrations of potassium were seen in stems and fruit, and again in roots and leaves. The stems and fruit potassium concentrations exceeded those in the roots and leaves (Fig. 2.4.3).

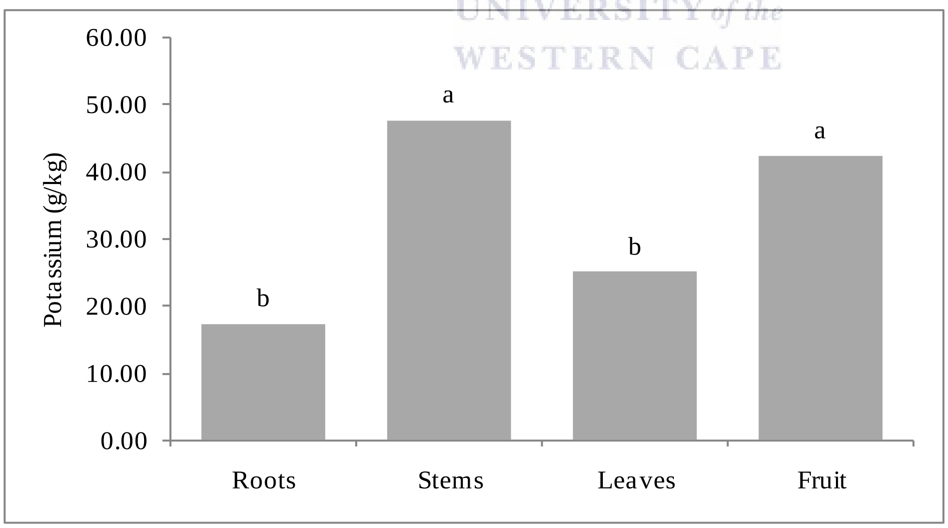

Figure 2.4.3 Potassium content of organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

Roots, stems and fruit showed no differences in calcium concentrations, whereas leaves had a larger amount of calcium in them (Figure 2.4.4).

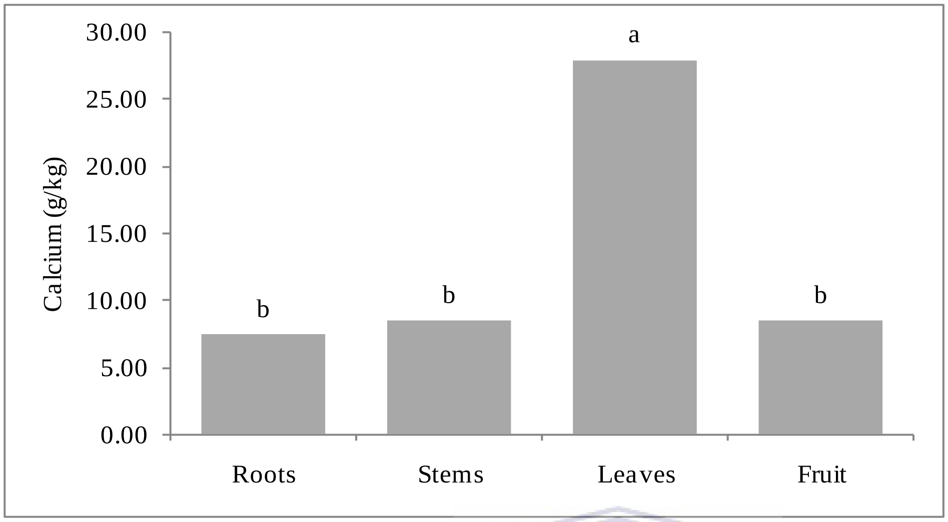

Figure 2.4.4: Calcium content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

The leaves showed the highest magnesium concentrations, followed by the fruits and then the roots and stems which were similar (Fig. 2.4.5).

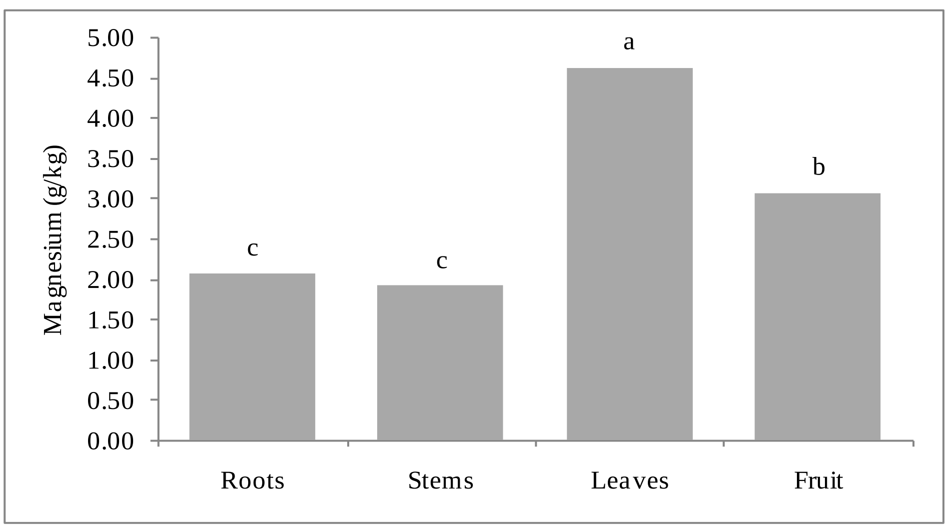

Figure 2.4.5: Magnesium content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

Figure 2.4.6 showed an interesting pattern, whereby a considerable amount of sodium was found the roots, and no significant differences were found in the much lower sodium concentrations in the stems, leaves or fruit.

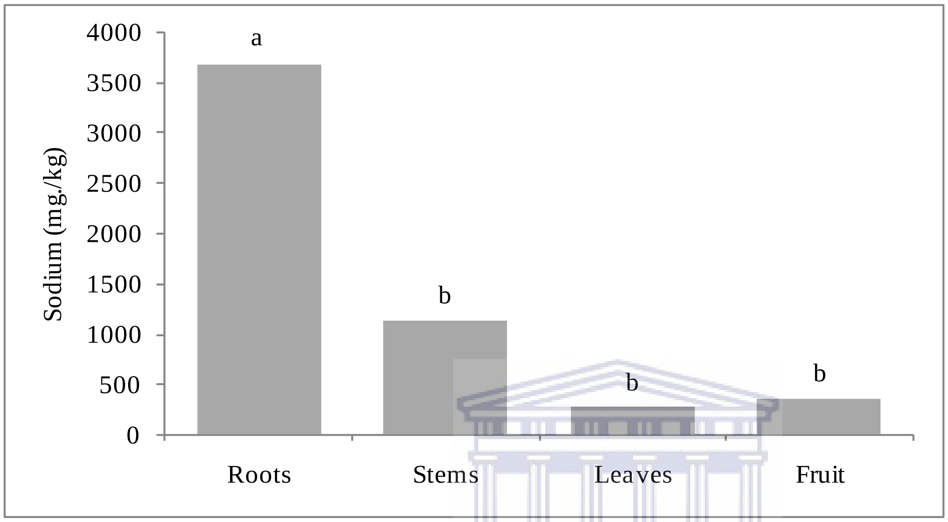

Figure 2.4.6: Sodium content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

Roots and leaves had higher manganese concentrations than the stems and the fruits (Fig. 2.4.7).

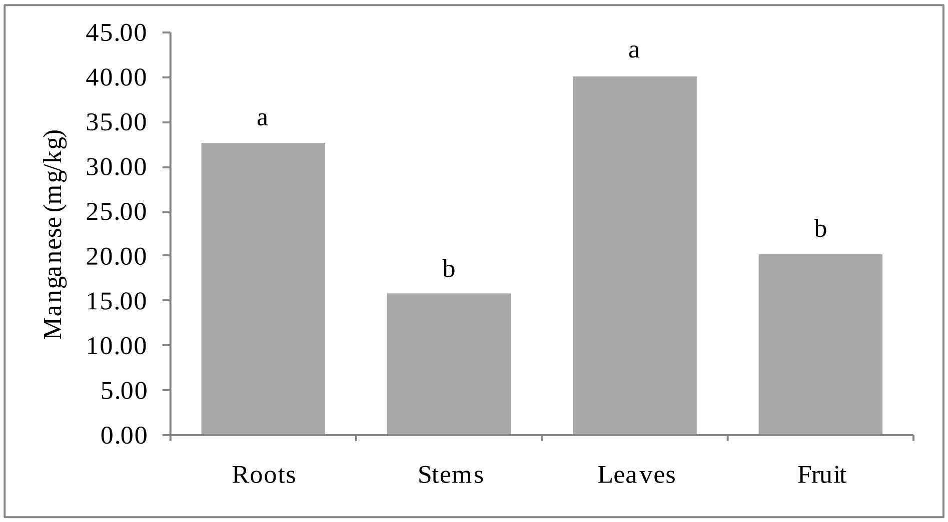

Figure 2.4.7: Manganese content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

Iron showed a similar result to that of sodium where the roots showed a higher concentration than was found in stems, leaves and fruit (Fig. 2.4.8).

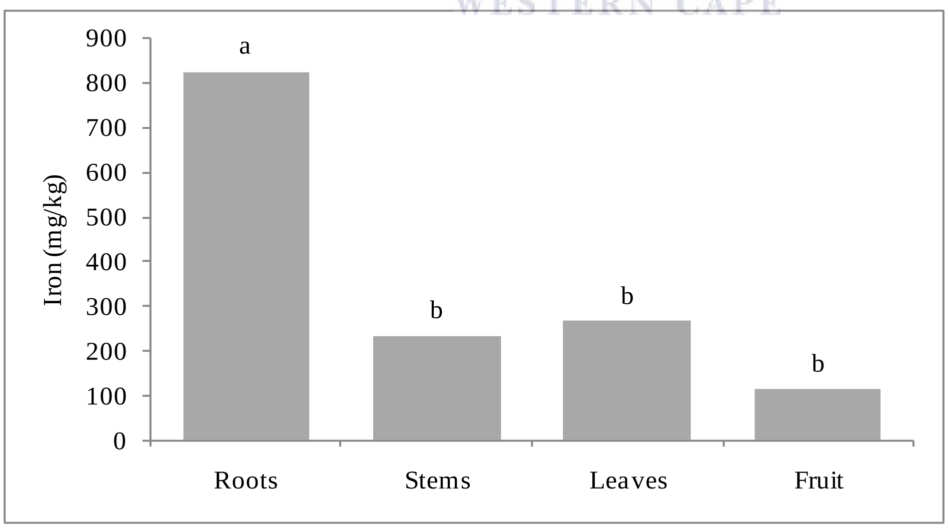

Figure 2.4.8: Iron content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

The copper concentration in the leaves was higher than that of the fruits (Fig. 2.4.9).

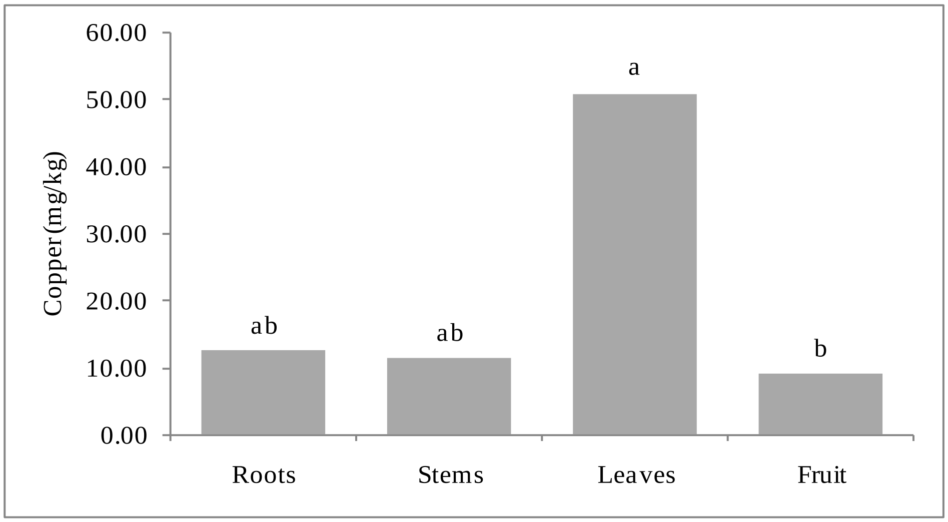

Figure 2.4.9: Copper content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

The zinc concentrations did not differ across the various organs, and the mean concentration found was 42.75 mg. kg^{-1}.

Boron concentrations were found to be significantly higher in leaves than in the fruits (Fig. 2.4.10). The fruit boron concentration exceeded that of the roots (Fig. 2.4.10).

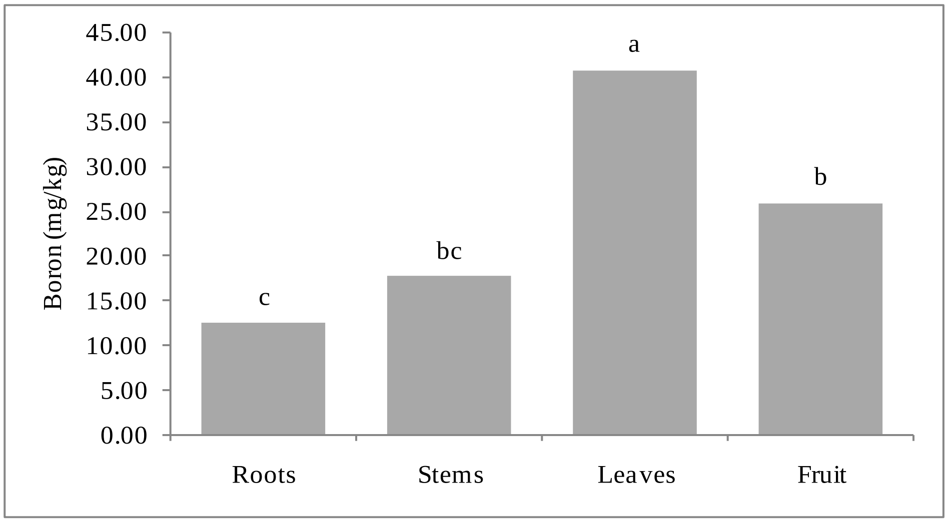

Figure 2.4.10: Boron content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

No significant difference was found in the cadmium concentrations between the various organs. The mean cadmium concentration was 0.658 mg. kg^{-1}, however cadmium reached 5.31 mg. kg^{-1} in a root sample. The highest cadmium concentration in an edible fruit was 0.49 mg. kg^{-1}.

