PHYTOREMEDIATION OF HEAVY METALS USING *Amaranthus dubius*

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Submitted in complete fulfillment for the Degree of Master of Technology (Biotechnology) in the Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa

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This study presents original work by the author. It has not been submitted in any form to another academic institution. Where use was made of the work of others, it has been duly acknowledged in the text. The research described in this dissertation was carried out in the Department of Biotechnology and Food Technology, Faculty of Engineering, Science and the Built Environment, Durban University of Technology, South Africa, under the supervision of **Prof Bharti Odhav** and **Prof Himansu Baijnath**

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Student's signature

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LIST OF ABBREVIATIONS

AA - Atomic Absorption

Ag - Silver

Al - Aluminium

As - Arsenic

BCF - Bioconcentration Factor

Cd - Cadmium

Cr - Chromium

Cu - Copper

ER - Endoplasmic Reticulum

Fe - Iron

GSH - Glutathione

Hg - Mercury

ICPMS - Inductively Coupled Plasma Mass Spectrometry

LS - Landfill Site

Ni - Nickel

NSS - Not Statistically Significant

Pb - Lead

PCs - Phytochelatins

RCA - Regular Cultivated Area

RDA - Recommended Daily Allowance

TEM - Transmission Electron Microscopy

TF - Translocation Factor

WWTS - Waste Water Treatment site

Zn - Zinc

ABSTRACT

Phytoremediation is an emerging technology where specially selected and engineered metal-accumulating plants are used for bioremediation. *Amaranthus dubius* (marog or wild spinach) is a popular nutritious leafy vegetable crop which is widespread especially in the continents of Africa, Asia and South America. Their rapid growth and great biomass makes them some of the highest yielding leafy crops which may be beneficial for phytoremediation.

This study was undertaken to evaluate the potential of *A. dubius* for the phytoremediation of Chromium (Cr), Mercury (Hg), Arsenic (As), Lead (Pb), Copper (Cu) and Nickel (Ni). Locally gathered soil and plants of *A. dubius* were investigated for the metals from a regularly cultivated area, a landfill site and a sewage site. Metals were extracted from the samples using microwave-digestion and analyzed using Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS). Further experiments were conducted with plants from locally collected seeds of *A. dubius,* in a tunnel house under controlled conditions. The mode of phytoremediation, the effect of the metals on the plants, the ability of the plant to extract metals from soil (Bioconcentration Factor - BCF), and the ability of the plants to move the metals to the aerial parts of the plants (Translocation Factor - TF) were evaluated for the different metals. Finally, *A. dubius* was micro-propagated in a tissue culture system with and without exposure to the metal, and the effect was studied by electron microscopy.

For a plant to be considered for phytoremediation it should be able to: to extract, degrade or stabilize the contaminant; have tolerance to high levels/concentrations of the contaminant; show a rapid growth rate and high biomass production, and be a cosmopolitan plant for growth and ease of harvesting.

The survey of the regularly cultivated area, landfill site and sewage site showed that soils were heavily contaminated with Cr, Hg, Cu and Ni. These levels were far above acceptable standards set for soils in the guidelines for Land Application and above the standards set for the Recommended Dietary (Daily) Allowance (RDA). These metals are toxic to humans even at low concentrations.

Naturally growing specimens of *A. dubius* from all three sites showed that they could tolerate Hg, sequester it from the soil and translocate it to the shoots. Cr could only be removed from the soil and stored in the roots, and limited amounts were translocated to the aerial parts. Pb, As, Ni, and Cu have some degree of transportability from the soil to the roots but not to aerial parts.

The results of this study under controlled conditions indicate that *A. dubius* can tolerate high Cr concentrations as indicated by the high BCF index. However, the ability of *A. dubius* to be considered for phytoextraction has to be viewed with caution, as the TF index indicates that only when the Cr concentration is 25 ppm is Cr being translocated to the aerial parts of the plant.

For Hg, the results show that the exposure of *A. dubius* to doses of up to 100 ppm show uniform growth and no visible phenotypic changes. In this study a measurement of the root length over a sixteen day period showed that the plant can tolerate doses of 75 ppm, but at 100 ppm there is a decrease in the root length. The ability of the plant to absorb Hg from the soil is well demonstrated by the high BCF index, however the TF > 1 was found only at 25 ppm. Thus it is evident from results that *A. dubius* can be used for phytoremediation when the concentrations of Hg are low.

For As, it was found that the plant samples in the soil containing 25 ppm showed no phenotypic changes, however, the samples exposed to 75 and 100 ppm showed toxic changes. The accumulation of As was different in comparison to Cr and Hg, in that the lowest was found in the roots and the highest levels were found in the leaves. The root length was found to increase for 25 ppm over a sixteen day period, but at 75 ppm this increase was only observed to day 12, thereafter there was a decrease in root length. The absorption of As from the soil by the roots is lower than that of leaves and shoots. Further evidence indicating that As is translocated in the leaves and shoots is indicated by the TF and BCF. As the objective of this study is to evaluate *A. dubius* for the phytoremediation of As, studies show that it can tolerate As levels of 75 ppm, and can also sequester and translocate most of the As to the aerial parts of the plant up to 100 ppm. Thus it can be concluded that *A. dubius* can phytoremediate As by hyperaccumulation.

In this study the effect of exposing *A. dubius* to Pb, Cu and Ni showed uniform growth rate and increasing root length at 25 and 75 ppm of Pb, and slightly lower growth rate at 100 ppm. The ability of the plant to take up Pb from the soil is only evident at 75 ppm which has a BCF value of 2.066. The ability to move the Pb to the aerial parts of the plant is limited to low concentration (25 ppm) where the TF > 1. Thus, *A dubius* shows limitations in its potential to remove Pb, Cu or Ni from contaminated sites.

Although experiments were not conducted with As, the results of the Cr uptake by callus cultures indicates that *A. dubius* can be micropropagated under laboratory conditions rendering it a viable proposition for commercialisation. *A. dubius* is a cosmopolitan weed with a rapid growth rate, yields a high biomass and is easy to harvest and the metal is sequestered from the soil and transported to the aerial parts. This study concludes that *A. dubius* can be defined as a hyperaccumulator of As.

1. INTRODUCTION

There has been an increasing concern with regard to the accumulation of heavy metals in the environment as they pose a threat to both human health and the natural environment. This is due to the fact that unlike many substances, metals are not biodegradable and hence accumulate in the environment. Contaminants such as mercury, arsenic, nickel, lead, cadmium and chromium enter the environment through industrial waste and landfill run off. Currently attempts are made to remediate the environmental heavy metals with conventional remediation technologies such as: solidification and stabilization; soil flushing; electro kinetics; chemical reduction/oxidation; soil washing; low temperature thermal desorption; incineration; vitrification; pneumatic fracturing; excavation/retrieval; and landfill disposal. However these are expensive and destructive. Thus the potential role of bioremediation, particularly by higher plants (phytoremediation) has gained significant interest.

Higher plants can accumulate heavy metals in different concentrations, but significant differences in metal accumulation exist between and within plant populations. In this study we chose *Amaranthus dubius* (marog or wild spinach) which is a popular nutritious leafy vegetable crop, rich in proteins, vitamins and minerals, and consumed virtually in the whole continent of Africa, Asia and South America. Their quick growth and great biomass makes them some of the highest yielding leafy crops which are beneficial for phytoremediation.

Initial experiments were designed to evaluate the levels of Cr, Hg, As, Pb, Cu and Ni from three different sites: (i) a regular cultivated area; (ii) a landfill site; and (iii) a waste water treatment site. *A. dubius* adapted to these conditions were harvested and the metal content within these plants and the soil in which they were growing analyzed. Subsequently, field experiments in a shade house were carried out to evaluate the phytoremediation strategy. The study was divided into two parts, in the first part the aim was to investigate the potential of wild *A. dubius*, growing in a regular cultivated area (RCA), a landfill site (LS) and a waste water treatment site (WWTS) to accumulate heavy metals from soil.

To achieve this aim the objectives were to:

- Identify suspected suitable sites in the Kwa Zulu Natal area
- Collect and analyze plants and soil for Cr, Hg, As, Pb, Cu and Ni from the 3 sites
- Evaluate the plant portal in which the respective metal is stored
- Determine the translocation of the metals from soil to plant
- Compare metal accumulation by wild *A. dubius* at each site

In the second part our aim was to investigate the factors that would affect bioremediation and the strategy used by the plant for metal accumulation. This was achieved by:

- Cultivating *A. dubius* under field conditions in a shade house
- Determining the beneficial or toxic effect of metals on *A. dubius*
- Determining the portal in which heavy metal is stored
- Determining the effect of metal concentration and exposure time to the metal uptake
- Determining the translocation and sequestration of the metal

Experiments were also carried out to see if callus cultures of *A. dubius* were capable of accumulating Cr. As the concentrations of callus was low its concentration could not be determined using Atomic Absorption Spectroscopy (AA) therefore bio-accumulation was visualized by Transmission Electron Microscopy (TEM)

The dissertation is arranged into five chapters and an appendix. Chapter one the introduction is followed by the chapter two which is a literature review. This gives an overview of phytoremediation and the strategies associated with it, the factors that affect heavy metal tolerance, translocation and sequestration and the advantages and limitations of phytoremediation, followed by a brief background of the current knowledge regarding the different metals used in this study. Chapter three describes the methodology used, chapter four covers the results and chapter five discusses the results.

