The Effects of Cadmium and Lead on *Phaseolus vulgaris*

Clarissa Brandt

A thesis submitted in partial fulfillment of the requirements for the degree of Magister Scientiae, in the Department of Biodiversity and Conservation Biology, in the Faculty of Natural Science, University of the Western Cape.

Date: November 2012

Supervisor: Professor Lincoln M. Raitt
The Effects of Cadmium and Lead on *Phaseolus vulgaris*

**KEYWORDS**

Heavy metals

*Phaseolus vulgaris*

Cadmium

Lead

Phytoremediation

EDTA

Phosphate
ABSTRACT

The Effects of Cadmium and Lead on *Phaseolus vulgaris*

Clarissa Brandt

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

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

2 Corinthians 9:6

First and foremost I would like give thanks and praise to my Heavenly Father for His boundless Love and Grace that has carried me throughout this project.

I would like to express my immense gratitude to Professor Lincoln M. Raitt for his admirable supervision, guidance, assistance, and encouragement throughout the project. I truly appreciate the extensive help that he has provided. Thank you Prof.

Lilburne Cyster, for his technical support with laboratory work, experiments, and the occasional words of encouragement.

Frikkie Calitz from Agricultural Research Council and staff of Bemlab for the statistical and laboratory assistance.

Finally, I would like to thank my parents, friends and family for their continuing support, understanding, encouragement and love during the course of this project.
<table>
<thead>
<tr>
<th>Chapter 1: Literature Review</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Introduction</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Heavy Metals</td>
<td>3</td>
</tr>
<tr>
<td>1.2.1 Sources of heavy metals</td>
<td>3</td>
</tr>
<tr>
<td>1.2.2 Toxicology of heavy metals</td>
<td>3</td>
</tr>
<tr>
<td>1.2.3 Effects of heavy metals on plants</td>
<td>4</td>
</tr>
<tr>
<td>1.3 Agriculture and Heavy Metals</td>
<td>6</td>
</tr>
<tr>
<td>1.4 Heavy Metals in vegetables</td>
<td>7</td>
</tr>
<tr>
<td>1.5 Tolerance to Heavy Metals</td>
<td>8</td>
</tr>
<tr>
<td>1.6 Monitoring of Heavy Metals</td>
<td>10</td>
</tr>
<tr>
<td>1.7 Cadmium</td>
<td>12</td>
</tr>
<tr>
<td>1.8 Lead</td>
<td>14</td>
</tr>
</tbody>
</table>
1.9 Heavy Metal Pollution in South Africa ................................................................. 15
1.10 Aims and Objectives ......................................................................................... 16
   1.10.1 Research Objectives ................................................................................. 16
   1.10.2 Research Questions ............................................................................... 16
1.11 Conclusion ..................................................................................................... 17
1.12 References ..................................................................................................... 18
Chapter 2: Heavy Metals in Phaseolus vulgaris on farms in Cape Town ............... 29
2.1 Introduction ..................................................................................................... 30
2.2 Materials and Methods ................................................................................... 33
   2.2.1 Sample collection and preparation ........................................................... 33
   2.2.2 Chemical Analysis ................................................................................... 33
2.3 Statistical Analyses ......................................................................................... 34
2.4 Results ........................................................................................................... 35
2.5 Discussion ....................................................................................................... 43
2.6 References ..................................................................................................... 46
Chapter 3: The Effect of Two Heavy Metals and Two Mitigation Techniques on the Growth and Chemical Composition of Phaseolus vulgaris var. Contender ......................................................... 49
3.1 Introduction ..................................................................................................... 50
   3.1.1 Cadmium ................................................................................................. 53
   3.1.2 Lead ........................................................................................................ 54
   3.1.3 Chelating Agents .................................................................................... 55
   3.1.4 EDTA .................................................................................................... 56
   3.1.5 Phosphate .............................................................................................. 57
3.2 Materials and Methods ........................................................................................................59
  3.2.1 Growing the plants ...........................................................................................................59
  3.2.2 Growth medium ...............................................................................................................60
  3.2.3 Experimental Design .......................................................................................................61
  3.2.4 Harvest ............................................................................................................................63
  3.2.5 Chemical Analysis .........................................................................................................63
    3.2.5.1 Digestion .....................................................................................................................63
    3.2.5.2 Nutrient concentration determination ........................................................................64
  3.2.6 Statistical Analysis .......................................................................................................64
3.3 Results ................................................................................................................................65
  3.3.1 The effect of heavy metals and mitigation treatments on the growth of bean plants .................................................................................................................................65
  3.3.2 The effect of heavy metals and mitigation treatments on the chemical composition of bean plants .................................................................................................................................69
3.4 Discussion .........................................................................................................................88
  3.4.1 Heavy metals, growth and chlorophyll .........................................................................88
  3.4.2 Mitigation, growth and chlorophyll ...............................................................................88
  3.4.3 Heavy metals and elemental content ............................................................................89
  3.4.4 Mitigation and elemental content ..................................................................................89
  3.4.5 Relative concentrations in roots and shoots .................................................................90
3.5 Conclusion ..........................................................................................................................91
3.6 References ..........................................................................................................................92
List of Figures