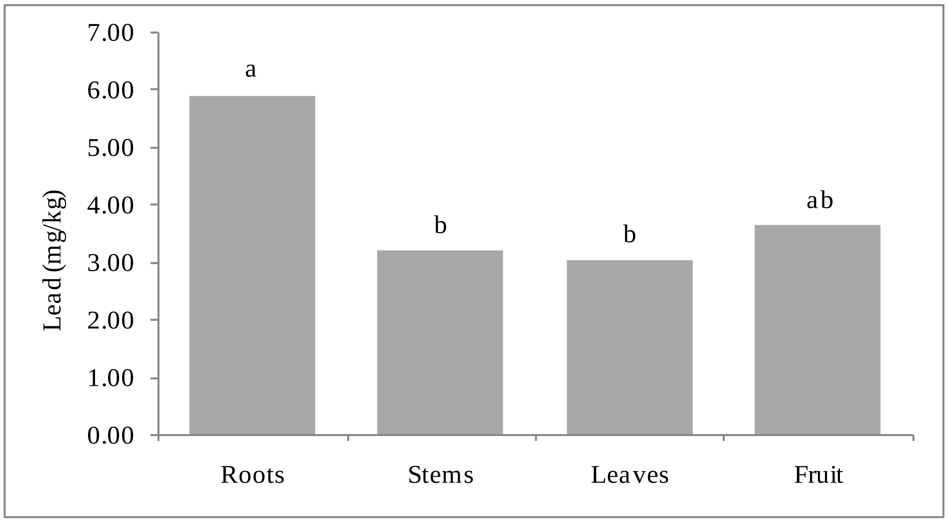

Figure 2.4.11: Lead content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.

Lead concentrations were higher in the roots than in the stems and leaves (Fig. 2.4.11). The highest lead concentration found was 5.924 mg. kg^{-1} in roots. The highest concentration in fruits was 3.640 mg. kg^{-1}. This was the same plant that gave high fruit cadmium concentrations.

2.5 Discussion

Similar trends were seen for all elements, whereby the highest concentrations were accumulated in the upper organs of the plants i.e. leaves and fruit. This shows that uptake and translocation of these elements were good.

Nitrogen, calcium, magnesium, manganese, copper and boron tended to be at higher concentrations in the leaves (Fig. 2.4.1, Fig. 2.4.4, Fig. 2.4.5, Fig. 2.4.7, Fig. 2.4.9 and Fig. 2.4.10) and high concentrations of nitrogen, phosphorus, and potassium were found in the fruits (Fig. 2.4.1, Fig. 2.4.2 and Fig. 2.4.3). This could be because these elements are relatively mobile in the plant (Hopkins and Hüner 2004).

The only element that showed a significant amount in the stems was potassium (Fig. 2.4.3). Iron and lead were found to be significantly higher in the roots than any other plant organ (Fig. 2.4.8 and Fig. 2.4.11). This corresponded to Begonia *et al*. (1998) and Chen *et al.* (2004), which stated that the mobility of lead from roots to shoots of plants is usually low. There are two possible reasons for this, first being that the layer of cuticle and waxes in leaves form a effective barrier against aerial lead penetration, as the protective layer stops lead ions on the leaf surface and does not let them into the leaves (Piechalak and Tomaszewska 2002). The second reason being, that lead is rapidly accumulated in the roots and only a small amount of the absorbed lead is translocated to the shoots (Li Li *et al*. 2008). The heavy metals zinc and cadmium showed no significant concentration differences in any plant organ.

Larcher (2001) reported on normal levels of elements in its plants (Table 2.1) and what is generally accepted to be a required level. Not all the results from samples collected from the farms correlated to the ranges proposed by Larcher (2001) (Table 2.2). It is noticeable that the elements in this study fell within the normal ranges.

Table 2.1 Normal ranges of various elements in plants and required levels modified from Larcher (2001), and average levels found in *Phaseolus vulgaris* in the field.

Element	Phytomass (Range) (g/kg) [a]	Requirement (g/kg) [b]	Field averages (g/kg) [b]
N	12 - 75	15 - 25	28
P	0.1 - 10	1.5 - 3	3.9
K	1 - 68	5 - 20	33.1
Ca	0.4 - 13	3 - 15	13.1
Mg	0.7 - 9	1 - 3	2.9
Na	0.02 - 1.5		1.4
Mn	0.003 - 1	0.03 - 0.05	0.027
Fe	0.002 - 0.7	ca. 0.1	0.36
Cu	0.004 - 0.02	0.005 - 0.01	0.021
Zn	0.001 - 0.4	0.01 - 0.05	0.043
B	0.008 - 0.2	0.01 - 0.04	0.024
Pb	Up to 0.02		0.004

[a] Larcher (2001)
[b] This study

However, when one considers the four heavy metals cadmium, copper, lead and zinc in the edible bean fruits (Table 2.2) it is clear that the average values for cadmium, lead and zinc are higher than the standard, and the highest lead concentration in a fruit was some twenty times higher . It has already been noted that heavy metal concentrations tend to be low in fruits (Yang *et al*. 2000). As with Meerkotter (2003 and 2012) and Soyagiso (2003), these heavy metal concentrations are alarmingly high in the edible portion.

Table 2.2 Heavy metal concentrations in bean fruits as compared with the standard.

Heavy metal	Mean measured	Highest measured	Standard [a]
Cd	0.26	0.49	0.1
Cu	9.1	17.0	30.0
Pb	3.64	10.19	0.3-0.5
Zn	43.5	62.0	40.0

[a] amendment to regulations made under the Foodstuffs, Cosmetics and disinfectants Act (Department of Health 2003)

2.6 References

Alam MGM, Snow ET and Tanaka A (2002) Arsenic and heavy metal contamination of vegetables grown in Samta Village, Bangladesh. *The Science of the Total Environment* **308** (2003): 83-96.

Alegria A, Barbera R and Farre R (1990) Influence of environmental contamination on Cd, Co, Cr, Cu, Ni, Pb and Zn content of edible vegetables: Safety and Nutritional Aspects. *Journal of Micronutrient Analysis* **8** (1990): 91-104.

Begonia GB, Davis MFT and Gray BCN (1998) Growth responses of Indian mustard and its phytoextraction of lead from a contaminated soil. *Bulletin of Environment Contamination Toxicology* **61**: 38-43.

Chen Y, Li X and Shen Z (2004) Leaching and uptake of heavy metals by ten different species of plants during an EDTA assisted phytoextraction process. *Chemosphere* **57**: 187-196.

Cieslinski G, Neilsen GH, Hogue EJ (1996) Effect of soil cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruit of strawberry plants (*Fragaria manassa* Duch.). *Plant and Soil* **180**: 267-276.

Codex Alimentarius Commission (1994) Contaminants, Joint FAO and WHO Food Standards Program, Vol.XV11.

DWAF (1998) Department of Water Affairs and Forestry, Second Edition, Waste Management Series, Minimum Requirements for Waste Disposal by Landfill.

Hopkins WG and Hüner NPA (2004) Introduction to Plant Physiology 3rd Edition. John Wiley and Sons Ltd, USA.

Kabata-Pendias A and Pendias H (1992) Trace elements in soils and plants- 2nd edition. CRC Press Inc, USA *In* Cieslinski G, Neilsen GH, Hogue EJ (1996) Effect of soil cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruit of strawberry plants (*Fragaria manassa* Duch.). *Plant and Soil* **180**: 267-276.

Kachenko AG and Singh B (2006) Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water, Air and Soil Pollution* **169**:101-123.

Larcher W (2001) Physiological Plant Ecology, 4th Edition. Springer-Verlag, Germany.

Li Li Y, Liu YG, Liu JL, Zeng GM, Li X (2008) Effects of EDTA on Lead uptake by *Typha orientalis* Presl. : A new lead-accumulating species in southern China. *Bulletin of Environmental Contamination and Toxicity* **81**: 36-41.

Lugon-Moulin N, Ryan L, Donini P and Rossi L (2006) Cadmium content of phosphate fertilizers used for tobacco production. *Agronomy for Sustainable Development* **26**: 151-155.

Meerkotter M (2012) Sources of heavy metals in vegetables in Cape Town and possible methods of remediation. Unpublished PhD thesis, University of the Western Cape, Cape Town.

Meerkotter M (2003) Heavy metals and vegetable farming in Cape Town. Unpublished MSc thesis. University of the Western Cape, Cape Town.

Moeletsi M, Mazema H and Halday I (2004) Strategy for mitigating the impacts of heavy metals in wastewater and the economic and environmental implications for the city of Cape Town. Proceedings of the 2004 Water Institute of Southern Africa (WISA) Biennial Conference.

Moore PD, Chapmen SB (eds) Methods in Plant Ecology- 2nd edition. Blackwell Scientific Publications, California, pp 302- 303. ISBN 0-632-00989-6.

Ott RL (1998) An introduction of statistical methods and data analysis. Duxbury Press, Belmort.

Piechalak A and Tomaszewska B (2002) Accumulation and detoxification of lead ions in legumes. *Phytochemistry* **60**: 153-167.

Piechalak A, Tomaszewska B and Baralkiewicz D and Malecka A (2002) Accumulation and detoxification of lead ions in legumes. *Phytochemistry* **60**: 153-162.

Schroeder HA and Balassa JJ (1963) Cadmium: Uptake by vegetables from Superphosphate in soil. *Science, New Series* **140** (3568): 819-820.

Seaward MRD and Richardson DHS (1990) Atmospheric sources of metal pollution and effects on vegetation. *In*: Piechalak A and Tomaszewska B (2002) Accumulation and detoxification of lead ions in legumes. *Phytochemistry* **60**: 153-167.

Shapiro SS and Wilk MB (1965) An analysis of variance test for normality (complete samples). *Biometrika* **52**: 591-611.

Sharma RK, Agrawal M and Marshall FM (2007) Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. *Environmental Pollution* (2007): 1-10.

Sogayiso M (2003) Contamination of vegetables with heavy metals. Unpublished BSc Honours project. University of the Western Cape, Cape Town.

Yang Y, Zhang F-S, Li H-F and Jiang R-F (2000) Accumulation of cadmium in the edible parts of six vegetable species grown in Cd contaminated soils. *Journal of Environmental Management* **90**: 1117-1122.

CHAPTER 3

The Effect of Two Heavy Metals and Two Mitigation Techniques on the Growth and Chemical Composition of *Phaseolus vulgaris* var. Contender.

3.1 Introduction

The consequences of excessive accumulation of heavy metals in agricultural soils are environmental contamination, which in turn leads to elevated heavy metal uptake by crops which ultimately affect food quality safety (Muchuweti *et al.* 2006). Large areas of agricultural soils are contaminated by heavy metals that mainly originate from mining, industrial emissions or sewage sludge (Schmidt 2003). The transfer of heavy metals from soils to plants is dependent on 3 factors: the total amount of available elements, the activity and ionic ratios of elements in the soil, and the rate of element transfer from solid to liquid phases and to plant roots (Schmidt 2003). Heavy metal uptake and translocation may differ greatly and depend on plant species and metals (Prasad 1999). Tolerance to these heavy metals involves exclusion or accumulation of heavy metals, the latter involves trapping, binding or complex formation in the tissue (Prasad 1999). Baker (1981) proposed the three basic uptake strategies. Excluder plants are plants that have a low uptake of the metal at high external metal concentrations (Baker 1981). Although they have a barrier to avoid uptake when metal concentrations become too high, the barrier loses its function and uptake increases extremely (Baker 1981). Accumulator and hyperaccumulator plants, as their names say, accumulate high concentrations of metals, and may have detoxification mechanisms in order to expel these metals (Baker 1981). Common strategies adopted by these accumulator plants are to immobilize the metals in the roots or accumulate high concentrations of metals in their leaves and then get rid of them by leaf fall (Prasad 1999). The use of biological materials to cleanup heavy metals contaminated soils has been focused on as an efficient and affordable form of bioremediation (Li Li *et al.* 2008). Phytoremediation is of growing interest because of its low environmental impact and cost-effectiveness (Shivendra and

Natalie 2002). Phytoremediation is the use of plants to remediate organic, inorganic or nuclear pollutants in contaminated sites (Lai and Chen 2004). Phytoremediation techniques include phytoextraction, phytostabilization, phytovolatilization, phytodegradation and phytofiltration (Lai and Chen 2004). Of these techniques, phytoextraction and phytostabilization are used to remediate inorganic-contaminated soil, especially soil contaminated by heavy metals (Lai and Chen 2004). Phytoextraction accumulates toxic metals from contaminated soil into the aboveground tissue of higher plants, which are then harvested and incinerated (Chaney *et al.* 1997). Phytostabilization uses the dense root system of the plant to physically stabilize the contaminated soil, protecting against erosion by wind and water, thereby reducing the risk to the environment by leaching of pollutants into groundwater (Chaney *et al.* 1997, Pulford and Watson 2003). Two strategies, one involving chelating agents and the other involving genetic engineering, are being developed to increase the phytoextraction of metals with higher biomass or by transgenic plants (Lai and Chen 2004). Various efforts have been made to develop technologies for the remediation of contaminated soils, including ex-situ washing with physico-chemical methods, and in-situ immobilization of metal pollutants (Rulkins *et al.* 1995). These methods of cleanup are generally very costly and often harmful to soil properties (i.e. texture, organic matter, and micro organisms) that are important for the restoration of contaminated soils (Azhar *et al.* 2006). Phytoextraction of contaminated soils has attracted considerable attention for its low cost of implementation and multiple environmental benefits (Salt *et al.* 1998). Two approaches have been proposed for the phytoextraction of heavy metals, the use of natural hyper-accumulator plants with exceptional metal accumulating capacity, and the utilization of high biomass plants with a chemically enhanced method of phytoextraction (Salt *et al.* 1998). Numerous efforts have been made to develop technologies for the remediation of contaminated soils, including ex situ washing with physio-chemical methods, and the in situ immobilization of metal pollutants (Chen *et al.* 2004). These methods of cleanup are generally very costly, and often harmful to properties of soil (i.e. texture, organic matter, micro organisms) that are desirable for the restoration of contaminated soils has attracted attention for its low cost of implementation and many environmental benefits (Salt *et al.* 1998). The success of a phytoextraction process is dependent on adequate plant yields and high-metal concentrations in the shoots of plants (Chen *et al.* 2004). The aim of phytoextraction is to reduce the levels of metals in contaminated soil to accepted levels within a reasonable time frame (Chen *et al.* 2004). A number of techniques have been developed that aim to remove heavy metals from contaminated soils, including ex-situ washing with physical-chemical methods (Anderson 1993) and in situ phytoextraction (McGrath 1998, Salt *et al.* 1998). In the ex situ washing methods, chelating agents or acids are used to improve heavy metal removals (Sun *et al.* 2001). Apart from ex-situ washing techniques, some of the in situ phytoextraction methods also use chelating agents to enhance heavy metal availability in soils (Sun *et al.* 2001). The process depends on the ability of the selected plants to grow and accumulate metals under the specific climate and soil conditions of the site being remediated (Chen *et al.* 2004).

The two heavy metals that were studied were cadmium and lead, and the two mitigation techniques that were assessed were EDTA and phosphate. Key questions that were asked and will be answered at the end of this chapter are the following: Did heavy metal treatments affect growth? Did the heavy metals (cadmium and lead) accumulate in the roots or in the shoots? What effects did the heavy metals have on nutrient uptake? Was there a mitigation effect? Did the mitigation treatments affect growth? Were phosphate and EDTA able to mitigate the effects of the heavy metals on growth, and on nutrient uptake?