2. LITERATURE REVIEW

2.1. PHYTOREMEDIATION

Phytoremediation, can be defined as the use of green plants to remove pollutants from the environment or to render them harmless (Berti and Cunningham, 2000; Salt et al., 1994). It is also referred to as green technology and can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Gratao et al., 2005; Salt et al., 1998). In this respect, plants can be compared to solar driven pumps capable of extracting and concentrating certain elements from their environment (Salt et al., 1995b). This technology is being considered as a new highly promising technology for the remediation of polluted sites. Over the last decade, there has been an increase in both industrial activities and urbanization. Both aquatic and terrestrial habitats are becoming progressively polluted due to the discharge of pollutants generated from various industries, transportation and fossil fuel burning. Many industries discharge their untreated wastewaters and other industrial wastes containing various proportions of heavy metals depending on what the industry produces, into natural body waters and on lands. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe) and the platinum group elements.

This led to huge increases in the amount of various wastes (solid, liquid and gaseous emissions) including heavy metals which led to potential increases in harmful toxic metals such as mercury, cadmium and lead, in many African countries, mining activities are also an important source of heavy metal input to the environment, for example, mercury in Algeria, arsenic in Namibia and South Africa, tin in Nigeria and Zaire and copper in Zambia. Other sources include activities such as leather tanning, electroplating, emissions from vehicular traffic, gas exhausts, crude oil hydrocarbon exploration and exploitation, energy and fuel production, downwash from power lines, intensive agriculture and sludge dumping (Pison and Menut, 2004). Current remediation strategies of heavy metals is primarily based on physicochemical technologies which are meant primarily for intensive *in situ* or *ex situ* treatment of relatively highly polluted sites, and thus are not very suitable for the remediation of vast, diffusely polluted areas where pollutants only occur at relatively low concentrations and superficially (Rulkens et al., 1998).

In this context, phytoremediation appears as a valid option since it is best suited for the remediation of these diffusely polluted areas and at much lower costs than other methods (Kumar et al., 1995). The idea of using plants to remove metals from soils came from the discovery of different wild plants, often endemic to naturally mineralized soils that accumulate high concentrations of metals in their foliage (Baker, 1987; Raskin et al., 1997).

2.1.1. STRATEGIES FOR PHYTOREMEDIATION

Utilizing the ability of certain trees, shrubs, and grass species to remove, degrade, or immobilize harmful chemicals can reduce risk from contaminated soil, sludges, sediments, and ground water through contaminant removal, degradation, or containment (Zavoda et al., 2001). In general, phytoextraction and phytovolatization are considered as the main options for the removal of heavy metals and other elemental compounds, whereas phytodegradation and phytostabilisation are applied mostly to organic contaminants (Meagher, 2000; Guerinot and Salt, 2001). Phytoremediation offers a cost-effective, nonintrusive, and safe alternative to conventional cleanup techniques (Glick, 2003). Phytoremediation can be accomplished by phytoextraction, phytodegradation, phytostabilization, phytovolatization and rhizofiltration.

- Phytoextraction: the use of plants to remove contaminants from soils. Pollutant-accumulating plants are utilized to transport and concentrate contaminants (metal or organic) from the soil into harvestable parts of the roots and aerial parts of the plant; the term is mostly used to refer to metal removal from soils (Kumar et al., 1995).

- Phytostabilization: the use of plants to reduce the bioavailability of pollutants in the environment. Plants stabilize pollutants in soils by chemically immobilizing the contaminants, thus rendering them harmless and reducing the risk of further environmental degradation by leaching of pollutants into the ground water or by airborne spread (Prasad and de Oliveira Freitas, 2003).

- Phytovolatization: the use of plants to volatilize pollutants. Plants extract volatile pollutants (e.g. selenium, mercury and arsenic) from the soil and biologically converts them to a gas which is released via transpiration from the foliage (Ghosh and Singh, 2005a; Ghosh and Singh, 2005b; Raskin et al., 1997).

- Phytodegradation: the use of plants to degrade organic pollutants. Plant roots are utilized to remediate contaminated soils by the breakdown of organic contaminants to simpler molecules which are stored in the plant tissue (Ghosh and Singh, 2005b).

- Rhizofiltration: the approach of using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants. This contaminated water is either collected from a waste site and brought to the plants, or the plants are planted in the contaminated area, where the roots then take up the water and the contaminants dissolved in it (Dushenkov et al., 1995).

Table one summarises the uses and mechanisms used for phytoextraction, phytovolatization, phytodegradation, phytostabilisation and rhizofiltration (Vidali, 2001).

Table 1: Overview of the applications of phytoremediation

Technique	Plant mechanism	Surface medium
Phytoextraction	Uptake and concentration of metal via direct uptake into the plant tissue with subsequent removal of the plants	Soils
Phytodegradation	Enhances microbial degradation in Rhizosphere	Soils, groundwater within rhizosphere
Phytostabilisation	Root exudates cause metal to precipitate and become less available	Soils, groundwater, mine tailing
Phytovolatization	Plants transpire selenium, mercury, and volatile hydrocarbons	Soils and groundwater
Rhizofiltration	Uptake of metals into plant roots	Surface water

2.1.1.1. PHYTOEXTRACTION

This method of phytoremediation involves the uptake of contaminants through the roots, with the contaminant being translocated to the aerial portions of the plant as shown in Figure 1 (Gleba et al., 1999). After a period of growth the plant is harvested, thereby removing the contaminant from the soil (Cluis, 2004). Plant roots generally contain higher metal concentrations than the shoots despite the translocation mechanisms, but an upper limit to the metal concentration within the root can occur. Root uptake of Pb by hydroponically grown plants reached a maximum concentration and did not increase further as the Pb concentration of the solution increased (Deepa et al., 2006). Metals are generally unevenly distributed throughout a plant, although in hyper-accumulators the metal content of the leaves is often greater than other portions of the plant; for example, the greatest proportion of Ni in *Alyssum heldreichii* was found in the leaves (Brooks, 1998). Calcium and Zn were found in both roots and shoots, although the shoots had higher concentrations of Zn (Brooks, 1998).

High concentrations of Zn were found in small hemispherical bodies located on the surface of some leaves of *Thlaspi caerulescens* which is considered to be the best known hyper-accumulator (Cluis, 2004). Site selection that is conducive with phytoextraction is of extreme importance. Phytoextraction is applicable only to sites that contain low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. Soil metals should also be bioavailable, or subject to absorption by plant roots. The land should be relatively free of obstacles, such as fallen trees or boulders, and have an acceptable topography to allow for normal cultivation practices, which employ the use of agricultural equipment. As a plant-based technology, the success of phytoextraction is inherently dependent upon several plant characteristics. The two most important characters include the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue (Cunningham and Ow, 1996; Deepa et al., 2006).

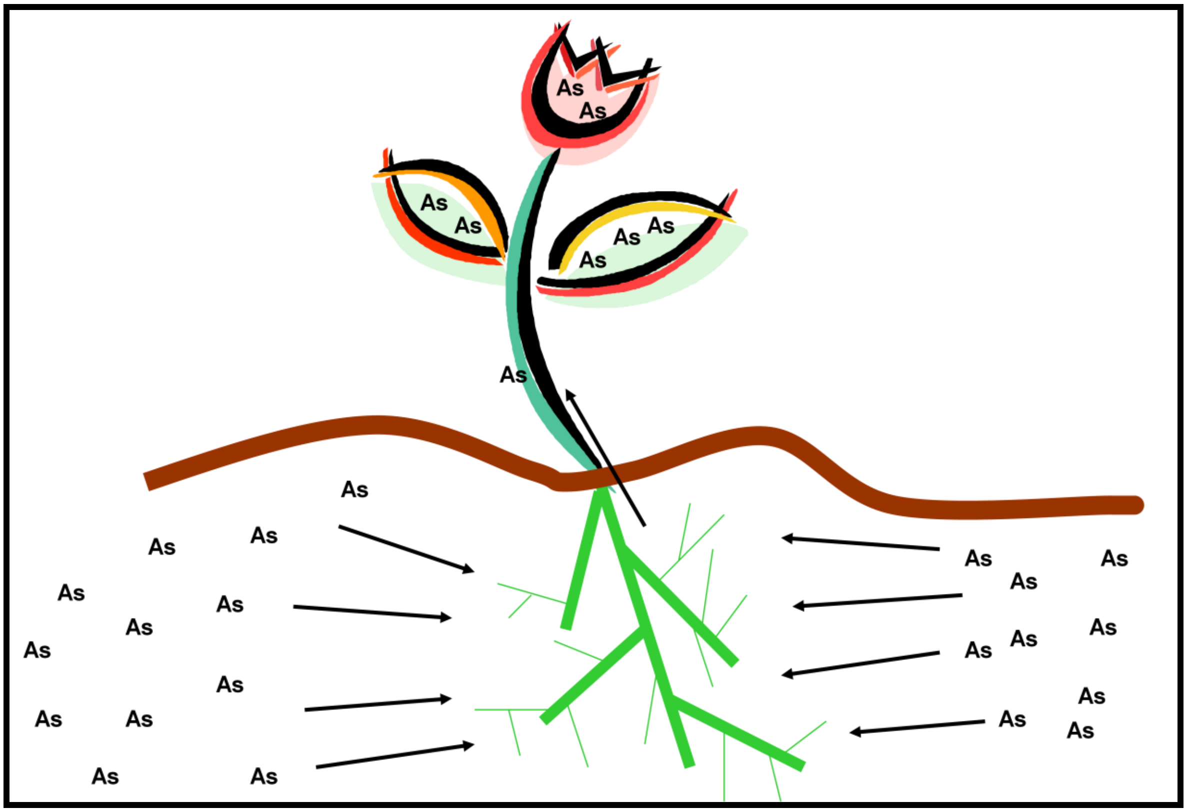

Figure 1: Phytoextraction of As from the soil to aerial parts of plant (leaves and stems).