Figure 2.1 The Kraaifontein Farming Area (x – areas sampled) Adapted from Google map…31
Figure 2.4.1: Nitrogen content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%................................................................................................................35
Figure 2.4.2: Phosphorus content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................36
Figure 2.4.3 Potassium content of organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................36
Figure 2.4.4: Calcium content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................37
Figure 2.4.5: Magnesium content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................37
Figure 2.4.6: Sodium content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................38
Figure 2.4.7: Manganese content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................39
Figure 2.4.8: Iron content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................39
Figure 2.4.9: Copper content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................40
Figure 2.4.10: Boron content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%........................................................................................................41
Figure 2.4.11: Lead content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at $p = 5\%$. 

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 $p = 5\%$. 

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 $p = 5\%$. 

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 $p = 5\%$. 

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 $p = 5\%$. 

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 $p = 5\%$. 

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 $p = 5\%$. 

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 $p = 5\%$. 

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 $p = 5\%$. 

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 $p = 5\%$. 
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%......................73

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%.................................................................................................................................................74

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%.................................................................................................................................................75

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%.................................................................................................................................................75

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%.................................................................76

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%.................................................................................................................................................77

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%...............................77

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%...............................78

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%.........................79
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 $p = 5\%$.

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 $p = 5\%$.

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 $p = 5\%$.

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 $p = 5\%$.

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 $p = 5\%$.

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 $p = 5\%$.

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 $p = 5\%$.

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 $p = 5\%$.
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%........86

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%........87
List of Tables

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…………………………….44

Table 2.2 Heavy metal concentrations in bean fruits as compared with the standard……………45

Table 3.1 The twenty five heavy metal and mitigation combinations used to grow *Phaseolus vulgaris* var. Contender bean plants…………………………………………………………………………………..62
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 $\geq 5 \text{ g/cm}^3$ (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 (Voutsas 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 (Voutsas 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 (Voutsas 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 (Voutsas 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 (Sandlio 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 Kopepe 1980, Krupa 1999). Up to 11% of the total cadmium content in plants can be found in stems and about 15%, depending on plant species and the ability to detoxicate this heavy metal, enters the leaves (Jastrow and Kopepe 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 (Sandallo et al. 2001).

Cadmium toxicity is also correlated with disturbances in the uptake and distribution of macro and micronutrients in plants (Sandallo 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 (Sandallo 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 (Sandallo 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 (Sandalia 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


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.](image)
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.

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 in the roots, and no significant differences were found in the much lower sodium concentrations in the stems, leaves or fruit.

![Sodium content of the organs of *Phaseolus vulgaris*. Bars with the same letter do not differ significantly at p = 5%.](image)

Roots and leaves had higher manganese concentrations than the stems and the fruits (Fig. 2.4.7).
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 2.4.7: Manganese content of the organs of Phaseolus vulgaris. Bars with the same letter do not differ significantly at p = 5%.

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).

![Graph showing copper content of organs of Phaseolus vulgaris](image)

**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).
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.

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

\(^a\) 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.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Mean measured</th>
<th>Highest measured</th>
<th>Standard(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.26</td>
<td>0.49</td>
<td>0.1</td>
</tr>
<tr>
<td>Cu</td>
<td>9.1</td>
<td>17.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Pb</td>
<td>3.64</td>
<td>10.19</td>
<td>0.3-0.5</td>
</tr>
<tr>
<td>Zn</td>
<td>43.5</td>
<td>62.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

\(^a\) amendment to regulations made under the Foodstuffs, Cosmetics and disinfectants Act (Department of Health 2003)
2.6 References


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...
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, microorganisms) 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
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 Ethylenediaminetetraacetic acid disodium salt dehydrate (Na$_2$-EDTA.2H$_2$O) (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.