3.1.1 Cadmium

Cadmium is a toxic trace pollutant (Sandalio *et al.* 2001), and is not essential for plants (Dixit *et al.* 2000). Lockwood (1976) described cadmium as a "non-essential heavy metal and a powerful enzyme inhibitor". Cadmium toxicity is usually associated with interrupting uptake and distribution of macronutrients and micronutrients in plants (Das *et al.* 1997, Sandalio *et al.* 2001) and its visible symptoms include stunting and chlorosis (Das *et al.* 1997). Cadmium has been shown to cause a reduction in biomass (Dixit *et al.* 2000) and a significant decrease in photosynthesis rate (Sandalio *et al.* 2001). Overnell (1975) reported that cadmium reduced concentrations of ATP and chlorophyll as well as decreasing oxygen production. In terms of its transport within plants, Perfus-Barbeoch *et al.* (2002) reported that cadmium is taken up by the roots via essential metal transporters and is partly translocated to the shoot. Benavides *et al.* (2005) describes its transport as follows: "Cadmium first enters the roots through the cortical tissue and is translocated to the above-ground tissues, and as soon as cadmium enters the roots, it can reach the xylem through an apoplastic and/or symplastic pathway, complexed by several ligands."

In terms of toxicity, dietary intake of cadmium poses a risk to animals and human health, as studies revealed that cadmium have shown to have carcinogenic effects and have been related to high prevalence of upper gastrointestinal cancer (Sharma *et al.* 2007).

3.1.2 Lead

Lead contamination in soils is a major environmental problem, and its normal sources can be classified in three categories: industrial, agricultural and urban activities (Shen *et al*. 2002). Lead is rapidly accumulated in the roots, if lead is bioavailable in the plant growth media and in general only a small proportion of absorbed lead is translocated to shoots (Li Li *et al.* 2008). Lead is one of the most persistent metals (Li Li *et al.* 2008) and frequently encountered heavy metals in polluted environments (Seaward and Richardson 1990). Severe lead contamination in soils and in ground and surface waters may cause a variety of environmental problems, including loss of vegetation, groundwater contamination, and lead toxicity in plants, animals and humans (Li Li *et al.* 2008). Lead, largely immobile in soils and the efficient uptake of metals by plants is often limited by the solubility and diffusion of heavy metals to the root surface of plants (Chen *et al.* 2004). Lead is rapidly accumulated in the roots, as roots are the main accumulation site of Pb^{2+}, and only a small proportion of absorbed lead is translocated to shoots (Li Li *et al.* 2008) as mobility of lead from roots to shoots of plants is usually low (Begonia *et al.* 1998). When lead enters the plant root, it encounters the neutral pH, high phosphate and high carbonate environment of the intercellular spaces (Li Li *et al.* 2008). Under these conditions, lead precipitates as phosphate or carbonate and does not reach the xylem for translocation (Malone *et al.* 1974, Gabrielle and Patrick 1996). The lead concentration in the underground parts is two to eight times higher than it in the leaves (Li Li *et al.* 2008). The layer of cuticle and waxes in leaves usually forms an effective barrier against atomic lead penetration. This protective layer stops Pb^{2+} ions on the root surface and does not let them into the leaves (Piechalak and Tomaszewska 2002). Differences in growth rate of root length are an important indicator of plant resistance to heavy metals (Li Li *et al.* 2008). Inhibition of root elongation and browning of roots occurred in plants grown in lead solutions (Azhar *et al.* 2006). When the chelator was added to the medium, a clear decrease in the lead toxic effect on plants was observed (Li Li *et al.* 2008). Increases in the concentration of lead in shoots were likely due to an increase in uptake rather than a decrease in the dry weights of the plants (Chen *et al.* 2004).

3.1.3 Chelating Agents

Plants produce peptides called phytochelatins that naturally bind and detoxify dangerous toxic metals such as lead, mercury and cadmium (Spiro 2003). These phytochelatins mediate the accumulation of the peptide-metal mix in the leaves of the plant, where they can be safely harvested (Spiro 2003). Synthetic chelating agents are widely used to supply plants with micronutrients in both soil and hydroponics (Huang *et al.* 1997) and the addition of synthetic chelators, soil acidiers, or commercial nutrients can enhance phytoremediation (Li Li *et al.* 2008) by increasing both the solubility of metal in soil solution and the concentration of metal in the shoots of plants (Huang and Cunningham 1996, Wu *et al.* 1999; Lai and Chen 2004) by increasing plant uptake and translocation of heavy metals from roots to green parts of the plants (Huang *et al.* 1997). Enhanced uptake and accumulation of metals by plants was obtained due to introducing a proper chelator into the environment (Li Li *et al.* 2008). However the application of synthetic chelating agents at high concentrations can also be toxic to plants (Cooper *et al.* 1999) and showed a reduction in the biomass of the plant and the total amount of metal removed (Lai and Chen 2004).

3.1.4 EDTA

EDTA is the most commonly used chelate because of its strong chelating ability for different heavy metals (Sun *et al*. 2001). In Li Li *et al.* (2008), it was found that EDTA increased the amount of Lead taken up by plants and also speeded up the metal translocation from roots to leaves. Laboratory studies have shown that EDTA is successful in removing Pb, Zn, Cu and Cd from contaminated soils (Sun *et al.* 2001; Lai and Shen 2004). EDTA was found to be the most effective phytoextraction (Blaylock *et al.* 1997) in enhancing the accumulation of heavy metals in the aerial parts of plants (Wu *et al.* 1999; Azhar *et al.* 2006). On addition of EDTA, an improvement in seedling growth was recorded (Azhar *et al.* 2006). Studies have shown that EDTA was the most effective chelator for lead (Huang and Cunningham 1996;Huang *et al.* 1997; Cooper *et al.* 1999; Lasat 2002; Michael *et al.* 2007) as it increased the amount of lead taken up by plants and also speeded up the metal translocation from roots to leaves (Li Li *et al.* 2008).The application of EDTA was helpful in reducing the toxic effect of lead due to its chelating property and shown to be beneficial in speeding up the phytoextraction of lead through hyper-accumulating plants (Azhar *et al.* 2006). The application of EDTA to the soil substantially increased the amount of lead extracted by the shoots of the plants (Chen *et al.* 2004). EDTA application slightly increased the concentrations of lead in the shoots; the total amount of metals extracted by shoots remained largely unchanged due to a reduction in the yield of the shoots as the rates at which EDTA was applied increased (Chen *et al.* 2004). The addition of EDTA eliminated to a great degree the inhibition of root elongation growth, lowered roots browning and resulted in a growing number of side roots (Li Li *et al.* 2008). EDTA has shown toxic effects by reducing shoot, root and dry matter stress tolerance indices that may be due to its chelating property (Azhar *et al.* 2006). EDTA is able to dissolve metals and enhance the uptake of metals by plants (Chen *et al.* 2004). The application of EDTA decreased the net production of shoot and root biomass and increased the accumulation of lead in the shoots of the plants (Chen *et al.* 2004).The phytotoxic effects of EDTA occurred at least partly in response to the uptake of lead by plants (Chen *et al.* 2004). Studies done by Chen *et al.* 2004 on peas had a greatest ability to uptake metal after the EDTA treatment. The use of EDTA has shown to increase the concentration of soluble Pb in Pb contaminated soils and subsequently enhance Pb uptake by plants with high biomass production (Blaylock *et al.* 1997; Huang *et al.* 1997). The Pb-EDTA complex is taken up by the plant, with transpiration as the driving force (Sun *et al.* 2001). Plants were very sensitive to the presence of EDTA (Chen *et al.* 2004). It appears that plants used for phytoextraction must first become well developed and established in contaminated soils before they are exposed to the increase stress of metals caused by the application of EDTA (Chen *et al.* 2004). It has been proposed that it would be advantageous if EDTA is applied to soil at a low rate to aid the breakdown barriers to the uptake of metals by plants and to enable metals to be translocated into the shoots (Chen *et al.* 2004). There are also concerns that the application of EDTA to soils has the potential risk that mobilized lead and other heavy metals will migrate from the soil to the groundwater (Chen *et al.* 2004) and should therefore be monitored.

3.1.5 Phosphate

Phosphate is an essential plant nutrient and used in agriculture as a fertilizer. Studies performed by Wang *et al*. (2008) found that phosphate fertilizers decreased lead concentration as well induced immobilization of cadmium, lead and zinc. In the same study, it was found that the addition of Triple Super Phosphate resulted in a decrease in cadmium and zinc uptake to the shoots. Hettiarachchi and Pierzynski (2002) and Cao *et al.* (2004) reported on a reduction of zinc concentrations when TSP was added. Triple Super Phosphate (TSP) fertilizer was administered to the plants, as this is the fertilizer used (in foliar spray form) by farmers in the Joostenbergvlakte farming (Meerkotter 2012, 2003).

3.2 Materials and Methods

A sand culture pot experiment was conducted in order to test the effectiveness of two mitigation treatments, Triple Super Phosphate and EDTA, in reducing the uptake of the heavy metals cadmium and lead. The extent to which they could mitigate growth effects and the uptake of the nutrient elements was also determined. The plant material used was the dwarf bean (*Phaseolus vulgaris* var. Contender).

3.2.1 Growing the plants

Performance tested seed, chemically treated and packaged by Starke Ayres was obtained from a local nursery. The seeds were sown in washed (99% pure) silica sand sourced from Consol glass, in seventy five 12.5 cm top diameter pots. This was to avoid complications from cation exchange in the soil. These were then watered every alternate day for two weeks with tap water. After two weeks they were then treated with Chemicult, a plant nutrient supplement (made up hydroponically using pack instructions) once a week, while still continuing to be watered every alternate day with tap water. Once the plant developed to their trifoliate stage, treatments were given.

The field capacity for the pots was calculated at 100 mL, and this replacement volume was applied at each watering, split into 50 mL each for treatment and mitigation agent. Metal treatment stock solutions and mitigation agents were applied in 25 combinations once a week for a period of seven weeks, including controls for both treatment and mitigation agent.

Stock solutions were prepared by dissolving the suitable amount of the specific metal nitrate; lead and cadmium, in 1 L of tap water. Granular triple super phosphate (TSP) was used, and the suitable amount of P needed was calculated from the percentage of P in the TSP fertilizer, which was 19.8 %. EDTA solutions were prepared using Ethylenediaminetetetraacetic acid disodium salt dehydrate (Na_2-EDTA.$2H_2O$) (99%).

3.2.2 Growth medium

There is a predominant theory in literature that heavy metals are accumulated more in the roots than the shoots (Yang *et al.* 2000) and this experiment is to test that very theory. The phosphate treatment serves as an immobilising and the EDTA as a mobilising agent. This is based on the theory that phosphate binds heavy metals in the soil, making them unavailable to plants. EDTA on the other hand is often used in bioremediation studies to enhance the uptake of heavy metals (Huang *et al.* 1997).

The WRC 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 kgs (WRC, 1997). Final concentrations at the legal limit and ten times the limit were used for each metal solution. The phosphate and EDTA concentrations worked out from their molar ratios with lead where EDTA has a 1:1 and Phosphate a 1: 4 molar ratio with the heavy metal. Several investigations into the immobilization of lead and cadmium by phosphate indicated that this ratio was effective in immobilizing lead and some other metals to a lesser extent (Chen *et al*. 2007, Wang *et al.* 2008). The amendments were tested at the following concentrations: Cadmium 2 and

20 mg. kg^{-1} of soil, Lead 6.6 and 66 mg. kg^{-1}, EDTA 4 and 12 mg. kg^{-1} soil and Phosphate 1 and 8 mg. kg^{-1} soil.

The experiment was conducted in growth cabinets of the Biodiversity and Conservation Biology department at University of the Western Cape's Bellville campus. These growth cabinets were set at 12 hour day-night cycles, day temperature at 23 ° C and night temperature at 11° C.

3.2.3 Experimental Design

A randomized block experiment of five heavy metal treatments together with five mitigation treatments was replicated five times. The 25 treatments are given in Table 3.1.

Table 3.1 The twenty five heavy metal and mitigation combinations used to grow *Phaseolus vulgaris* var. Contender bean plants.

Pot	Heavy Metal	Mitigation
1	Control	Control
2	Control	Low EDTA (4 mg/kg)
3	Control	High EDTA (12 mg/kg)
4	Control	Low Phosphate (1 mg/kg)
5	Control	High Phosphate (8mg/kg)
6	Limit Cd (2mg/kg)	Control
7	Limit Cd (2mg/kg)	Low EDTA (4 mg/kg)
8	Limit Cd (2mg/kg)	High EDTA (12 mg/kg)
9	Limit Cd (2mg/kg)	Low Phosphate (1 mg/kg)
10	Limit Cd (2mg/kg)	High Phosphate (8mg/kg)
11	10x Limit Cd (20 mg/kg)	Control
12	10x Limit Cd (20 mg/kg)	Low EDTA (4 mg/kg)
13	10x Limit Cd (20 mg/kg)	High EDTA (12 mg/kg)
14	10x Limit Cd (20 mg/kg)	Low Phosphate (1 mg/kg)
15	10x Limit Cd (20 mg/kg)	High Phosphate (8mg/kg)
16	Limit Pb (6 mg/kg)	Control
17	Limit Pb (6 mg/kg)	Low EDTA (4 mg/kg)
18	Limit Pb (6 mg/kg)	High EDTA (12 mg/kg)
19	Limit Pb (6 mg/kg)	Low Phosphate (1 mg/kg)
20	Limit Pb (6 mg/kg)	High Phosphate (8mg/kg)
21	10X Limit Pb (66 mg/kg)	Control
22	10X Limit Pb (66 mg/kg)	Low EDTA (4 mg/kg)
23	10X Limit Pb (66 mg/kg)	High EDTA (12 mg/kg)
24	10X Limit Pb (66 mg/kg)	Low Phosphate (1 mg/kg)
25	10X Limit Pb (66 mg/kg)	High Phosphate (8mg/kg)

3.2.4 Harvest

After 49 days the plants were harvested, divided into shoots and roots and the dry mass of the shoots recorded. A chlorophyll index meter was also used to record the chlorophyll index in young fully expanded leaves. They were then placed in brown paper bags and oven dried at 70 ºC for approximately two days. Thereafter they were ground and plant material was digested for analysis of heavy metal, cadmium and lead concentrations. Where there was sufficient material (shoots), other plant nutrients were also determined.

3.2.5 Chemical Analysis

3.2.5.1 Digestion

A sulphuric - peroxide digestion method was used (Moore and Chapman 1986). A 0.21 g mass of Se and 14 g of $Li_2SO_4.H_2O$ were added to 420ml of 100 vol H_2O_2. The solution was mixed well and 350 ml of conc. H_2SO_4 was carefully added to it. The mixture was cooled down by placing it on ice during the addition of the acid. After every session of use, the digestion mixture was stored in a fridge at approximately 4 °C.

A 0.4 g sample of dry ground material was weighed into cigarette paper and placed in a digestion tube. A 4.5 ml sample of the digestion mixture was then added. The mixture was digested in a heating block in a fume cupboard at 150 °C for the first hour and thereafter gradually increased till 380 °C until an almost colourless solution was obtained. Glass funnels were used to cover the opening of the digestion tube to minimize the loss of fumes. In the instances where all the digestion solution evaporated out of the digestion tube, another 4.5 ml of the digestion mixture was added. After digestion, the solution was transferred into a 100 ml volumetric flask after filtering and diluted to volume. Four blank solutions where only the cigarette paper was digested were also made.