In phytoextraction as with the excavation of soil from a contaminated site, the disposal of contaminated material is of great concern. Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal (Kumar et al., 1995). However in some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation (Cunningham and Ow, 1996; Comis, 1996). Phytoextraction should be viewed as a long-term remediation effort, requiring many cropping cycles to reduce metal concentrations (Kumar et al., 1995) to acceptable levels. The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years. This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal- contaminants (Kumar et al., 1995; Blaylock et al., 1997).

Ebbs et al. (1997) reported that *Brassica juncea,* while having one-third the concentration of Zn in its tissue, is more effective at Zn removal from soil than *Thlaspi caerulescens*, a known hyperaccumulator of Zn. This advantage is due primarily to the fact that *Brassica juncea* produces ten-times more biomass than *Thlaspi caerulescens* (Ebbs et al., 1997).

Plants being considered for phytoextraction must be tolerant of the targeted metal, or metals, and be efficient at translocating them from roots to the harvestable above-ground portions of the plant (Blaylock and Huang, 2000). Other desirable plant characteristics include the ability to tolerate difficult soil conditions (*i.e.,* soil pH, salinity, structure, water content), the production of a dense root system, ease of care and establishment, and few disease and insect problems. Although some plants show promise for phytoextraction, there is no plant which possesses all of these desirable traits with the 'perfect plant' continuing to be the focus of many plant-breeding and genetic-engineering research efforts.

2.1.1.2. PHYTODEGRADATION

The plant takes up the contaminant through its roots from where the contaminant is translocated to the aerial portions of the plant. The difference between phytoextraction and phytodegradation is that in the latter the contaminant is converted to a less toxic form during translocation to the aerial portions of the plant (Figure 2). Phytodegradation is also known as phytotransformation, and is a contaminant destruction process.

Plant-produced enzymes metabolize contaminants which may be released into the rhizosphere, where they can remain active (Singh and Labana, 2003). Plant-formed enzymes have been discovered in plant sediments and soils. These enzymes include dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase (Dietz and Schnoor, 2001).

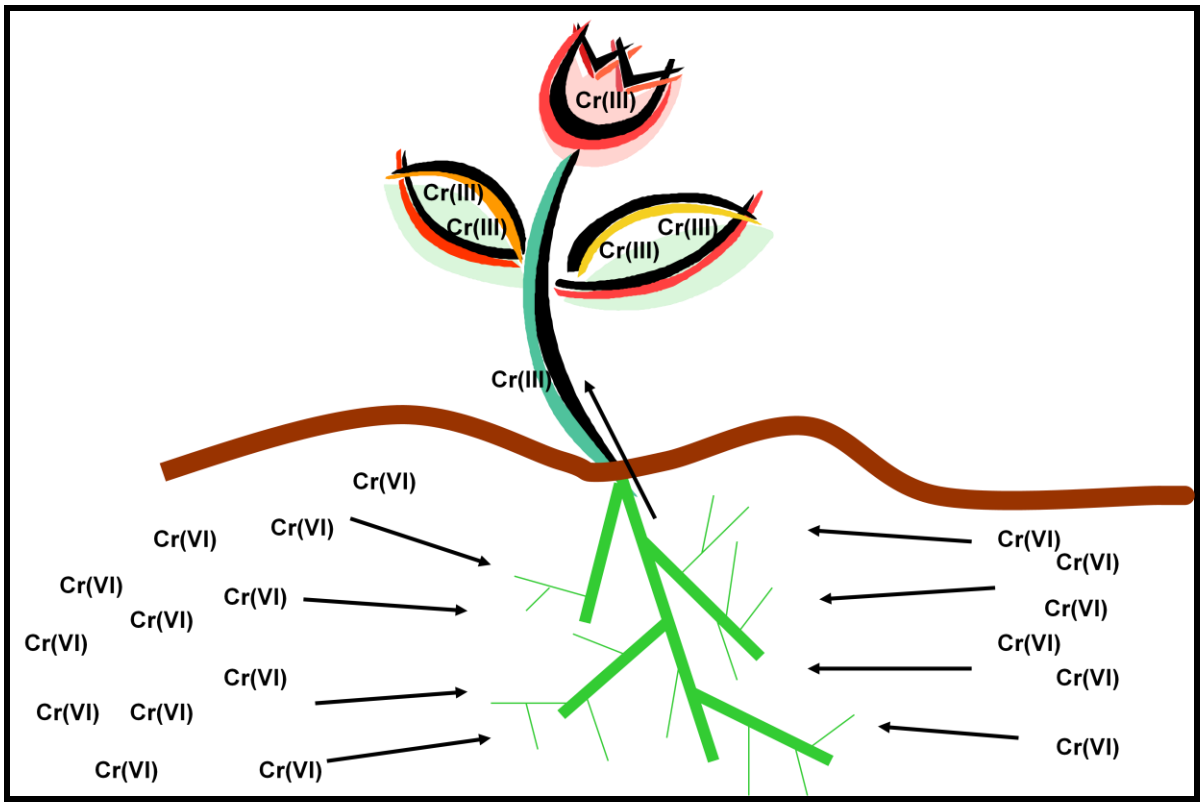

Figure 2: Phytodegredation of Cr (VI) from the soil to Cr (III) in the aerial parts of the plant.

2.1.1.3. PHYTOSTABILISATION

The traditional means by which metal toxicity is reduced is by in-place inactivation, a remediation technique that employs the use of soil amendments to immobilize or fix metals in soil. Although metal migration is minimized, soils are often subject to erosion and still pose an exposure risk to humans and other animals. Phytostabilization, known as phytorestoration, is a plant-based remediation technique that stabilizes wastes and prevents exposure pathways via wind and water erosion (Prasad and de Oliveira Freitas, 2003). With this method of phytoremediation the plant root system releases chemicals into the surrounding soil which bind to the contaminant making it less bioavailable to the surrounding environment (Figure 3). Phytostabilization is also known as in-place inactivation or phytoimmobilization. A study by Salt et al. (1995b) showed that *Brassica juncea* has the potential for effective phytostabilization. In a laboratory study, leachate from sand planted with seedlings of *Brassica juncea* contained 22 g/mL lead, compared to 740 g/mL lead from sand without plants.

Another study indicated that *Brassica juncea* roots reduced Cr (VI) to Cr (III) (Gleba et al., 1999). In comparison to other modes of phytoremediation the purpose of phytostabilization is not to remove contaminants from the soil, but merely to stabilize them thus removing the risk to human life and the environment. Although phytostabilization is most effective at sites having fine-textured soils with high organic-matter content, it is also suitable for treating a wide range of sites where large areas of surface contamination exist (Cunningham et al., 1995; Berti and Cunningham, 2000). Despite this some highly contaminated sites are not always suitable for phytostabilization, because plant growth and survival is not a possibility.

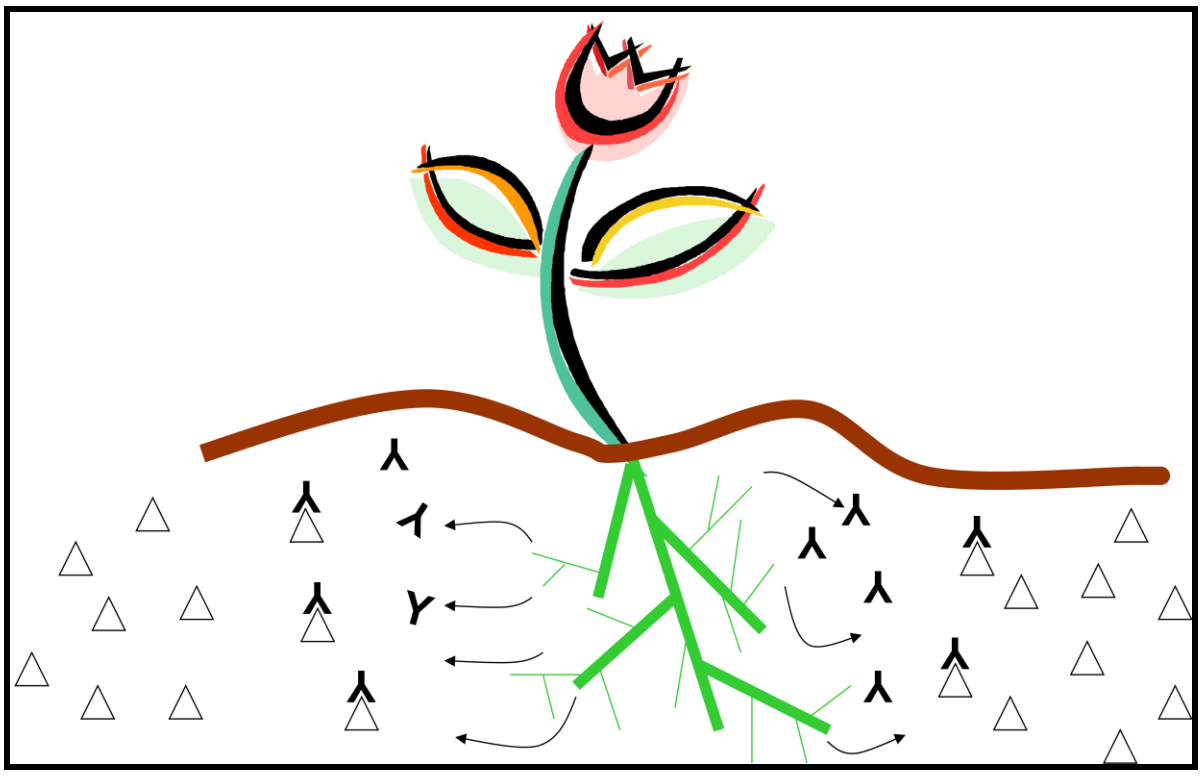

Figure 3: Phytostabilisation of metal contaminants from the soil.