<table>
<thead>
<tr>
<th>Pot</th>
<th>Heavy Metal</th>
<th>Mitigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>Low EDTA (4 mg/kg)</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>High EDTA (12 mg/kg)</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>Low Phosphate (1 mg/kg)</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>High Phosphate (8mg/kg)</td>
</tr>
<tr>
<td>6</td>
<td>Limit Cd (2mg/kg)</td>
<td>Control</td>
</tr>
<tr>
<td>7</td>
<td>Limit Cd (2mg/kg)</td>
<td>Low EDTA (4 mg/kg)</td>
</tr>
<tr>
<td>8</td>
<td>Limit Cd (2mg/kg)</td>
<td>High EDTA (12 mg/kg)</td>
</tr>
<tr>
<td>9</td>
<td>Limit Cd (2mg/kg)</td>
<td>Low Phosphate (1 mg/kg)</td>
</tr>
<tr>
<td>10</td>
<td>Limit Cd (2mg/kg)</td>
<td>High Phosphate (8mg/kg)</td>
</tr>
<tr>
<td>11</td>
<td>10x Limit Cd (20 mg/kg)</td>
<td>Control</td>
</tr>
<tr>
<td>12</td>
<td>10x Limit Cd (20 mg/kg)</td>
<td>Low EDTA (4 mg/kg)</td>
</tr>
<tr>
<td>13</td>
<td>10x Limit Cd (20 mg/kg)</td>
<td>High EDTA (12 mg/kg)</td>
</tr>
<tr>
<td>14</td>
<td>10x Limit Cd (20 mg/kg)</td>
<td>Low Phosphate (1 mg/kg)</td>
</tr>
<tr>
<td>15</td>
<td>10x Limit Cd (20 mg/kg)</td>
<td>High Phosphate (8mg/kg)</td>
</tr>
<tr>
<td>16</td>
<td>Limit Pb (6 mg/kg)</td>
<td>Control</td>
</tr>
<tr>
<td>17</td>
<td>Limit Pb (6 mg/kg)</td>
<td>Low EDTA (4 mg/kg)</td>
</tr>
<tr>
<td>18</td>
<td>Limit Pb (6 mg/kg)</td>
<td>High EDTA (12 mg/kg)</td>
</tr>
<tr>
<td>19</td>
<td>Limit Pb (6 mg/kg)</td>
<td>Low Phosphate (1 mg/kg)</td>
</tr>
<tr>
<td>20</td>
<td>Limit Pb (6 mg/kg)</td>
<td>High Phosphate (8mg/kg)</td>
</tr>
<tr>
<td>21</td>
<td>10X Limit Pb (66 mg/kg)</td>
<td>Control</td>
</tr>
<tr>
<td>22</td>
<td>10X Limit Pb (66 mg/kg)</td>
<td>Low EDTA (4 mg/kg)</td>
</tr>
<tr>
<td>23</td>
<td>10X Limit Pb (66 mg/kg)</td>
<td>High EDTA (12 mg/kg)</td>
</tr>
<tr>
<td>24</td>
<td>10X Limit Pb (66 mg/kg)</td>
<td>Low Phosphate (1 mg/kg)</td>
</tr>
<tr>
<td>25</td>
<td>10X Limit Pb (66 mg/kg)</td>
<td>High Phosphate (8mg/kg)</td>
</tr>
</tbody>
</table>
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 °C 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 Li₂SO₄·H₂O were added to 420ml of 100 vol H₂O₂. The solution was mixed well and 350 ml of conc. H₂SO₄ 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 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](image)

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%.

<table>
<thead>
<tr>
<th>Fresh Mass (g)</th>
<th>20mg Cd - Shoot</th>
<th>2mg Cd - Shoot</th>
<th>Control - Shoot</th>
<th>6.6mg Pb - Shoot</th>
<th>66mg Pb - Shoot</th>
<th>20mg Cd - Root</th>
<th>2mg Cd - Root</th>
<th>Control - Root</th>
<th>6.6mg Pb - Root</th>
<th>66mg Pb - Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot</td>
<td>f</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>ef</td>
<td>c</td>
<td>de</td>
<td>c</td>
<td>cd</td>
</tr>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
### 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).

![Graph showing the effect of mitigation treatment on cadmium concentrations](image)

**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](image)

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)

![Graph showing the effect of mitigation treatment on iron concentrations of bean shoots. Bars with the same letter do not differ significantly at p = 5%.](image)

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 1592.21 mg kg⁻¹ and the shoot cadmium concentration was 428.99 mg 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 fertilizers 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


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


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


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.