3.2.5.2 Nutrient concentration determination

Analyses of sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and magnesium (Mg) were done using a solar atomic absorption spectrophotometer and nitrogen (N) concentrations were determined using a nitrogen distillation unit.

3.2.6 Statistical Analysis

Analyses of variance (ANOVA) were run to determine whether there were statistically significant differences ($p \leq 0.05$) in the data between the different treatments and also between the root and shoot samples using the Statistical Package for the Social Sciences. The package was also used to determine the differences in the other essential plant elements. After differences were found, a Tukey Post Hoc test was done to determine between which treatments these differences occurred.

3.3 Results

3.3.1 The effect of heavy metals and mitigation treatments on the growth of bean plants.

Figure 3.1 shows the effect of the heavy metal treatments on the fresh mass of the bean plants. It is clear that there was considerable inhibition of growth at the highest cadmium

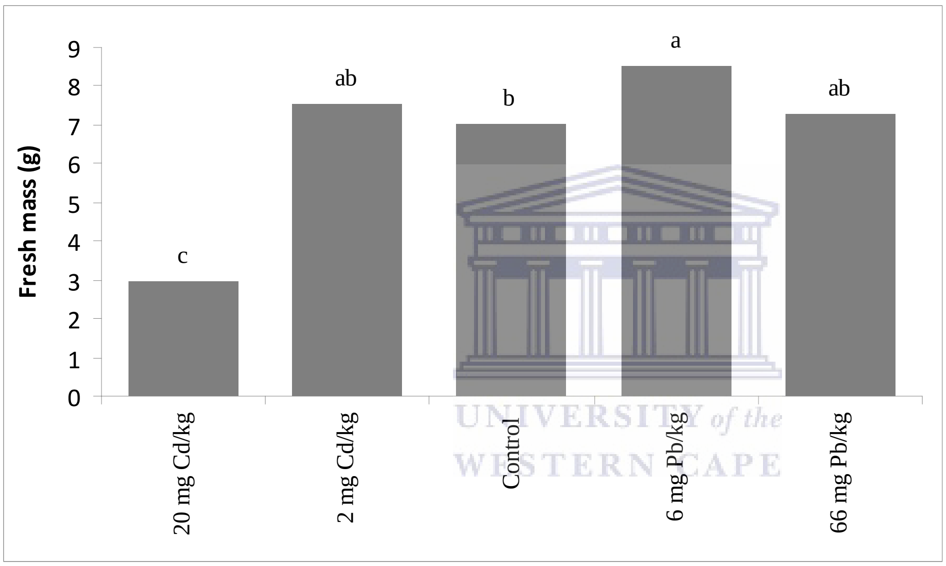

Figure 3.1 The effect of heavy metal treatment (mass per kg soil) on the fresh mass of the bean plants. Bars with the same letter do not differ significantly at p = 5%.

concentration, and the lowest lead treatment resulted in increased fresh mass when compared with the control treatment.

In Figure 3.2 it can be seen that the dry mass of the bean plants treated with the highest cadmium concentration was lower than that of the plants treated with the lowest cadmium concentration and lowest lead concentration.

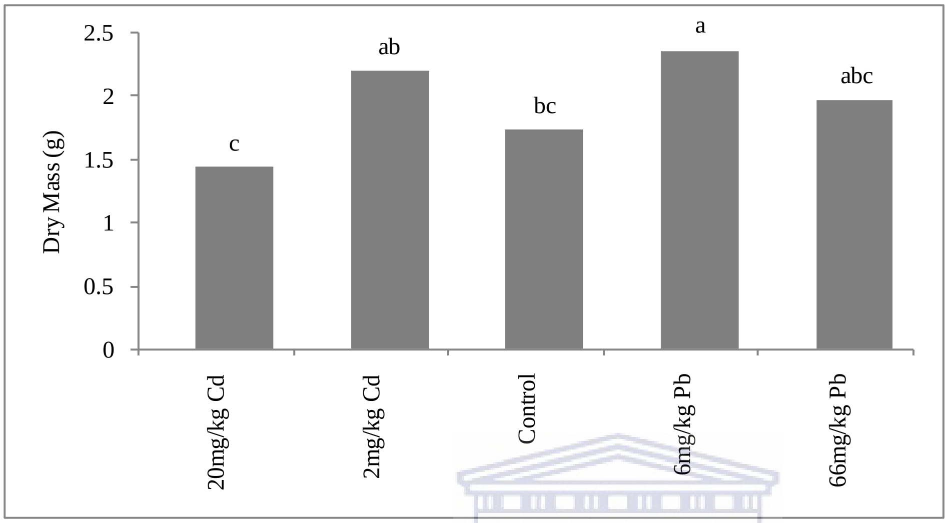

Figure 3.2 The effect of heavy metal treatment (mass per kg soil) on the dry mass of the bean plants. Bars with the same letter do not differ significantly at p = 5%.

No differences in either fresh mass or dry mass of the bean plants were found between the different mitigation treatments.

From Figure 3.3, the comparison of the fresh mass of both shoots and roots under the different heavy metal treatments is given. Again the lowest lead treatment shows

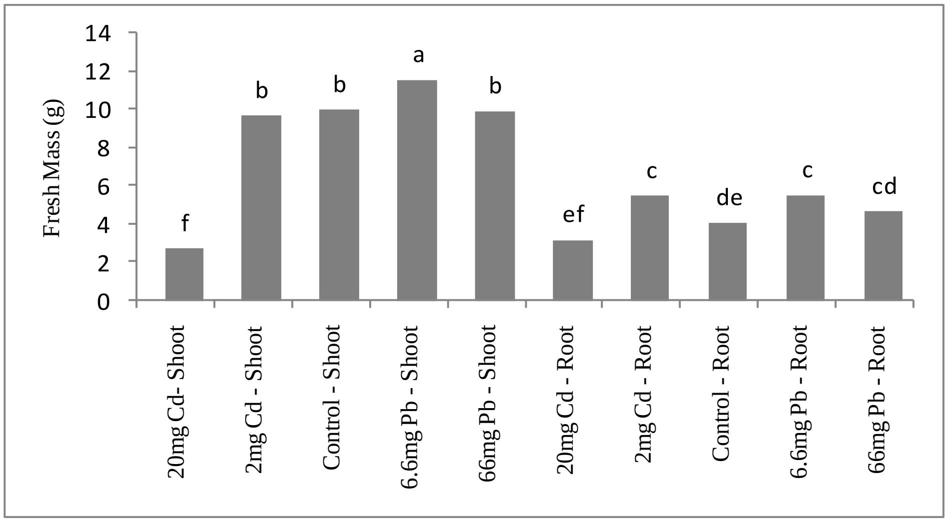

Figure 3.3 The effect of heavy metal treatments (mass per kg soil) on the fresh mass of the shoots and roots of bean plants. Bars with the same letter do not differ significantly at p = 5%.

an increase in shoot fresh mass as compared to that of the control and the highest cadmium concentration reduced shoot growth. Because the shoots are considerably heavier than the roots, this is similar to the results for the whole plant (Fig 3.1). The root yield was lower than the shoot yield, except in the case of the highest cadmium treatment. In this case the shoot fresh mass was lower than all the root fresh mass values except again for the highest cadmium treatment. The low cadmium and lead treatments resulted in a higher root fresh mass than the control.

The dry mass of shoots and roots shows a similar pattern (Fig. 3.4) but both the lower cadmium and the lower lead treatments show higher shoot dry mass than the control. There are no significant differences in dry mass amongst the roots, which in turn are lower than all but the highest shoot cadmium treatment's dry mass but then it is only from the highest root lead concentration that it does not differ.

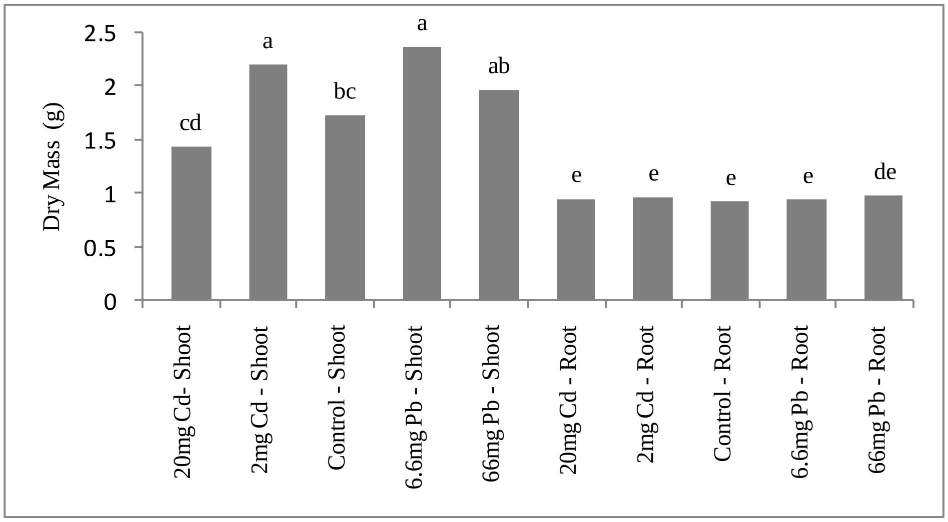

Figure 3.4 The effect of heavy metal treatments (mass per kg soil) on the dry mass of the shoots and roots of bean plants. Bars with the same letter do not differ significantly at p = 5%.

There was an effect of the mitigation treatment on the fresh mass (Fig. 3.5) though not on the dry mass of the shoots. The high phosphate treatment resulted in a higher fresh mass than the control and the high EDTA treatment.

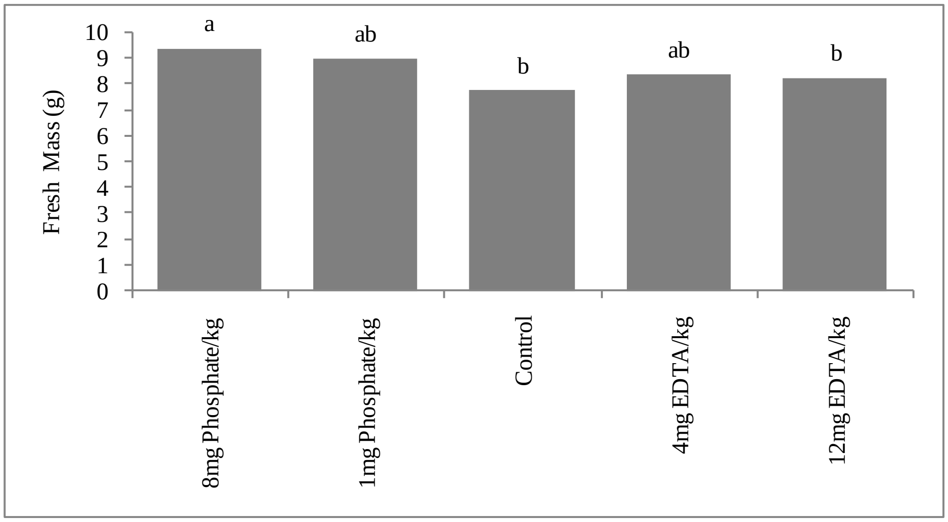

Figure 3.5 The effect of mitigation treatment (mass per kg soil) on the fresh mass of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

In summary, low lead treatments tended to increase fresh and dry mass over that of the control, while high cadmium reduced fresh mass. The mitigation treatments had little effect on the growth of the bean plants.

3.3.2 The effect of heavy metals and mitigation treatments on the chemical composition of bean plants.

The heavy metals also had an effect on the chlorophyll index (Fig 3.6), though the mitigation treatments did not. The highest Cd treatment reduced the chlorophyll index below that of the control. The chlorophyll index from the cadmium treatments was lower than that of the lead treatments.

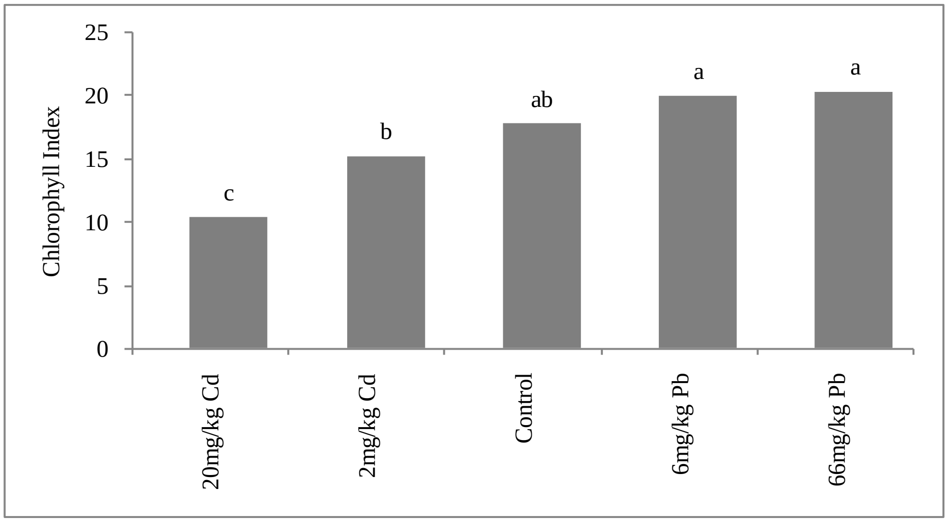

Figure 3.6 The effect of heavy metals on the chlorophyll index of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

When comparing the effect of the mitigation treatments on the root cadmium and lead concentrations, the same pattern was seen in the high Phosphate and high EDTA concentrations, as well as the low phosphate and low EDTA concentrations, where the concentrations were considerably more in the high than in the lower treatments (Figure 3.7 and Fig. 3.8).

Figure 3.7 shows that the low EDTA treatment reduced the cadmium concentration in the bean roots, all other treatments resulted in a higher cadmium concentration than in the control. The highest concentration was found with the highest phosphate concentration.

In case of the lead content (Fig 3.8), both EDTA treatments and the lower phosphate treatment reduced the root lead concentration below that of the control. The high phosphate treatment, as in the case of cadmium, caused a marked increase in the root lead content.