Plants chosen for phytostabilization should be poor translocators of metal contaminants to above ground plant tissues that could be consumed by humans or animals. The lack of appreciable metals in shoot tissue also eliminates the necessity of treating harvested shoot residue as hazardous waste (Flathman and Lanza, 1998). Selected plants should be easy to establish and care for, grow quickly, have dense canopies and root systems, and be tolerant of metal contaminants and other site conditions which may limit plant growth.

The research of Smith and Bradshaw, (1972), led to the development of two cultivars of *Agrostis tenuis* Sibth and one of *Festuca rubra* L which are now commercially available for the phytostabilization of Pb, Zn, and Cu contaminated soils. At sites which support plant growth, site managers must be concerned with the migration of contaminated plant residue off site or disease and insect problems which limit the longevity of the plants (Schnoor, 2000). Phytostabilization has advantages over other soil-remediation practices in that it is less expensive, less environmentally evasive, easy to implement, and offers aesthetic value (Berti and Cunningham, 2000; Schnoor, 2000). When decontamination strategies are impractical because of the size of the contaminated area or the lack of remediation funds, phytostabilization is advantageous (Berti and Cunningham, 2000). It may also serve as an interim strategy to reduce risk at sites where complications delay the selection of the most appropriate technique for the site.

2.1.1.4. PHYTOVOLATIZATION

This method relies upon the ability of the plants to absorb contaminants through its roots and convert them into a less toxic form which is released into the atmosphere via transpiration (Figure 4). Phytovolatization is primarily a contaminant removal process, transferring the contaminant from the original medium (ground water or soil water) to the atmosphere. However, metabolic processes within the plant might alter the form of the contaminant, and in some cases transform it to less toxic forms. Examples include the reduction of highly toxic mercury species to less toxic elemental mercury, or transformation of toxic selenium (as selenate) to the less toxic dimethyl selenide gas by *Brassica juncea* (Adler, 1996; De Souza and Pickering, 2002). Mercury and selenium are toxic (Suszcynsky and Shann, 1995), and there is a doubt as to whether the volatization of these elements into the atmosphere is safe (Watanabe, 1997). Selenium phytovolatization has been given the most attention to date (McGrath, 1998; Lewis et al., 1966), because this element is a serious problem in many parts of the world where there are areas of selenium-rich soil (Brooks, 1998). Although there have been no efforts to genetically engineer plants which volatilize toxic compounds, it is likely that researchers will pursue this possibility in the future. According to Brooks (1998), the release of volatile selenium compounds from higher plants was first reported by Lewis et al. (1966).

This reports that members of the family Brassicaceae are capable of releasing up to 40 g selenium.$ha^{-1}day^{-1}$ as various gaseous compounds. There are some aquatic plants, such as *Typha latifolia* L., which are also good for Se phytoremediation (Pilon-Smits et al., 1999). Unlike plants that are being used for Se volatilization, those which volatilize Hg are genetically modified. *Arabidopsis thaliana* L., and *Nicotiana tabacum* L., have been genetically modified with bacterial organomecurial lyase *(MerB)* and mercuric reductase *(MerA)* genes (Heaton et al., 1998; Rugh et al., 1998). These plants absorb elemental Hg (II) and methyl mercury (MeHg) from the soil and release volatile Hg(O) from the leaves into the atmosphere (Heaton et al. 1998). The phytovolatization of Se and Hg into the atmosphere has several advantages. Volatile Se compounds, such as dimethylselenide, are 1/600 to 1/500 as toxic as inorganic forms of Se found in the soil (De Souza et al., 2000). The volatization of Se and Hg is also a permanent site solution, because the inorganic forms of these elements are removed and the gaseous species are not likely to be redeposited at or near the site (Atkinson et al., 1990). Furthermore, sites that utilize this technology may not require much management after the original planting.

Phytovolatization also has the added benefits of minimal site disturbance, less erosion, and no need to dispose of contaminated plant material (Heaton et al., 1998; Rugh et al., 2000). Heaton et al. (1998) suggest that the addition of Hg(O) into the atmosphere would not contribute significantly to the atmospheric pool. However, those who support this technique also agree that phytovolatization would not be wise for sites near population centers or at places with unique meteorological conditions that promote the rapid deposition of volatile compounds (Heaton et al., 1998; Rugh et al., 1998).

Unlike other remediation techniques, once contaminants have been removed via volatization, there is a loss of control over their migration to other areas. Despite the controversy surrounding phytovolatization, this technique is a promising tool for the remediation of Se and Hg contaminated soils.

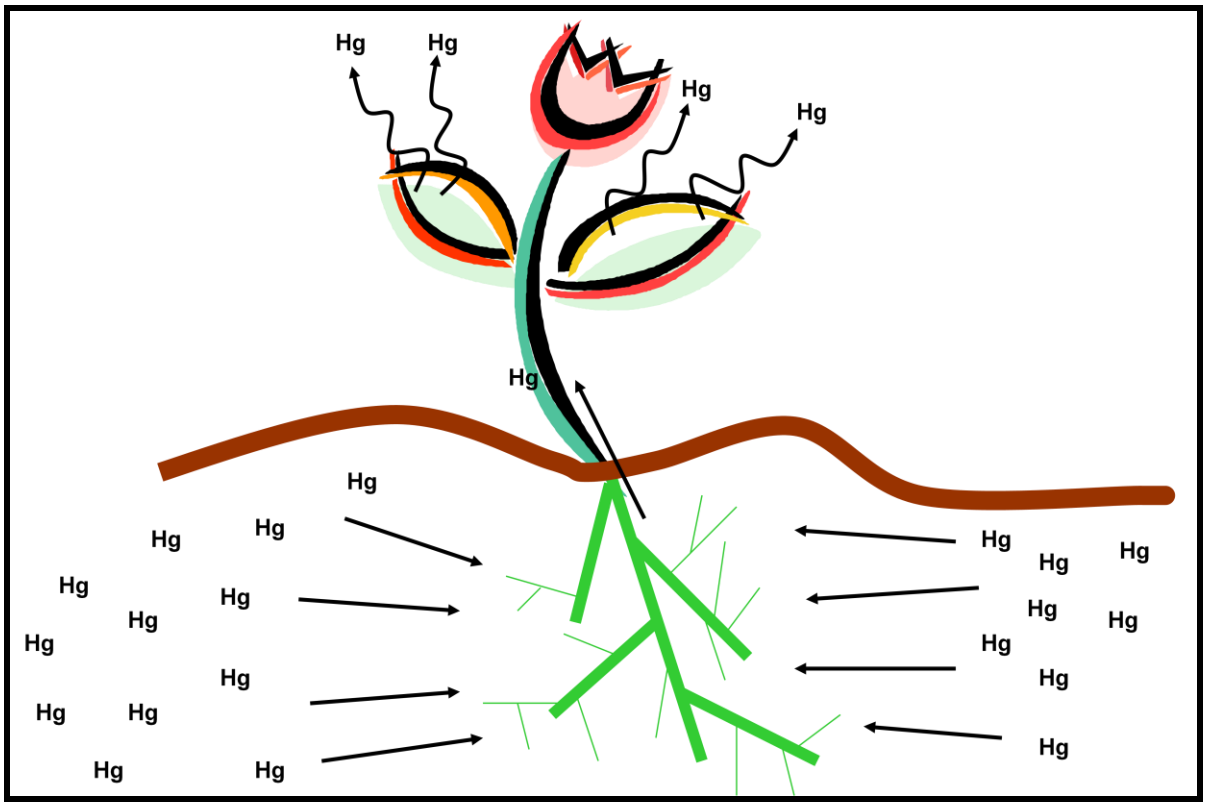

Figure 4: Phytovolatization of Hg from the soil.

2.1.1.5. RHIZOFILTRATION

Rhizofiltration is a phytoremediative technique designed for the removal of metal contaminants from aquatic environments. The process involves the growth of plants in metal polluted waters where the plant absorbs and concentrates the metals in roots and shoots (Zhu et al., 1999; Dushenkov et al., 1995). Changes in the rhizosphere pH and root exudates also contribute to the precipitation of metals onto the root surface (Prasad and de Oliveira Freitas, 2003). As the plant becomes saturated with the metal contaminants either the roots or the whole plants are harvested for disposal. Rhizofiltration is a cost-competitive technology for the treatment of surface water or groundwater containing low, but significant concentrations of heavy metals such as Cr, Pb, and Zn (Kumar et al., 1995). The commercialization of this technology is driven by economics, applicability to many problem metals, ability to treat high volumes, lesser need for toxic chemicals, reduced volume of secondary waste, possibility of recycling, and the likelihood of regulatory and public acceptance (Kumar et al., 1995; Dushenkov et al., 1995). However, the application of this plant-based technology may be more challenging and susceptible to failure than other methods of similar cost.

The production of hydroponically grown transplants and the maintenance of successful hydroponic systems in the field requires the expertise of qualified personnel, and the facilities and specialized equipment required can increase overhead costs. Perhaps the greatest benefit of this remediation method is related to positive public perception. The use of plants at a site where contamination exists conveys the idea of cleanliness and progress to the public in an area that would have normally been perceived as polluted.

2.1.2. FACTORS AFFECTING HEAVY METAL TOLERANCE

In order to be realistic about phytoremediation, focused studies on factors regulating phytoremediation are necessary. Several factors would accelerate phytoremediation technology (Figure 5). The main ones are hyperaccumulation of metals, tolerance and high biomass production; bioaccumulation coefficient; chelator (natural or synthetic) induced hyperaccumulation; genetic engineering and production of transgenic plants having tolerance and metal accumulation ability for use in phytoremediation. An understanding of these factors would influence the metal bioaccumulation coefficient, which in turn will depend upon heavy metal availability in the soil; absorption, transport and sequestration etc., and development of low cost technologies for chelate-induced hyperaccumulation.