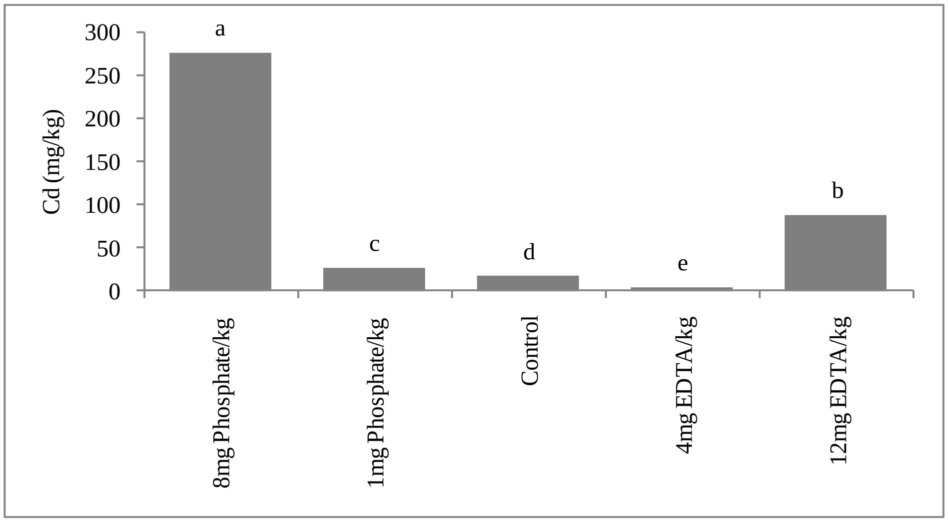

Figure 3.7 The effect of mitigation treatment (mass per kg soil) on the cadmium concentrations of the bean roots. Bars with the same letter do not differ significantly at p = 5%.

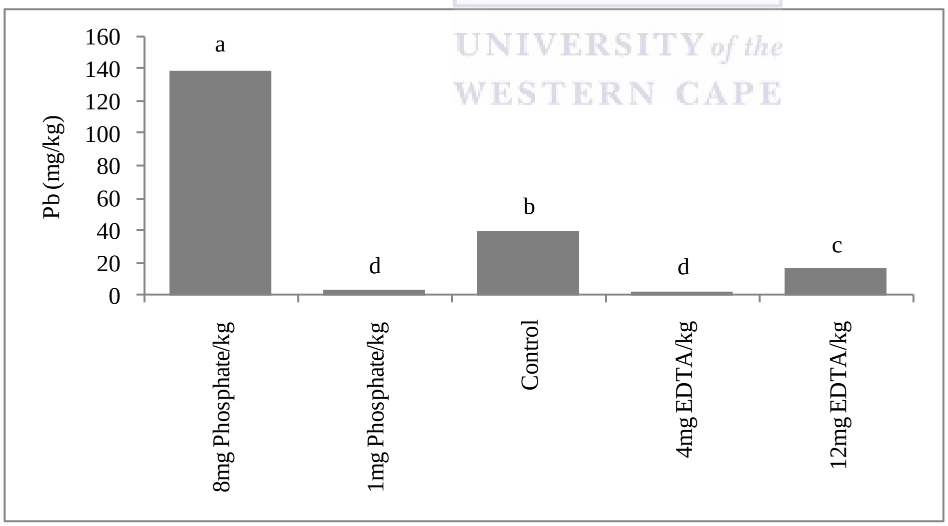

Figure 3.8 The effect of mitigation treatment (mass per kg soil) on the lead concentrations of the bean roots. Bars with the same letter do not differ significantly at p = 5%.

In contrast with the mitigation treatment effects in the roots, low level mitigation treatments enhanced shoot cadmium concentrations and high level treatments reduced them (Fig. 3.9).

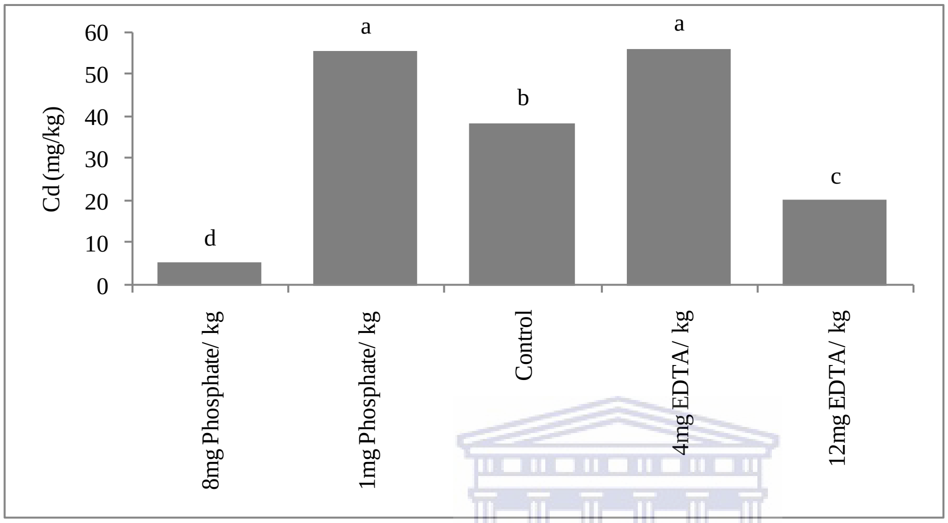

Figure 3.9 The effect of mitigation treatment (mass per kg soil) on the Cadmium concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

However both high level mitigation treatments resulted in increased shoot lead concentrations (Fig 3.10)

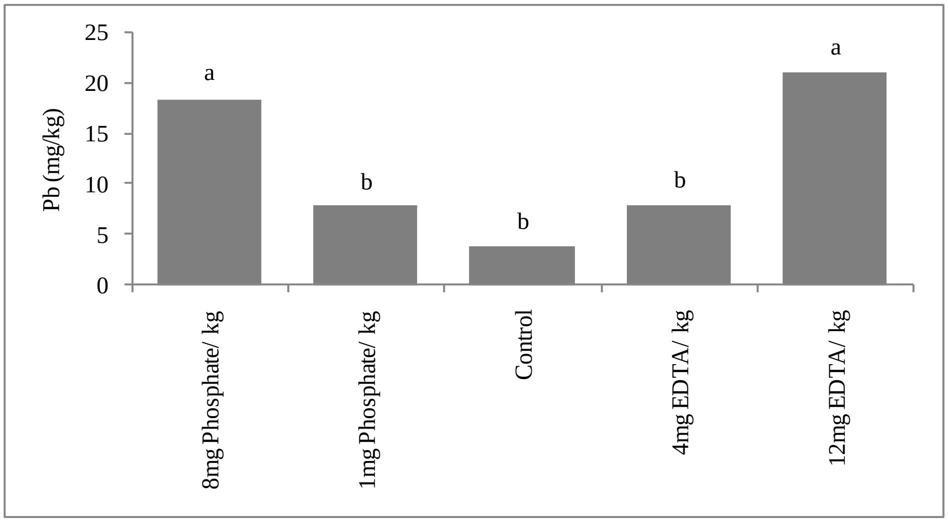

Figure 3.10 The effect of mitigation treatment (mass per kg soil) on the lead concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The highest cadmium treatment resulted in a higher nitrogen concentration in the bean shoots (Fig 3.11). The higher lead treatment affected the nitrogen content slightly (Fig 3.11).

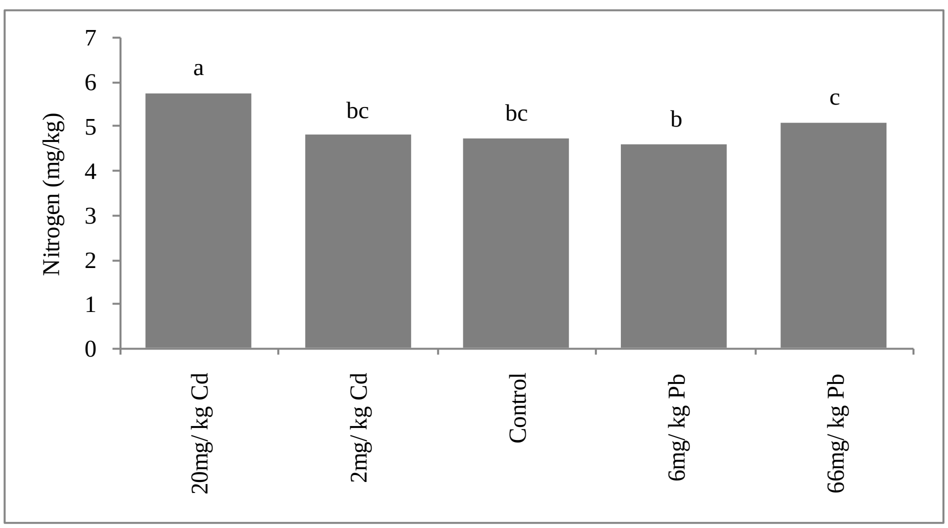

Figure 3.11 The effect of heavy metal treatment (mass per kg soil) on the nitrogen concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The highest lead treatment resulted in a lower shoot phosphorus concentration than was found with the other heavy metal treatments (Fig 3.12).

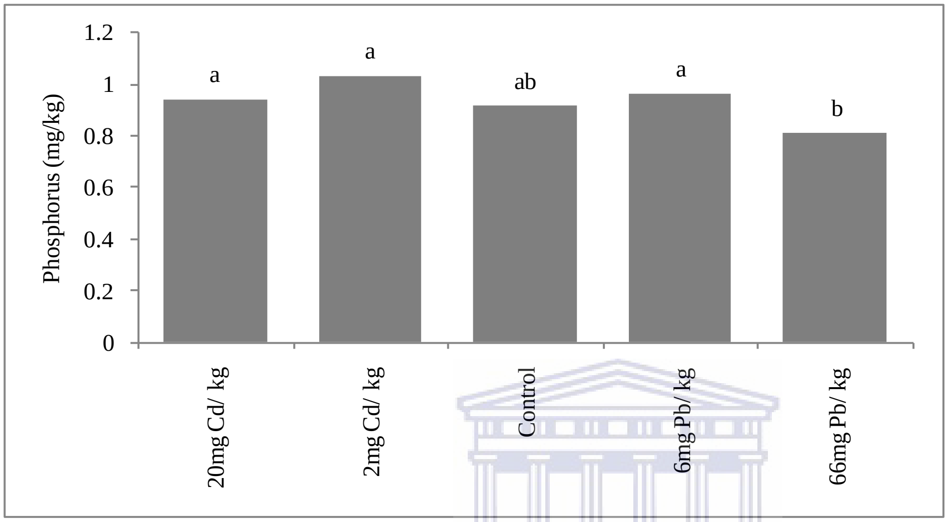

Figure 3.12 The effect of heavy metal treatment (mass per kg soil) on the phosphorus concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The highest phosphate treatment gave higher shoot phosphate content than the highest EDTA treatment (Fig 3.13).

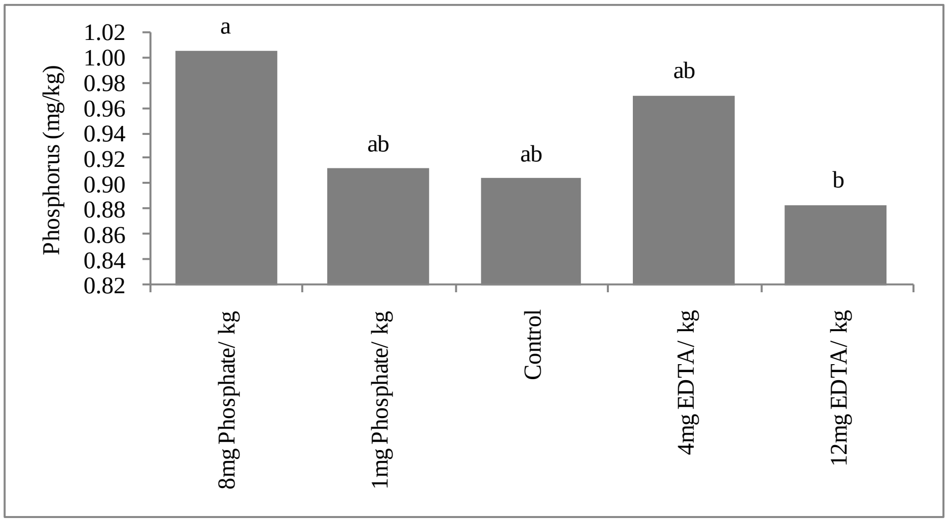

Figure 3.13 The effect of mitigation treatment (mass per kg soil) on the phosphorus concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The highest phosphate treatment also resulted in enhanced potassium content (Fig 3.14)

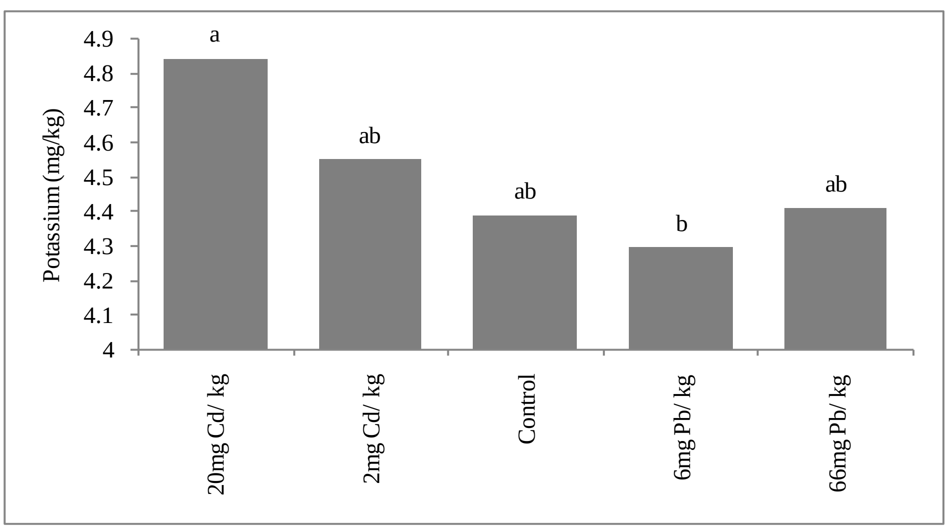

Figure 3.14 The effect of heavy metal treatment (mass per kg soil) on the potassium concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The calcium content (Fig 3.15) was higher with the highest cadmium treatment, and lower with the highest lead treatment.

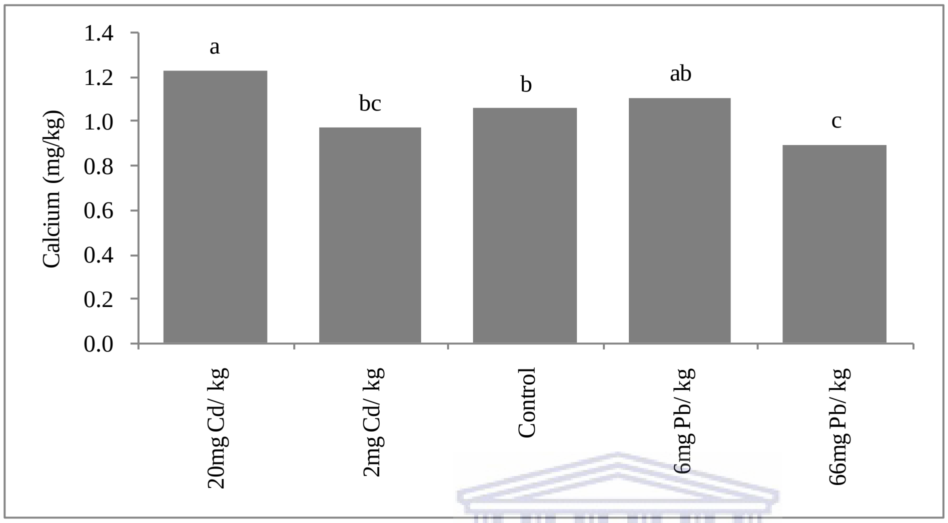

Figure 3.15 The effect of heavy metal treatment (mass per kg soil) on the calcium concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The highest cadmium treatment resulted in higher shoot magnesium content than in the case of the highest lead treatment (Fig 3.16).

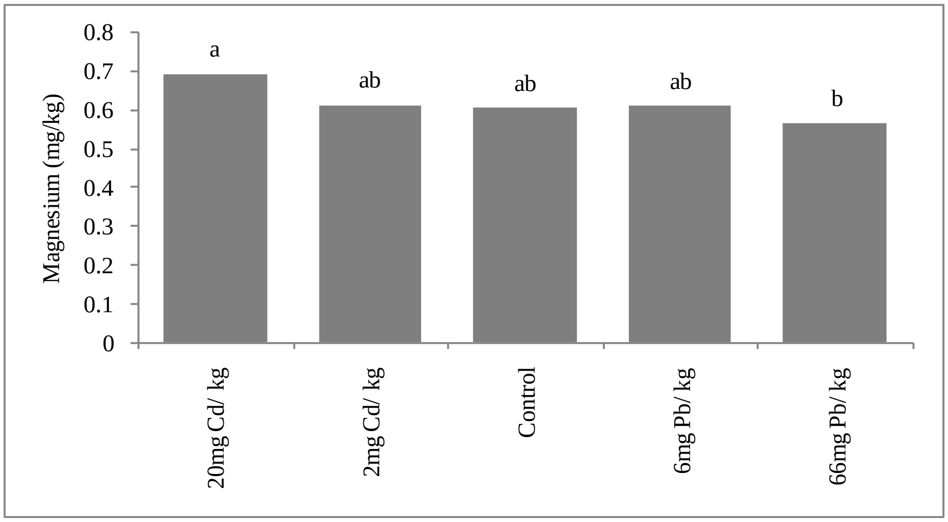

Figure 3.16 The effect of heavy metal treatment (mass per kg soil) on the magnesium concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The shoot sodium content was much higher with high cadmium treatment than in all other treatments (Fig. 3.17).

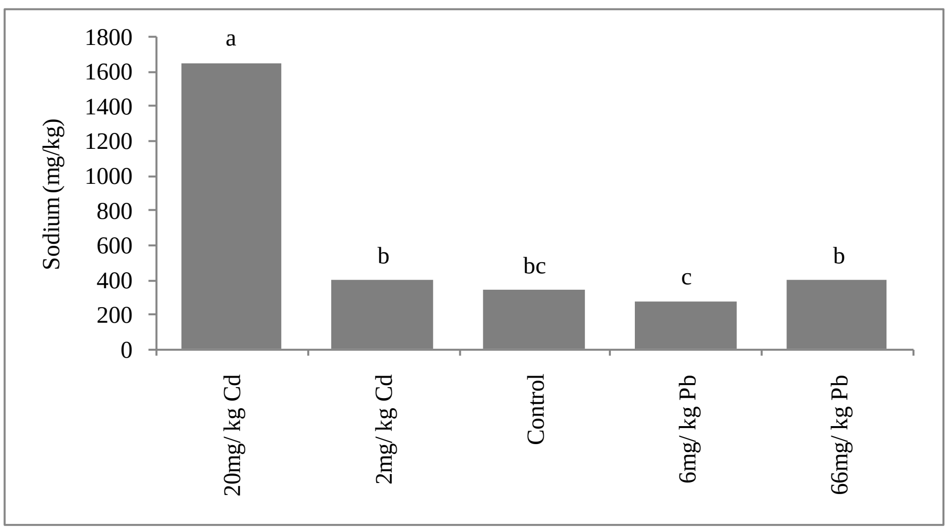

Figure 3.17 The effect of heavy metal treatment (mass per kg soil) on the sodium concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

Low phosphate and both of the EDTA treatments resulted in a higher shoot sodium content (Fig. 3.18).

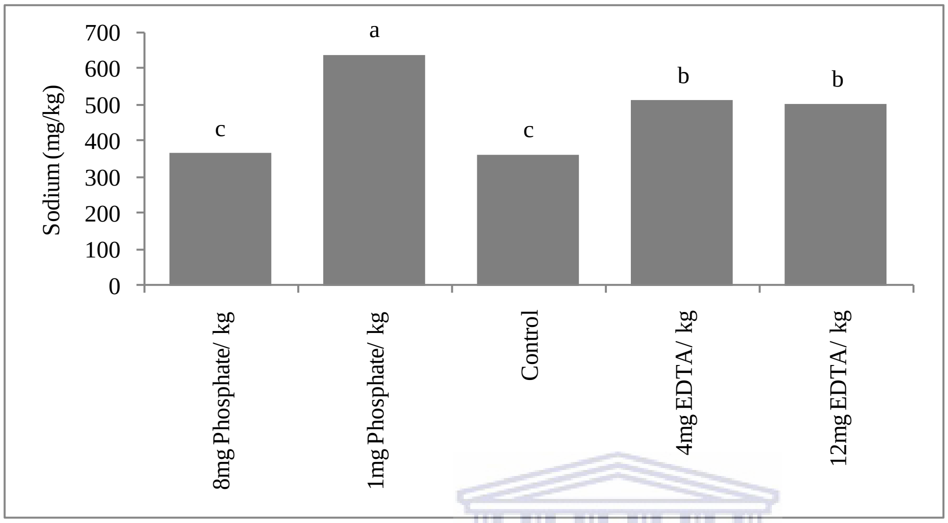

Figure 3.18 The effect of mitigation treatment (mass per kg soil) on the sodium concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The low phosphate treatment resulted in an increased shoot Zinc concentration (Fig. 3.19).

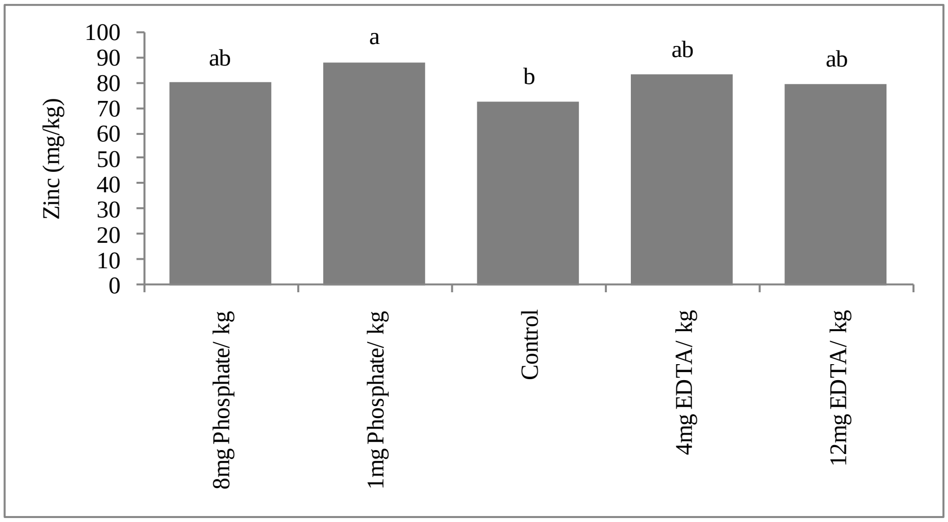

Figure 3.19 The effect of mitigation treatment (mass per kg soil) on the zinc concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The lower lead treatment enhanced the boron content of the shoots over that found with both high level heavy metal treatments (Fig. 3.20).

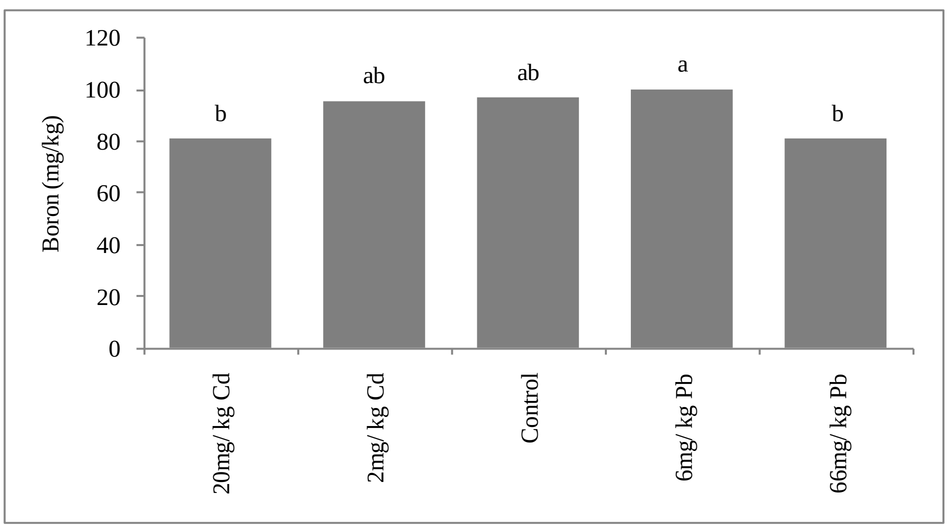

Figure 3.20 The effect of heavy metal treatment (mass per kg soil) on the boron concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The high lead treatment lowered the shoot Manganese level (Fig. 3.21).

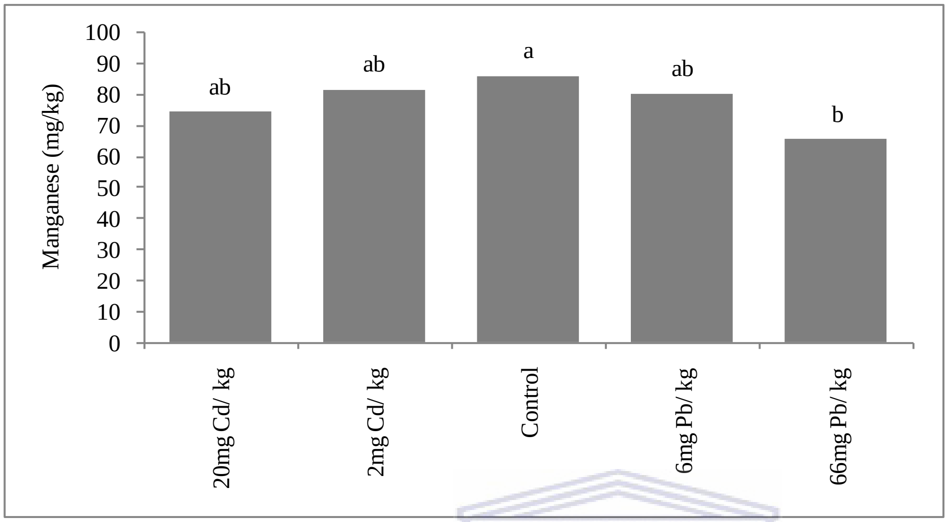

Figure 3.21 The effect of heavy metal treatment (mass per kg soil) on the manganese concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The high Phosphate mitigation treatment resulted in a higher shoot Manganese concentration (Fig. 3.22).

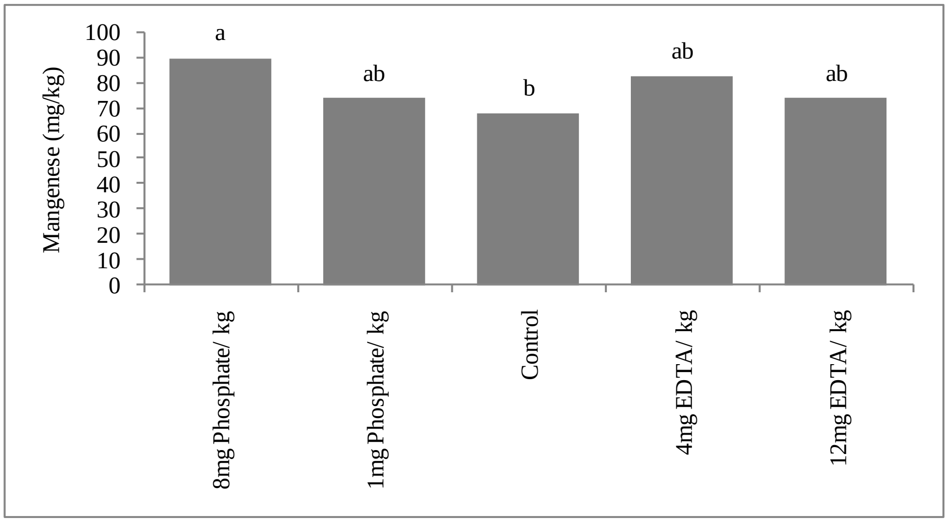

Figure 3.22 The effect of mitigation treatment (mass per kg soil) on the manganese concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The high cadmium treatment increased the shoot iron content above all other treatments (Fig. 3.23).

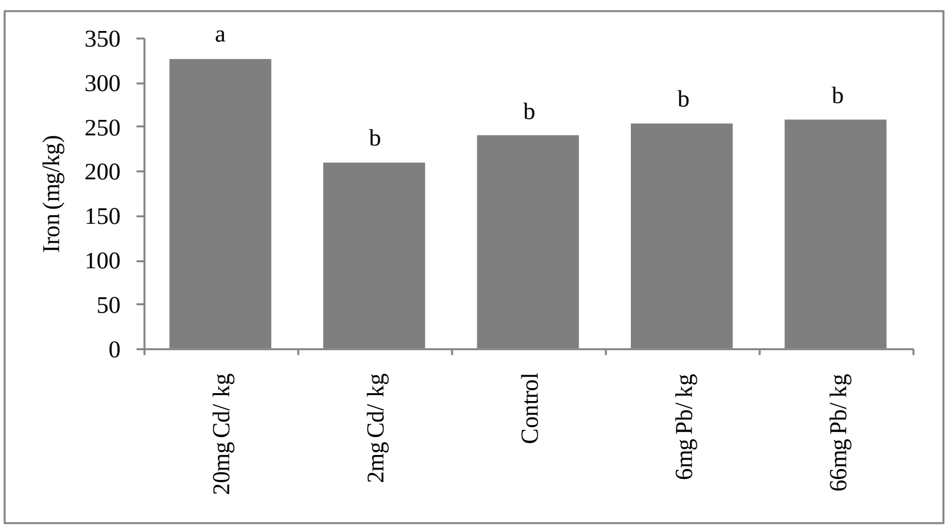

Figure 3.23 The effect of heavy metal treatment (mass per kg soil) on the iron concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

Low Phosphate and both EDTA mitigation treatments increased the shoot Iron content (Fig. 3.24)

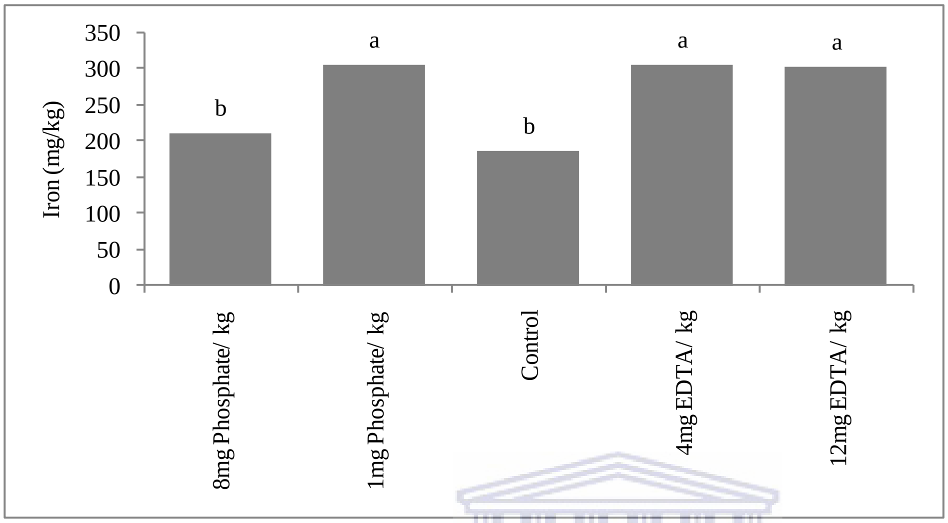

Figure 3.24 The effect of mitigation treatment (mass per kg soil) on the iron concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The high Cadmium treatment resulted in a higher shoot copper concentration than in the case of the two lead treatments (Fig. 3.25).