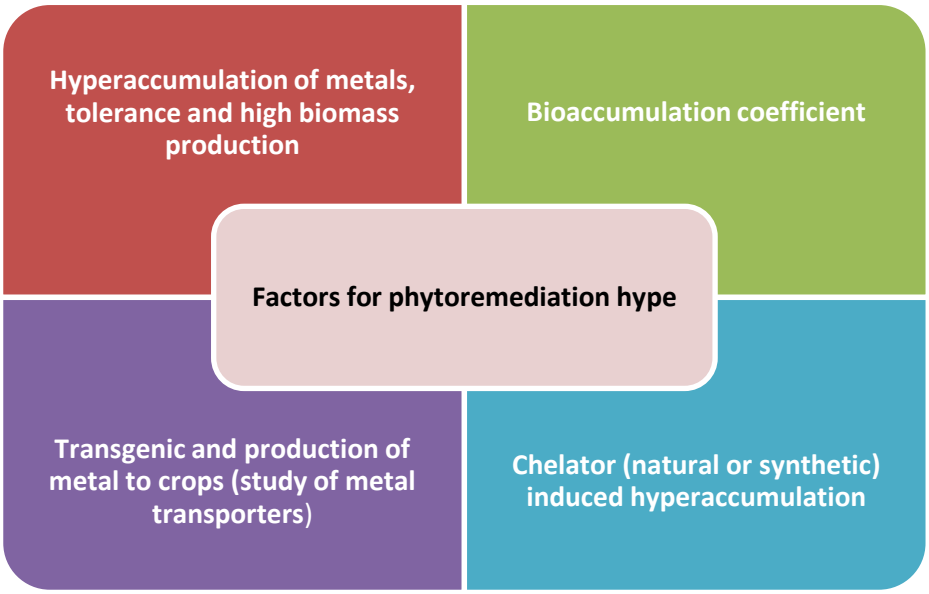

Figure 5: Factors increasing the hype for the use of phytoremediation/green technology.

Remediation of contaminated sites using plants has been put into practice but each metal and site uses different plants or plant properties. Faster growth rate, high biomass, hardiness, and tolerance to pollutants are some of the favorable plant properties being exploited for remediation. Among these, plant uptake of water and contaminants, plant-microbe interactions, enhanced microbial activity in the rhizosphere, fate and transport of contaminants in plant root zone, further translocation, and tolerance mechanisms, are of paramount importance in improving phytoremediation technologies.

2.1.3. TRANSLOCATION AND SEQUESTRATION OF METALS

Once taken up by root cells, metal ions find their way to the shoot and then to their final intracellular destination, such as the vacuoles, by a process called translocation. It is believed that the increased tolerance of hyper-accumulators is associated with the presence of high-affinity chelating molecules in the cytoplasm. For example, phytochelatins (cysteine and gluthanione rich compounds) help to sequester metals such as Ag, Cd, Cu, and Ni and thus protect cells from their harmful effects on surrounding proteins. In *Thlaspi caerulescens*, Zn is believed to be complexed with histidine in root cells and organic acids in the shoot and finally the complexed metals are transported and sequestered in the vacuoles, which account for the plant hypertolerance to metals. After chelation by glutathione (GSH) or phytochelatins (PCs), a transporter (usually an ABC-type) actively transports the metal-chelate complex to vacuoles where it may undergo further complexation with sulphide.

2.1.4. METAL HYPERACCUMULATORS FOR PHYTOREMEDIATION

Phytoremediation is a broad expression comprising different strategies used by plants to decontaminate soil and water, namely rhizofiltration, phytostabilization, phytodegradation, phytoextraction, and phytovolatization. Numerous plant species have been identified for the purpose of phytoremediation with certain plant species, known as hyper-accumulators, being attractive candidates as they are able to accumulate potentially phytotoxic elements to concentrations 50-500 times higher than average plants. The high bioconcentration factor and the efficient root to-shoot transport system endowed with enhanced metal tolerance provide hyper-accumulators with a high potential detoxification capacity.

As previously discussed, plants used for phytoextraction must be fast growing and have the ability to accumulate large quantities of environmentally important metal contaminants in their shoot tissue (Kumar et al., 1995; Cunningham and Ow, 1996; Blaylock et al., 1997). Many plant species have been screened to determine their usefulness for phytoextraction. Researchers initially applied hyperaccumulators to clean metal polluted soils (Chaney et al., 1997). At present, there are nearly 400 known hyperaccumulators (Salt and Kramer, 2000), but most are not appropriate for phytoextraction because of their slow growth and small size. Several researchers have screened fast-growing, high- biomass accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots (Kumar et al., 1995; Salt et al., 1995b; Blaylock et al., 1997; Huang et al., 1997). Many metal-tolerant plant species, particularly grasses, escape toxicity through an exclusion mechanism and are therefore better suited for phytostabilization than phytoextraction (Baker, 1981). However, *Hordeum vulgare* L. (barley) and *Avena sativa* L. (oats) are tolerant of metals such as Cu, Cd, and Zn, and accumulate moderate to high amounts of these metals in their tissues. A list of known metal hyperaccumulating plant species together with the metal, contaminant and medium they act in is given in Table 2. Experiments that utilize seeds of these metal hyperaccumulating species have more precision than those conducted with seeds from commercially available sources.

The majority of hyperaccumulating species discovered so far are restricted to tropical areas (Baker and Brooks, 1989). The first hyperaccumulators characterized were members of the families Brassicaceae and Fabaceae. Although the number of metal-accumulating species identified to date has been reported to be 397, there is a continuous search for novel phytoextracting plants which are adapted to particular ecosystems and climates (Salt et al., 1998). Studies have demonstrated that the ability to accumulate heavy metals varies greatly between species and between cultivars within a species (Salt et al., 1995a). Particular emphasis has been placed on the evaluation of shoot metal-accumulation capacity of the cultivated *Brassica* (mustard) species because of their relation to wild metal-accumulating mustards (Kumar et al., 1995). Although the largest numbers of temperate-climate hyperaccumulating species belong to the Brassicaceae (Baker and Brooks, 1989), in the tropics the Euphorbiaceae is the best represented group (Ensley, 1997). Other field trials for plants with the potential for phytoremediation of metals have been reported (Table 3).

Table 2: Hyperaccumulators and common plants employed in phytoremediation research

Hyperaccumulator	Metal	Plant	Contaminant	Medium
Thlaspi caerulescens	zinc (Zn) cadmium (Cd)	indian mustard	heavy metals, selenium and radionuclides	soil
Berkheya coddii	nickel (Ni)	poplar	chlorinated solvents and nitrates	soil and groundwater
Astragalus racemosus	selenium (Se)	cotton wood	chlorinated solvents, metals, nitrates	groundwater
Pteris vittata	arsenic (As)	duck weed	explosive waste	groundwater
Ipomoea alpina	copper (Cu)	mulberry	PAHs	soil
Haumaniastrum robertii	cobalt (Co)	sunflower	Radionuclides	groundwater
Iberis intermedia	thallium (Tl)	grasses	heavy metals and petroleum	soil
Gypsophila sphaerocephala	boron (B)	alfalfa, juniper	petroleum hydrocarbons	soil and groundwater

Table 3: Plants with the potential for the phytoextraction of various metals

Metal	Plant species	Reference
Cr	*Alternanthera sessilis* *Pistia stratiotes*	(Moodley et al., 2007)
	Brassica juncea	(Gleba et al., 1999)
	Arabidopsis thaliana	(Baker et al., 1991)
Hg	*Azolla caroliniana*	(Bennicelli et al., 2004)
As	*Pteris vitatta*	(Ma et al., 2001) (Wei et al., 2006)
Pb	*Alternanthera sessilis* *Pistia stratiotes*	(Moodley et al., 2007)
	Zea mays	(Huang and Cunningham, 1996)
	Alyssum murale	(Baker et al., 1991)
Cu	*Thlaspi rotundifolium*	(Reeves and Brooks, 1983)
	Alyssum lesbiacum	(Baker et al., 1991)
Ni	*Alternanthera sessilis* *Pistia stratiotes*	(Moodley et al., 2007)
	Brassica campestris	(Glick, 2003)
	Sibertia acuminate	(Cunningham et al., 1995)
	Thlaspi caerulescens	(Baker et al., 1991)

2.1.5. LIMITATIONS AND ADVANTAGES OF PHYTOREMEDIATION

Phytoremediation is not a ready fix method for contaminated soils. As with all new technologies it is still poorly understood, and before this technology can become efficient and cost effective on a commercial scale there are limitations that need to be overcome (Khan et al., 2000). For example, there is very little known about the biochemical, molecular and physiological processes involved in the hyper-accumulation process (Salt et al., 1994). For phytoremediation to be effective and to occur within a reasonable time frame, plant yield (biomass production) and contaminant accumulation have to be dramatically enhanced. This may be achieved by cultivation of rapidly growing plants or genetic engineering of plants with as yet unidentified hyper-accumulating genes. Other limitations of phytoremediation include the possible contamination of the food chain as a result of grazing on heavy metal contaminated vegetation. However, phytoremediation interests in the mining sector is growing due to the recovery of rare and expensive trace metals from harvested biomass (phytomining) and the low cost use of plants to remediate mining areas (Tyrer, 2006). Other limitations and advantages of phytoremediation can be seen in Table 4 (Chaney et al., 1997; Ghosh and Singh, 2005a; Ghosh and Singh, 2005b).

Table 4: Advantages and limitations of phytoremediation

Advantages	Limitations
• Low input costs & aesthetically pleasing (no excavation) • Soil stabilization & reduced leaching of water and transport of inorganics in the soil • Generation of a recyclable metal rich plant residue • Applicability to a range of toxic metals and radionuclides • Minimal environmental disturbance • Elimination of secondary air or water-borne wastes • Enhanced regulatory and public acceptance	• The plant must be able to grow in the contaminated soil or material • The plant can accumulate inorganics that it can reach through root growth and are soluble in soil water • Process can take years for contaminant concentrations to reach regulatory levels (long-term commitment) • The contaminant must be within (or drawn toward) the root zones of plants that are actively growing • It must not present an imminent danger to human health or further environmental harm • Climatic conditions are a limiting factor • Introduction of non-indigenous species may affect biodiversity

2.2. HEAVY METALS

There has been an increasing concern with regard to the accumulation of toxic heavy metals in the environment and their impact on both public health and the natural environment (Gardea-Torresdey et al., 2004). The accumulation of heavy metals in soil is becoming a serious problem as a result of industrial and agricultural practices to name but a few of the causes of pollution today. Fertilizers from sewage sludge, mining waste and paper mills all contribute to the continuous deposition of heavy metals into soils. Another point of concern is the effect of leaching on these contaminated sites which in turn contaminate water tables (Gratao et al., 2005). Living organisms require a trace amount of some heavy metals which include copper (Cu), iron (Fe), nickel (Ni) and zinc (Zn) and are often referred to as essential elements (Odjegba and Fasidi, 2004). However there are some non-essential heavy metals which are of great concern due to their presence in areas of heavy metal pollution such as chromium (Cr), mercury (Hg) and lead (Pb) (Kamal et al., 2004). The capacity of plants to concentrate metals has usually been considered a detrimental trait since some plants are directly or indirectly responsible for a proportion of the dietary uptake of toxic heavy metals by humans (Chaney et al., 1997; Cunningham et al., 1995). The dietary intake of heavy metals through consumption of contaminated crop plants can have long-term effects on human health (Ow, 1996).

2.2.1. PLANT TOLERANCE

Plant tolerance to heavy metals depends largely on plant efficiency in the uptake, translocation, and further sequestration of heavy metals in specialized tissues or in trichomes and organelles such as vacuoles. The uptake of metals depends on their bioavailability, and plants have evolved mechanisms to make micronutrients bioavailable. Chelators such as siderophores, organic acids, and phenolics can help release metal cations from soil particles, increasing their bioavailability. For example, organic acids (malate, citrate) excreted by plants act as metal chelators. By lowering the pH around the root, organic acids increase the bioavailability of metal cations. However, organic acids may also inhibit metal uptake by forming a complex with the metal outside the root.

Citrate inhibition of aluminum (Al) uptake and resulting Al tolerance in several plant species is an example of this mechanism. Copper tolerance in *Arabidopsis* is also the result of a similar mechanism. The presence of rhizosphere microbes may also affect plant uptake of inorganics. For example, rhizosphere bacteria can enhance plant uptake of mercury and selenium. However, the exact mechanisms of these plant-microbe interactions are largely unknown. It is possible that the microbe-mediated enhanced uptake may be due either to a stimulatory effect on root growth or to microbial production of metabolites that could affect plant gene expression of transporter proteins, or to a microbial effect on the bioavailability of the element.

2.2.2. CHROMIUM

The impact of Cr contamination in the physiology of plants depends on the metal speciation, which is responsible for its mobilization, subsequent uptake and resultant toxicity in the plant system (Shanker et al., 2005). Chromium and its compounds have multifarious industrial uses. They are extensively employed in leather processing and finishing (Nriagu, 1988), in the production of refractory steel, electroplating cleaning agents and in the production of chromic acid and specialty chemicals. Hexavalent chromium compounds are used in industry for metal plating, cooling tower water treatment, hide tanning and, until recently, wood preservation. These anthropogenic activities have led to the widespread contamination that Cr shows in the environment and have increased its bioavailability and biomobility. A detailed review on the critical assessment of Cr in the environment has been published by (Kimbrough et al., 1999). The leather industry is the major cause for the high influx of Cr to the biosphere, accounting for 40% of the total industrial use (Barnhart, 1997). The stable forms of Cr are the trivalent Cr (III) and the hexavalent Cr (VI) species, although there are various other valence states which are unstable and short lived in biological systems. Chromium (VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate (CrO_4^{-2}) or dichromate ($Cr_2O_7^{-2}$) ions (Lytle et al., 1998). Chromium (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments. Chromium toxicity in plants is observed at multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis (Salt et al., 1994; Zavoda et al., 2001).

The toxic properties of Cr (VI) originate from the action of this form itself as an oxidizing agent, as well as from the formation of free radicals during the reduction of Cr (VI) to Cr (III) occurring inside the cell. Chromium (III), on the other hand, apart from generating reactive oxygen species, if present in high concentrations, can cause toxic effects due to its ability to coordinate various organic compounds resulting in inhibition of some metalloenzyme systems. The differential toxicity of these two species can be explained by translocation and partitioning: Cr (VI) is actively taken up by a metabolic driven process, whereas Cr (III) is probably passively taken up and retained by cation exchange sites; in addition, Cr (VI) competes with various elements of similar electronic structure; hence, it seems that Cr (VI) has an advantage at the entry level into the plant system (Pulford et al., 2001). The pathway of Cr (VI) transport is an active mechanism involving carriers of essential anions such as sulphate (Cervantes et al., 2001). Iron, S and P are known also to compete with Cr for carrier binding.

Salt et al. (1994) demonstrated that *Brassica juncea* (Indian mustard) could efficiently accumulate palladium (Pd), zinc (Zn), cadmium (Cd), nickel (Ni), copper (Cu), and Cr(VI) from soils or water in both roots and stems. Kumar et al. (1995) demonstrated the ability of six Brassica species, *B. nigra*, *B. oleracea*, *B. campestris*, *B. carinata*, *B. juncea*, and *B. napus* to efficiently accumulate heavy metals. They found that Cr had the highest phytoextraction coefficient, followed by Cd, Ni, Zn, and Cu. Lytle et al. (1998) found that *Eichhornia crassipes* dosed with Cr(VI), accumulated Cr(III) in root and stem tissues. After Cr (VI) was reduced to Cr (III) in the fine lateral root, Cr (III) was translocated to leaf tissues. Zhu et al. (1999) reported that *Eichhornia crassipes* was a good accumulator of Cd and Cr, but a poor accumulator of arsenic (As) and Ni. Pulford et al. (2001) investigated the concentrations of Cr and Zn in various tissues of various tree species, grown in both the field and hydroponic systems. They found that Cr was taken up mainly in the roots, whereas Zn was strongly translocated from the roots to the stems. Zavoda et al. (2001) found that *Helianthus annuus* (Dwarf sunflowers) accumulated Cd > Ni > Cr and *Brassica juncea* accumulated Cr > Ni > Cd.

2.2.3. MERCURY

Mercury (Hg) is a global environmental pollutant that is present in soil, water, air and biota. The naturally occurring Hg can be released into the atmosphere and then exchanged between the soil and water systems by processes such as wind erosion, degassing from Hg mineralized soil and rock formations, volcanic eruptions and other geothermal activities (Ebinghaus et al., 1999). Anthropogenic sources of Hg can be attributed to the combustion of fossil fuels, wood, sewage sludge and crematories, high temperature processes, e.g. smelting, cement and lime production and manufacturing/commercial activities: e.g. metal processing, gold extraction (Porcella et al., 1996). Mercury and its compounds are persistent, bioaccumulative and toxic, and they pose a risk to both humans and the ecosystem. Exposures to Hg, e.g. breathing Hg-contaminated air, eating Hg-contaminated food products, eating and touching Hg contaminated soil may result in devastating neurological damage, kidney damage, and even death. Historic and recent industrial activities, including the mining of gold, silver and mercury itself, have caused mercury contamination of terrestrial and aquatic ecosystems (Porcella et al., 1996). Mercury contaminated soil is believed to contribute to human health risks and phytotoxicity of plants. Therefore, the numerous Hg-contaminated sites that exist in the world have given rise to a great concern for remediation (Wang, 2004). A few remediation techniques have been used in practice so far for removal of Hg from contaminated soil, e.g. washing soil with halogenated substances and heating soil to more than 600°C (Hempel and Thoeming, 1999). However, these techniques are relatively expensive and cause further disturbance to the already damaged environment.

All physiological and biochemical processes in plants may be negatively affected by Hg when plants are exposed to Hg-contaminated soil, water or air (Patra et al., 2004). Elemental Hg (Hg0) does not react with most biomolecules unless first oxidized to Hg^{2+}, and this may be catalytically driven by peroxidase or catalase (Du and Fang, 1983; Ogata and Aikoh, 1984). Mercury cations have a high affinity for sulphydryl (-SH). Because almost all proteins contain sulphydryl groups or disulphide bridges (-S-S-), Hg can disturb almost any function in which proteins are involved in plants (Clarkson, 1972). Organic Hg is 1–2 orders of magnitude more toxic to some eukaryotes and is more likely to biomagnify across trophic levels than ionic Hg (Hg^{2+}) (Bizily et al., 2003).