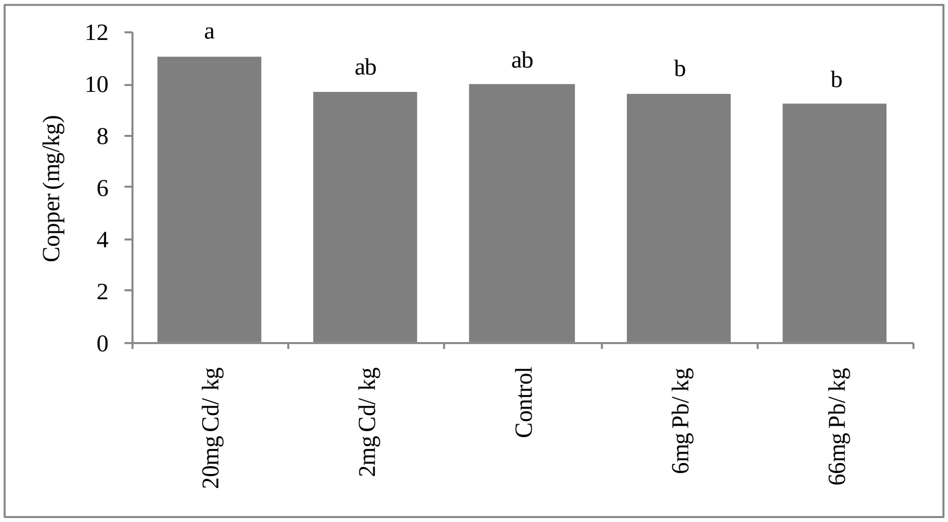

Figure 3.25 The effect of heavy metal treatment (mass per kg soil) on the copper concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

The low EDTA treatment increased the shoot Copper concentration (Fig. 3.26).

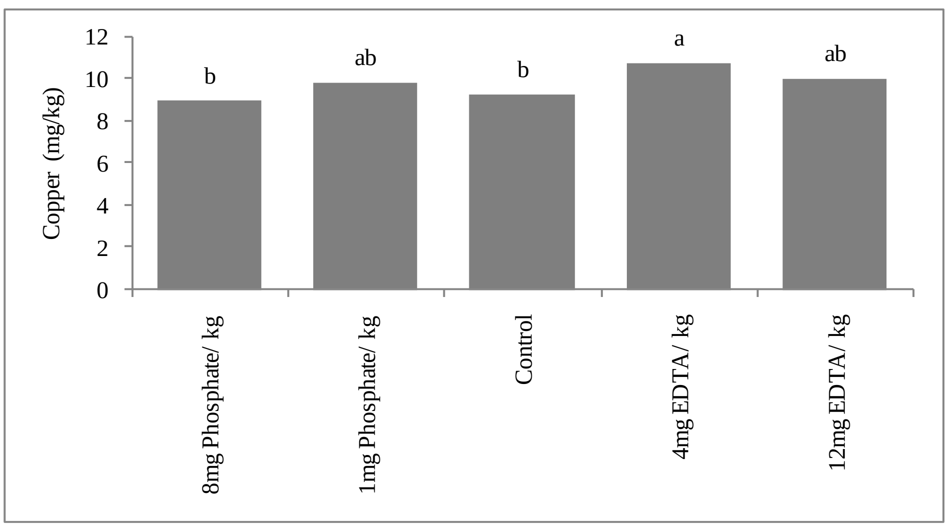

Figure 3.26 The effect of mitigation treatment (mass per kg soil) on the copper concentrations of the bean shoots. Bars with the same letter do not differ significantly at p = 5%.

When comparing roots and shoots, in general the heavy metals are found to be higher in the roots. Figure 3.27 shows cadmium levels to be higher with cadmium treatments, and with cadmium and control, the concentration was higher in the roots than in the shoots. Figure 3.28 shows the same pattern with the lead treatments. As seen in the case of cadmium, root lead concentrations were found to be higher than shoot lead levels (Fig 2.28). With 20 mg Cd per kg soil, the root cadmium concentration was 1592.21mg.kg $^{-1}$ and the shoot cadmium concentration was 428.99mg.kg $^{-1}$, compared with the control at 1.58 mg.kg $^{-1}$. The same trend was seen when comparing the lead concentrations. The root lead concentration was 1233.33 mg.kg $^{-1}$ (Fig 3.28), the contents for the highest treatments and the control were 54.46 mg.kg $^{-1}$ and 2.34 mg.kg $^{-1}$ respectively.

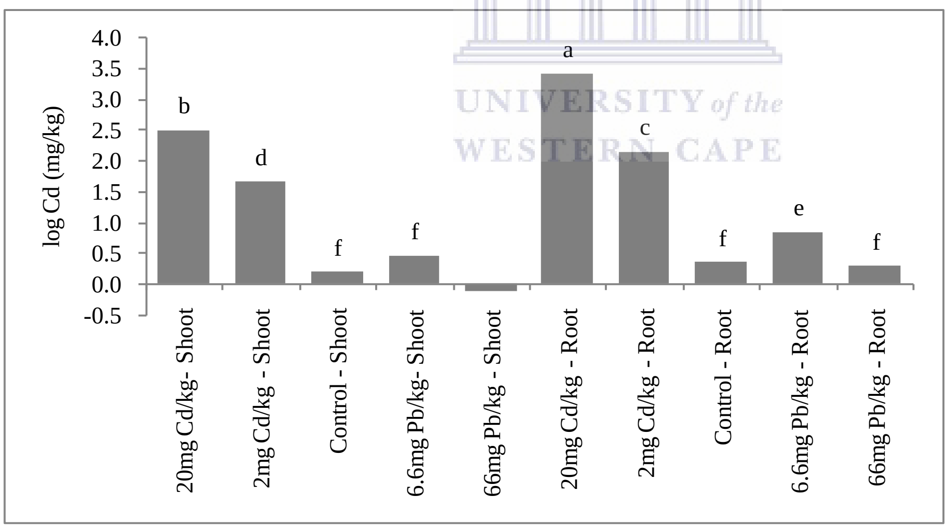

Figure 3.27 The effect of heavy metal treatment (mass per kg soil) on the cadmium concentrations on the bean shoots and roots. Bars with the same letter do not differ significantly at p = 5%.

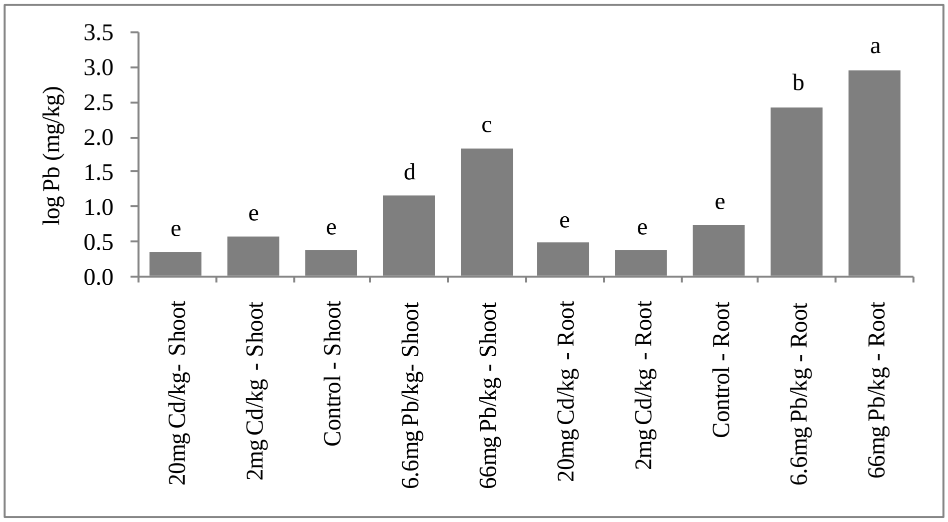

Figure 3.28 The effect of heavy metal treatment (mass per kg soil) on the lead concentrations on the bean shoots and roots. Bars with the same letter do not differ significantly at p = 5%.

When observing the effects of the mitigation treatments in the roots and shoots, the root Cadmium levels are higher than the shoot Cadmium levels with every treatment except the high EDTA (Fig. 3.29). In general, mitigation treatments enhanced Cadmium concentrations over that of the control. The Phosphate treatments resulted in higher Cd levels in the roots than did the EDTA treatments.

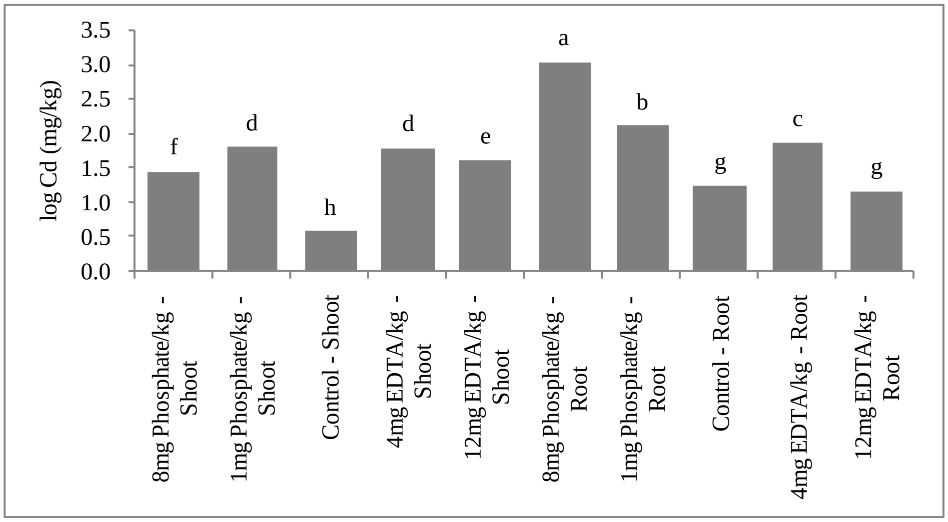

Figure 3.29 The effect of mitigation treatment (mass per kg soil) on the cadmium concentrations on the bean shoots and roots. Bars with the same letter do not differ significantly at p = 5%.

Lead uptake was affected very differently by the mitigation treatments to that of cadmium. Root concentrations tended higher than shoot concentrations again in the control and low level mitigation treatments, but root control lead levels were relatively high (Fig. 3.30). This mitigation tended to reduce lead levels and enhance cadmium concentrations.

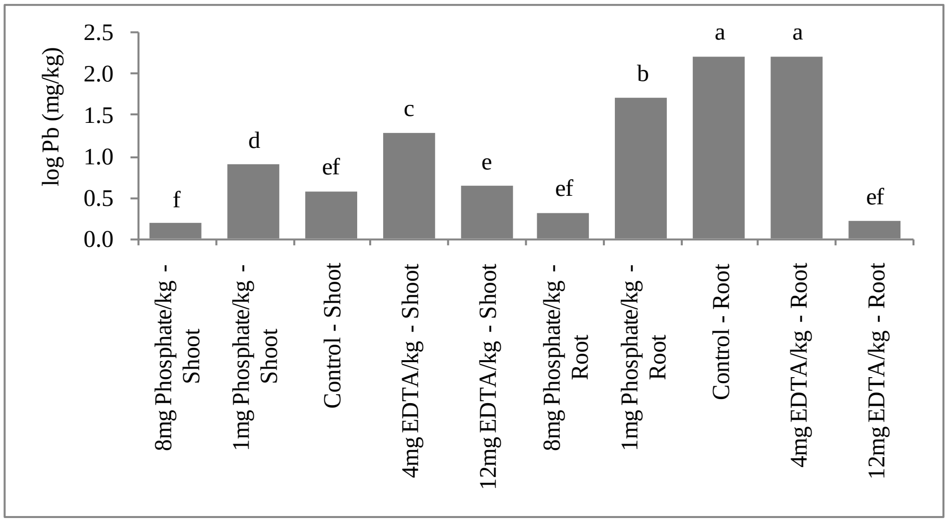

Figure 3.30 The effect of mitigation treatment (mass per kg soil) on the lead concentrations on the bean shoots and roots. Bars with the same letter do not differ significantly at p = 5%.

3.4 Discussion

3.4.1 Heavy metals, growth and chlorophyll

Heavy metal toxicity affects photosynthesis, as it inhibits both light and dark reactions (Prasad 1999). The inhibition of chlorophyll synthesis and increase in chlorophyll degradation leads to chlorosis of leaves observed after heavy metal treatment (Prasad 1999). Baszynski (1986) observed reduced dry matter production up to 50% in crop yield. Similar results were seen in this experiment, as high cadmium concentrations reduced the chlorophyll index (Fig. 3.6). Cadmium also reduced the fresh mass of the bean plants (Fig 3.1 and Fig 3.3).

The lead treatments did not reduce the growth or chlorophyll index (Fig 3.1, Fig 3.3 and Fig. 3.6). Li Li *et al*. (2008) noted that high lead content does not necessarily affect growth.

3.4.2 Mitigation, growth and chlorophyll

An experiment done by Wang *et al.* (2008) saw that the addition of a phosphate fertilizer caused an increase in biomass. This experiment showed a similar result (Fig. 3.3). This is to be expected as phosphate is a nutrient macroelement that is often limiting (Hopkins and Hüner 2004).

Azhar *et al.* (2006) reported that EDTA reduced shoot, root and dry matter. This was not seen in this study, as dry and fresh mass of roots and shoots showed no negative effect (Fig. 3.5). Neither the phosphate, nor the EDTA had an effect on the chlorophyll index.

3.4.3 Heavy metals and elemental content

Cadmium can alter the uptake of minerals by plants through its effects on the availability of minerals from the soil or through a reduction in the population of soil microbes (Benavides *et al*. 2005). Most of the elements increased with the higher cadmium treatment. With the higher level cadmium treatment, increased uptake of nitrogen and calcium was seen (Fig. 3.11 and Fig.3.15). Nitrogen's mobility within a plant is very high. As it is mobilized, it gets exported from the older leaves to the younger developing leaves (Hopkins and Hüner 2004). Nitrogen concentrations were found to be higher in shoots and were elevated in the higher cadmium treatment. Phosphorus is easily distributed and mobilized within a plant (Hopkins and Hüner 2004). Phosphorus uptake was higher with both cadmium treatments (Fig 3.19). Iron is considered a micronutrient essential for plant growth and survival, as it is required for the functioning of an array of enzymes, especially those which take part in oxidation and reduction processes as well as electron transport in photosynthesis (Prasad 1999). However, iron may be referred to as a heavy metal when the concentration limit is exceeded. Results showed that cadmium increased iron uptake in the plant as the iron content was higher when treated with the higher level of cadmium (Fig 3.23) it was particularly notable that high cadmium levels resulted in dangerously high sodium concentrations reaching the shoots (Fig 3.17).

3.4.4 Mitigation and elemental content

Chen *et al.* (2007) and Wang *et al*. (2008) reported that the addition of Triple superphosphate reduced cadmium uptake in shoots. This result was also seen in the experiment (Fig. 3.7). Zinc concentrations increased with the addition of the low phosphate treatment (Fig. 3.19). Wang *et* *al*. (2008) also reported that phosphate fertilzers decreased lead concentrations. This was partially seen in the study as the lower level of phosphate showed a decrease in lead in the roots, however with the higher level of phosphate treatment, high concentrations of lead was seen (Fig 3.8 and Fig 3.10).

Blaylock *et al.* (1997); Huang *et al.* (1997) and Li Li *et al.* (2008) all reported that EDTA increased the amount of lead taken up by plants. The high level EDTA treatment showed a similar result in the shoots (Fig. 3.10). EDTA is able to complex metals and enhances the uptake of metals by plants (Chen *et al.* 2004). This indeed was seen in the zinc, iron and copper concentrations (Fig. 3.19, Fig. 3.24 and Fig. 3.26) where an increase of concentrations was seen with the addition of EDTA.

3.4.5 Relative concentrations in roots and shoots

When comparing the overall effect of heavy metals on roots and shoots, the administration of cadmium increased the amount of cadmium in the roots and shoots, and the same result was found when lead was given (Fig. 3.27 and Fig. 3.28). When mitigation treatments were given however, phosphate enhanced cadmium levels in roots and shoots (Fig. 3.29). With lead, a different picture was seen as root concentrations were higher than that of the shoots. This correlated with the studies done by Begonia *et al.* (1998); Chen *et al.* (2004) and Li Li *et al.* (2008) that all reported that lead is accumulated most in the roots, and translocation to the shoots is minimal.

3.5 Conclusion

The results showed that cadmium and lead had no effect on root dry mass however, the concentrations of cadmium and lead were significantly higher in the roots than in the shoots and were more pronounced when looking at the lead concentrations.

When observing the mitigation treatments, it was seen that they had no effect on root fresh mass or on root dry mass, and neither on the root lead content. There was also no effect on mitigation treatments on fresh mass or dry mass weights.

When comparing the results found between roots and shoots, there was no effect of heavy metals on the dry mass weight and mitigation treatments had no effect on the dry mass or fresh mass weight in the roots. With respect to the shoots, mitigation treatments had no effect on the dry mass weight, neither on the chlorophyll index.

3.6 References

Anderson WC (1993) Soil washing/ soil flushing. *Amer. Acad. Environmental Engineers*. Annaplois, MD.

Azhar N, Yasin Ashraf M, Hussain M and Hussain F (2006) Phytoextraction of Lead (Pb) by EDTA application through sunflower (*Helianthus annuus* L.) cultivation: seedling growth studies. *Pakistan Journal of Botany* **38**(5) 1551-1560.

Baker AJM (1981) Accumulation and excluders-strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**: 643-654.

Baszynski T (1986) Interference of Cd in functioning of the photosynthetic apparatus of higher plants. *Acta Soc Bot Pol* **55**: 291-304.

Begonia GB, Davis MFT and Gray BCN (1998) Growth responses of Indian mustard and its phytoextraction of lead from a contaminated soil. *Bulletin of Environment Contamination Toxicology* **61**: 38-43.

Benavides MP, Gallego SM and Tomaro ML (2005) Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology* **17** (1): 21-34.

Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.* **31**:860-865.

Cao XD, Ma QY, Rhue DR and Appel CS (2004) Mechanisms of lead, copper and zinc retention by phosphate rock. *Environmental Pollution* **131**: 435-444.

Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS and Baker AJM (1997) Phytoremediation of soil metals. *Environmental Biotechnology* **8**: 279-284.

Chen Y, Li X and Shen Z (2004) Leaching and uptake of heavy metals by ten different species of plants during an EDTA assisted phytoextraction process. *Chemosphere* **57**: 187-196.

Chen SB, Xu MG, Ma YB, Yang JC (2007) Evaluation of different phosphate amendments on availability of metals in contaminated soil. *Ecotox Environ Safe* **67**: 278–285.

Cooper EM, Sims JT, Cunningham SD, Huang JW and Berti WR (1999) Chelate-assisted phytoextraction of lead from contaminated soils. *Journal of Environmental Quality* **28**: 1709-1719.

Dixit V, Pandey V and Shyam R (2000) Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *Journal of Experimental Botany* **52** (358): 1101-1109.

Duxbury T (1985) Ecological aspects of heavy metal responses in micro-organisms. *Advance in Microbiological Ecology* **8**: 185-235.

Gabriel T and Patrick JT (1996) Uptake and localization of lead in corn (*Zea mays* L.) seedlings, a study by histochemical and electron microscopy. *Sci. Total Environ.* **188**: 71-85.

Hettiarachchi GM and Pierzynski GM (2002) In situ stabilization of soil lead using phosphorus and manganese oxide: influence of plant growth. *J. Environ. Qual.* **31**: 564-572.

Hopkins WG and Hüner NPA (2004) Introduction to Plant Physiology 3rd Edition. John Wiley and Sons Ltd, USA.

Huang JW and Cunningham SD (1996) Lead phytoextraction: species variation in lead uptake and translocation. *New Phytologist* **134**: 75-84.

Huang JW, Chen J, Berti WB and Cunningham SD (1997) Phytoremediation of lead-contaminated soils, role of synthetic chelates in lead phytoextraction. *Environ. Sci. Technol.* **31**: 800-805.

Lai H-Y and Chen Z-S (2004) Effects of EDTA on solubility of cadmium, zinc and lead and their uptake by rainbow pink and vetiver grass. *Chemosphere* **55**: 421-430.

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. *Journal of Environmental Quality* **31**: 109-120.

Li Li Y, Liu YG, Liu JL, Zeng GM, Li X (2008) Effects of EDTA on Lead uptake by *Typha orientalis* Presl. : A new lead-accumulating species in southern China. *Bulletin of Environmental Contamination and Toxicity* **81**: 36-41.

Malone C, Koeppe DE and Miller RJ (1974) Localization of lead accumulated by corn plants. *Plant Physiol.* **53**:388-394.

McGrath SP (1987) Long-term studies of metal transfers following applications of sewage sludge. *In*: Sun B, Zhao FJ, Lombi E, McGrath SP (2001) Leaching of heavy metals from contaminated soils using EDTA. *Environmental Pollution* **113**: 111-120.

McGrath SP (1998) Phytoextraction for soil remediation. *In*: Sun B, Zhao FJ, Lombi E, McGrath SP (2001) Leaching of heavy metals from contaminated soils using EDTA. *Environmental Pollution* **113**: 111-120.

Meerkotter M (2012) Sources of heavy metals in vegetables in Cape Town and possible methods of remediation. Unpublished PhD thesis. University of the Western Cape, Cape Town.

Meerkotter M (2003) Heavy metals and vegetable farming in Cape Town. Unpublished MSc thesis. University of the Western Cape, Cape Town.

Michael WHE, Mathias E and Andreas S (2007) Chelate assisted in phytoextraction of heavy metals from soil. Effect, mechanism, toxicity and fate of chelating agents. *Chemosphere* **68**: 989-1003.

Moore PD, Chapmen SB (eds) (1986) Methods in Plant Ecology- 2nd edition. Blackwell Scientific Publications, California, pp 302- 303. ISBN 0-632-00989-6.

Muchuweti M, Birkett JW, Chinyanga E, Zvauya R, Scrimshaw MD and Lester JN (2006) Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agriculture, Ecosystems and Environment* **112**: 41-48.

Perfus-Barbeoch L, Leonhardt N, Vavasceur A and Forestier C (2002) Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. *The Plant Journal* **32**: 539-548.

Piechalak A and Tomaszewska B (2002) Accumulation and detoxification of lead ions in legumes. *Phytochemistry* **60**: 153-167.

Prasad MNV (1999) Phytoremediation of metals and radionuclides in the environment: The case for natural hyperaccumulators, metal transporters, soil-amending chelators and transgenic plants; chapter 14 *in* Heavy Metal Stress in plants, Prasad MNV (ed.), Springer-Verlag, Berlin

Pulford ID and Watson C (2003) Phytoremediation of heavy metal contaminated land by trees- a review. *Environ. Int.* **29**: 529-540.

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: Using plants to remove pollutants from the environment. *Current opinion in Biotechnology* **8**: 221-226.

Rulkins WH, Grotenhuis JTC and Tichy R (1995) Methods for cleaning contaminated soils and sediments. *In*: Azhar N, Yasin Ashraf M, Hussain M and Hussain F (2006) Phytoextraction of Lead (Pb) by EDTA application through sunflower (*Helianthus annuus* L.) cultivation: seedling growth studies. *Pakistan Journal of Botany* **38**(5) 1551-1560.

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **49**: 643-668.

Sandalio LM, Dalurzo HC, Gomez M, Romero-Puertas MC and delRio LA (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany* **52** (364): 2215-2126.

Schmidt U (2003) Enhancing Phytoextraction: The effect of chemical soil manipulation on mobility, plant accumulation and plant leaching of heavy metals. *Journal of Experimental Quality* **32**: 1939-1954.

Seaward MRD and Richardson DHS (1990) Atmospheric sources of metal pollution and effects on vegetation.

Sharma RK, Agrawal M and Marshall FM (2007) Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. *Environmental Pollution* (2007): 1-10.

Shen Z-G, Li X-D, Wang C-C, Chen H-M and Chua H (2002) Lead phytoextraction from contaminated soil with high biomass plant species. *Journal of Environmental Quality* **31**: 1893-1900.

Shivendra VS and Natalie LS (2002) Characterization of a lead hyperaccumulator shrub, *Sesbania drummondii*. *Environmental science technology* **36**: 4676-4680.

Spiro B (2003) Assessment of the distribution of heavy metals in plants, waters and soils around a Cu smelter and mining town, Karabash, Urals, Russia. *Geophysical Research Abstracts* **5** (06775).

Sun B, Zhao FJ, Lombi E, McGrath SP (2001) Leaching of heavy metals from contaminated soils using EDTA. *Environmental Pollution* **113**: 111-120.

Wang B, Xie Z, Chen J, Jiang J and Su Q (2008) Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica* *chinensis* L.) in a mining tailing contaminated soil. *Journal of Environmental Sciences* **20**: 1109-1117.

Wu J, Hsu FU and Cunningham SD (1999) Chelate-assisted Pb phytoremediation: Pb availability, uptake and translocation constraints. *Environ. Sci. Technol.* **33**: 1898-1904.

Yang Y, Zhang F-S, Li H-F and Jiang R-F (2000) Accumulation of cadmium in the edible parts of six vegetable species grown in Cd contaminated soils. *Journal of Environmental Management* **90**: 1117-1122.

CHAPTER 4

Summary and Recommendations

4.1 Field study

The results from this study showed that although elemental concentrations fell within the normal ranges (listed by Larcher, 2001), the average values of the heavy metals cadmium, lead and zinc were higher than the legal standard in the edible bean fruits (Table 2.1). This is alarming as it is those very fruits that end up on the food market, and are ultimately consumed.

Heavy metal contamination in legume crops has consequences as consumer safety is compromised and horticultural yield may be negatively affected. The findings of this thesis could possibly provide valuable information to the small-scale farmers for the safe and beneficial cultivation of legume crops and increase awareness regarding heavy metal contamination in these plants, as it is these crops will ultimately end up on the food market.

4.2 Pot experiment

Heavy metals increased the shoot dry mass but had no effect on the root dry mass. The results also showed that administering heavy metal resulted in changes in mineral uptake. Some mitigation effect was seen, as concentrations of heavy metals were lower in plants that were treated with EDTA and phosphate, this however was seen in the fresh mass of the shoots, but not on the dry mass of the shoots. In terms of the roots, EDTA seemed to have a better effect on mitigating cadmium than on lead.

4.2.1 Heavy metals, growth and chlorophyll

High cadmium concentrations reduced the chlorophyll index (Fig. 3.6). Cadmium also reduced the fresh mass of the bean plants (Fig 3.1 and Fig 3.3). The lead treatments did not reduce the growth or chlorophyll index (Fig 3.1, Fig 3.3 and Fig. 3.6).

4.2.2 Mitigation, growth and chlorophyll

The high phosphate treatment increased fresh mass of shoots (Fig. 3.5). This is to be expected as phosphate is a nutrient macroelement that is often limiting (Hopkins and Hüner 2004). EDTA showed no negative effect on dry or fresh mass of roots and shoots. Neither the phosphate, nor the EDTA had an effect on the chlorophyll index.

4.2.3 Heavy metals and elemental content

A number of the elements increased in concentration with the higher cadmium treatment. With the higher level cadmium treatment, increased uptake of nitrogen, calcium, sodium and iron was seen (Fig 3.11, Fig 3.15, Fig 3.17 and Fig 3.23). On the other hand, high lead treatment reduced the shoot calcium and manganese concentration, but did not affect the other elements.

4.2.4 Mitigation and elemental content

The high lead treatments with both phosphate and EDTA increased root cadmium concentrations (Fig 3.7). However where high phosphate treatment increased root lead concentrations, they were reduced by the higher EDTA treatment (Fig 3.8). Both higher level mitigation treatments reduced the cadmium concentrations in the shoots (Fig 3.9) but increased the shoot lead concentrations (Fig 3.10). Both sodium and iron concentrations increased in the shoots with low phosphate and both EDTA treatments (Fig 3.18 and Fig 3.24).

4.2.5 Relative concentrations in roots and shoots

Both cadmium and lead showed higher concentrations in the roots than in the shoots, but more cadmium than lead reached the shoots (Figure 3.27 and Figure 3.28). With mitigation treatments, the root concentrations remained higher than the shoot concentrations (Figure 3.29 and Figure 3.30)

4.3 Recommendations

Optimized agricultural practices are essential for quality control of cultivated legume crops. Findings confirmed the necessity to monitor heavy metal contamination via liaison with small sector farmers and promote the production of safe and good quality food.

Monitoring programmes for contaminants and toxins should be implemented to improve food safety, warn of actual and potential food scares, and facilitate assessment of potential health hazards. Further greenhouse and field experiments should be carried out to provide further insight into heavy metal uptake and means of mitigation in plants.