The biophysical behaviour of organic Hg is thought to be due to its hydrophobicity and efficient membrane permeability. Mercury compounds can also bind to RNA, several synthetic polyribosomes, and DNA. This result is consistent with previous studies on *Pisum sativum* L., *Mentha spicata* L., and *Picea abies* (L.) Karst (Beauford et al., 1977). The low translocation of Hg to the shoots is probably due to the high affinity of roots for Hg. The Hg trapped in the roots was mainly bound to root cell walls, about 80% of accumulated Hg was bound in the cell wall of the willow roots. Many studies have shown that plant roots accumulate Hg when exposed to Hg-contaminated soils (Coquery and Welbourn, 1994). Laboratory studies illustrated that plant roots absorbed Hg from solution and roots accumulated much greater amount of Hg than shoots (Godbold and Hütterman, 1988). Both field and laboratory studies have demonstrated that plants accumulate more Hg when it is introduced in organic form than in inorganic form (Godbold and Hütterman, 1988). Leaves can absorb gaseous Hg via stomata, which has been shown in previous laboratory studies (Du and Fang, 1982; Ericksen et al., 2006). Du and Fang (1982) reported that uptake of Hg0 by the leaf increased with increasing Hg vapour concentration, temperature, and illumination. Leaves can also absorb Hg after deposition of particulate Hg on the leaf surface and release gaseous Hg into the atmosphere (Hanson et al., 1995). Furthermore, Hanson et al. (1995) reported that at low external Hg concentrations in the air, the release of Hg from leaf to air was higher than the leaf Hg absorption from the air in the tree species *Picea abies* L., *Liriodendron tulipifera* L., *Quercus alba* L., and *Acer rubrum* L. Similar results were also found by Ericksen et al., (2006). This evidence suggests that foliage can manage both uptake and volatilization of gaseous Hg.

2.2.4. ARSENIC

Arsenic (As) is a naturally occurring element which is widely distributed in the environment (Gonzaga et al., 2006). Arsenic has recently drawn attention due to its chronic and epidemic toxic effects to humans through widespread contamination of water and food crops through natural release of the element from aquifer rocks in Bangladesh (Hopenhayn, 2006) and West Bengal, India (Chowdhury et al., 2000). Geogenic arsenic contamination in aquifer rocks has also been reported in Thailand (Visoottiviseth et al., 2002), Vietnam, Inner Mongolia, Greece, Hungary, USA, Ghana, Chile, Argentina and Mexico (Smedley and Kinniburgh, 2002).

Sources of As are both natural (soil and rock erosion, dissolution of minerals and ores, volcanic activity and biological activity) and anthropogenic (municipal wastes incineration, coal and oil fired power plant, smelting of non-ferrous metals, cement works, lead and copper alloy production, use of As-containing pesticides and herbicides) (Minganti et al., 2004).

Arsenic contaminated soils, sediments, and sludge are the major sources of arsenic contamination of the food chain, surface water, groundwater, and drinking water (Frankenberger and Arshad, 2002). Other potential sources of As contamination are the chemicals used extensively in agriculture as pesticides, insecticides, defoliants, wood preservatives, and soil sterilants (Nriagu and Azcue, 1994). Arsenic contamination is a severe environmental problem facing the world (Wei and Chen, 2006). Techniques currently available for the remediation of As contaminated soils are expensive and time-consuming, often hazardous to workers, and capable of producing secondary wastes (Lombi et al., 2000). Arsenic hyperaccumulators usually have the ability to uptake large concentration of arsenic, even in a low level arsenic soil, illustrating efficient bioaccumulation, which is an important factor in phytoremediation (Ma et al., 2001; Chen et al., 2005; Tu et al., 2002). Arsenic, accumulated into plants primarily through their root system, is not readily translocated to the shoots (Salt et al., 1994; Kumar et al., 1995; Rahman et al., 2007).

Recently, the fern *Pteris vittata* (Chinese brake fern) was identified as an arsenic hyperaccumulator (Ma et al., 2001; Wei et al., 2002). Another fern, *Pityrogramma calomelanos* (silver fern) has also been discovered as an arsenic hyperaccumulator in Thailand, and the fern showed great potential in phytoremediation of arsenic contaminated soils (Francesconi et al., 2002; Visoottiviseth et al., 2002). Chinese brake fern has great arsenic tolerance and accumulating ability, it grew healthily both in tailings with as high as 23 400 mg As kg-1 in the field and in soils spiked with 1500 mg As kg-1 in greenhouse conditions (Ma et al., 2001). Field test has also shown that it has great potential in phytoremediation of arsenic contamination in soils (Salido et al., 2003). Besides, greenhouse experiments have also shown that *Pteris cretica* (Cretan brake fern) was an arsenic hyperaccumulator (Meharg, 2002; Zhao et al., 2002; Wei and Chen, 2006).

2.2.5. LEAD

The toxic effects of lead have been known for centuries. Its many useful properties gave rise to a dramatic escalation of lead use around the time of the industrial revolution, when lead poisoning was common amongst workers in the smelting, painting, plumbing, printing and other industries. With the advent of motor vehicles early in the 20th century, and the use of lead in petrol, environmental lead contamination increased substantially. Lead continues to be widely used, especially in developing countries, for example in petrol, paints and pigments, ammunition, cabling, television sets, computers, protective gear, ceramics, cosmetics, and many other ways. Children are at particularly high risk of exposure to environmental lead, because of their elevated rates of development, ingestion and metabolism, and because of their developmental and behaviour patterns (Landrigan, 1999). Hand-to-mouth activity, as part of normal play and development, constitutes the main pathway of childhood exposure to lead rich dust and soil. Even at relatively low concentrations, lead has been shown to interfere with human biochemical pathways, causing wide-ranging health effects. At levels around 10 g/dl biochemical and neurobehavioural effects (such as decrements in intelligence scores, shortened concentration spans, reading and language problems) have been demonstrated. Other health effects associated with elevated lead levels include anaemia, hearing loss, and abnormal development of tissues and organs, such as the kidneys, heart and brain. At extremely high levels of exposure, ataxia, cerebral oedema, paralysis, coma and death may result (Needleman and Bellinger, 1991). Several epidemiological studies undertaken in cities over the past two decades indicate that large proportions (over 90%) of South African children have had, and in some areas continue to have, unacceptably high blood lead levels. Extensive work has been done on heavy metal uptake capacity of Indian Mustard (*Brassica juncea*) a high biomass crop plant (Salt et al., 1995a; Pickering et al., 2000). The efficiency of Indian mustard to take up lead was enhanced with treatment with chelates (Vassil et al., 1998; Wu et al., 1999). A few plant species have been reported to accumulate lead to high concentrations in the above-ground parts and these plants can be called as hyperaccumulators (Kumar et al., 1995; Brooks, 1998; Blaylock et al., 1997; Huang et al., 1997; Salt et al., 1997; Barlow et al., 2000; Jarvis and Leung, 2002).

2.2.6. COPPER

Copper (Cu) is one of the metals widely generated as waste by industrial activities. Copper is one of the most abundant trace metals and is a micronutrient of great importance in agricultural production and occurs as Cu^{+} and as Cu^{2+} (Luo and Christie, 1998). Copper in soil occurs almost exclusively in the divalent form, which is isomorphous with Zn^{2+}, Mg^{2+} and Fe^{2+}. Soil Cu may be derived from the natural weathering of residual or transported parent material and from a number of anthropogenic sources. Potentially toxic Cu levels in soils are generally associated with sewage sludge amendments and cupric fungicide treatment as copper salts (Rejeb et al., 1999). Copper mining and smelting, brass manufacture, electroplating and excessive use of Cu based agrochemicals form the other sources of Cu to soil and it may reach very high concentration in localized areas. The average abundance of copper in the earth's crust is recorded as 24 to 55 ppm (Deepa et al., 2006). Copper in particular has been associated with stomach and intestinal distress, as well as anemia in humans.

Deram et al. (1997) found that accumulation of Cu, Co and Ni in *Arrhenatherum elatius* increased significantly because acid-extractable Cu concentration (1 mol/L hydrochloric acid) increased from 200 to 7500 mg/kg, Co from 40 to 175 mg/kg and Ni from 8 to 1276 mg/kg in the supporting soils after addition of 4 g/kg EDTA. *Elsholtzia splendens* (*Elsholtzia haichowensis*) had been identified as a Cu tolerant and accumulating plant species in mining areas and high Cu level nutrient solution (Yang et al., 2002). *Elsholtzia splendens* grows abundantly over copper mining areas and was first recognized for its value in exploration of copper ores in the 1950s.

2.2.7. NICKEL

Nickel (Ni) is an essential element that can be toxic and possibly carcinogenic in high concentrations. Ni is ubiquitously distributed in nature. It is found in different concentrations in all soil types of diverse climatic regions (Srivastava et al., 2005). Naturally derived soils from serpentine rocks are rich in Ni, but due to various industrial and anthropogenic activities such as mining, refining of Ni ores, burning of fossil fuels and residual oil and sewage sludge other areas have also become prone to Ni contamination (Nriagu, 1988). The normal range of Ni in soil is 2 to 750 ppm, with a critical soil concentration at 100 ppm (Gardea-Torresdey et al., 2005). Exposure to Ni compounds causes irreversible damage to the central nervous system, cardiovascular system, lungs and gastrointestinal tract (Axtell et al., 2003). Nickel has been classified among the essential micronutrients and remains associated with some metallo-enzymes, but Ni is toxic at elevated concentrations in plants (Srivastava et al., 2005). In plants Ni is responsible for chlorosis, yellowing and necrosis of leaves, deformation of plant parts, stunted growth and generation of free radicals (Halliwell and Gutteridge, 1999).

One of the most persuasive ecological explanations for hyperaccumulation of Ni and other toxic metals appears to be the defensive role against herbivores or pathogens (Martens and Boyd, 2002). This function, which might be similar in other hyperaccumulators, can be improved if the metal is localized in the outer layers of leaves and roots. Like in other Ni accumulators, such as *Hybanthus floribundus*, *Senecio coronatus* and *Thlaspi montanum* variety *siskiyouense*, and *A. bertolonii*, Ni has been evidenced in leaf epidermal cells as a red-stained nickel-dimethylglyoxime complex (Martens and Boyd, 2002; Leon et al., 2005). Several *Alyssum* species are known to hyperaccumulate nickel. These species can potentially be used to remediate Ni-contaminated soils.

2.3. AMARANTH SPECIES AND PHYTOREMEDIATION

The Amaranthaceae family contains about 60 genera and more than 800 species of herbaceous plants and a few shrubs, trees, and vines, native to tropical America and Africa. Globe amaranth (*Gomphrena*) and cockscomb (*Celosia*) are cultivated as ornamentals. The large genus *Amaranthus* contains the ornamentals love-lies-bleeding (*A. caudatus*) and Joseph's-coat (*A. tricolor*), as well as many weedy plants known as pigweed, especially *A. retroflexus*.

Amaranthus (Marog) is a popular nutritious leafy vegetable crop, rich in proteins, vitamins and minerals and is consumed virtually in the whole continent of Africa, Asia and South America. Their quick growth and great biomass makes them some of the highest yielding leafy crops which would be beneficial as a primary food source, thus preventing starvation and malnutrition in the third world countries. Grubben (1976) mentioned that because of their high yield, ability to grow in hot weather conditions, high nutritive value and their pleasant taste and the fact that they grow all year; makes the Amaranth a popular vegetable.

However there are disadvantages to Amaranths namely; (i) It is regarded as an aggressive and invasive weed in cultivated farm and disturbed lands; (ii) it contains high levels of nitrogen and when eaten in excess by ruminants, the consumed herbs are converted to highly toxic nitrates by microorganisms in the rumen resulting in severe poisoning, bloating or even death; and (iii) Amaranth being a C4 plant is an excellent indicator of heavy metal pollution and problems may arise if contaminated plants are cooked and eaten by humans.

In some places amaranths are considered to be cosmopolitan weeds in that they are capable of growing anywhere. The common names for amaranths are African spinach, Indian spinach, amaranth, bush greens, Chinese spinach and green leaf to mention a few (Hutchings, 1996). There has been phytoremediation work done using the amaranth family with the more common species being *Amaranthus hybridus* (Jonnalagadda and Nenzou, 1997), which was used to determine the effect of coal mine contamination on the uptake and distribution of lead, cadmium, mercury, nickel, manganese and iron; *Amaranthus tricolor* and *Amaranthus retroflexus* (Bigaliev et al., 2003) were used for the uptake of cadmium, mercury, zinc and

copper; and *Amaranthus spinosus* (Prasad and de Oliveira Freitas, 2003) was used for the accumulation of cadmium, zinc and iron. The species chosen for this project was *Amaranthus dubius* due to the limited work done on this species and the availability of plant material (Figure 6, 7, 8). Plant characteristics are listed in Table 5.

Figure 6: Inflorescence of *A. dubius*

Figure 7: *A. dubius* portals - (A) Leaf, (B) Stem, (C) Inflorescence, (D) Root structure.

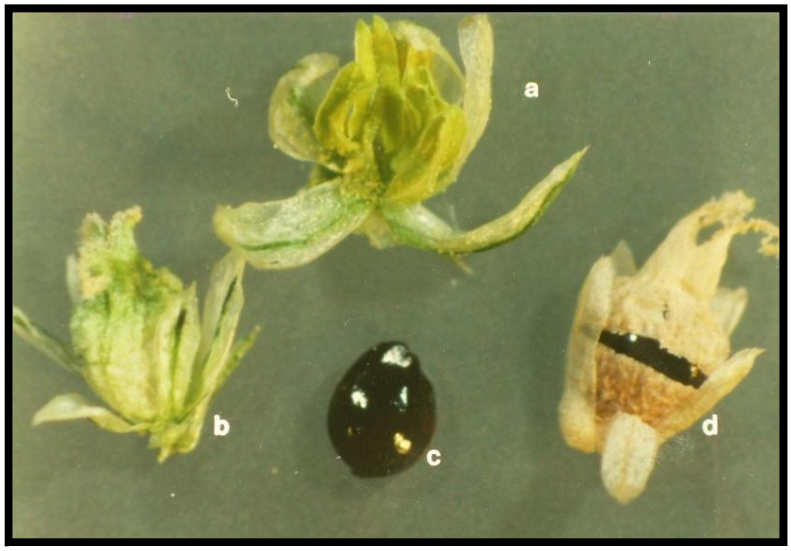

Figure 8: Reproductive structures of *A. dubius* (x 23.5) – (a) ♂Flower, (b) ♀Flower, (c) Seed, (d) Fruit

Table 5: Morphological description of *Amaranthus dubius*

Family	**Amaranthaceae**
Genus	*Amaranthus* **Species:** *dubius*
Common Names	**English**: Marog, Green pig weed **Zulu:** Umfino Imbuya **Indian**: Green herbs **Afrikaans:** Groot meerjarige
Habitat	Annual, spikes up to 1m tall with erect numerous branches, glabrous, or with some short pubescence near inflorescence. Widely distributed, common around human habitation in rural and peri rural areas. Young stem glossy green tinged with maroon streaks.
Root	Pinky red branched tap root with white adventitious roots
Stem	Erect branches, stem woody – old stem flakey and brownish green
Leaf	Leaves with petiole very variable in length 25-85mm long. Lamina ovate to elliptic, 15-80mm long and 35 to 55mm wide. Sub-glabrous, broadly cuneate at base, obtuse at apex with minute micron, green with dark blotches
Inflorescence	Green terminal panicle with leafless spiciform branches with numerous clusters of axillary flowers below.
Male Flower	At upper one third of inflorescence
Perianth	5 straw coloured perianth segments
Androecium	5 ovate to broadly branched segments 1.25mm to 2.5mm long
Female Flower	Has 5 oblong spathulate perianth segments 1.5 to 2.5mm long whitish green midrib, rounded or often shortly mucronate at apex
Gynoecium	About as long as or slightly shorter than perianth, rounded to pyriform, circumscissile
Fruit	Smooth to only slightly wrinkled. Seeds glossy ellipsoid, 0.8 to 1.0mm in diameter, chestnut black. Surface almost smooth, very faintly reticulate

3. METHODOLOGY

3.1. METAL BIOABSORBTION BY *Amaranthus dubius* FROM A REGULARLY CULTIVATED AREA (RCA), LANDFILL SITE (LS) AND WASTE WATER TREATMENT SITE (WWTS)

3.1.1. SITE DESCRIPTION AND SAMPLE PROCUREMENT

Duplicate samples of soil and *A. dubius* plants (500g dry weight) were collected from the 3 sites: (i) a regular cultivated area (RCA); (ii) a landfill site (LS); and (iii) a waste water treatment site (WWTS) in June 2005. Each replicate consisted of subsamples collected from different points in their respective sampling area. The species (*A. dubius*) studied was identified using taxonomic keys and a specimen was deposited in the Ward Herbarium, University of Kwa-Zulu Natal, Westville Campus.

A site in the Merebank area that is regularly cultivated for various crops was chosen as this site is used for subsistence farming by members of the local community, with the soil regularly disturbed as a result of crop rotation. An aerial photo and a zoomed sampling area are shown in Figure 9 - (i) and (ii). This site is surrounded by formal housing settlements, paper and pulp industry (Mondi), petroleum industry (SAPREF) and an international airport (Durban International).

The Springfield Landfill Site [Figure 10 (i)] is a highly disturbed area, as a result of the constant turning of soil due to solid waste dumping. The samples in this study were collected from an area that showed minimum disturbance with the landfill site being surrounded by both formal housing and informal settlements [Figure 10 (ii)].

The Northern Waste Water Works [Figure 11 (ii)] is a catchment area which is mainly domestic in character. However, leachates from the Bisasar Road landfill site is also pumped into the treatment works. Specimens collected were growing in matured, dry sludge, the sampling area is indicated in Figure 11 (i). The site is surrounded by both formal housing and informal settlements.

Figure 9: i – Regular cultivated area (A) surrounded by a formal housing settlement (B), paper and pulp industry (C), petroleum industry (D) and Durban International Airport (E)
ii – zoomed view of regular cultivated area (A) – sampling area.

Figure 10: i – Springfield Landfill Site (A) surrounded by formal housing (B) and informal housing (C) settlements
ii – a zoomed in view of the sampling area.

Figure 11: i – Northern Waste Water Works (A) surrounded by formal housing (B) and informal housing (C) settlements
ii – a zoomed in view of the sampling area.

3.1.2. EXTRACTION OF HEAVY METALS

3.1.2.1. METAL EXTRACTION USING THE MICROWAVE DIGESTION PROCEDURE

The heavy metals from soils and plants were extracted using a microwave digestion procedure described by (Zunk and Planck, 1990), as this technique was found superior to the standard wet digestion process described by Wang et al. (1999) and Somer and Nakisci Unlu, (2006) has been used when the concentration of metals in the sample is low. A mls 1200 mega – high performance microwave digestion unit [Milestone Microwave Laboratory Systems – supplier (Italy)] was used. Samples of ±0.5 g were weighed out in the digestion vessel and 5 ml of 69.5% HNO_3 and 2 ml of 30% H_2O_2 added (Roy et al., 2005). The vessels were placed into a sample holder and then into the microwave digestor. The microwave digestor's parameters were set as 5 steps with the samples in the vessels being exposed to different power ranges for different periods of time with the power ranging from 0 - 600 W and time from 1 - 5 min (Table 6). The samples were then removed after 1 h, allowed to cool for ± 45 min, decanted into a 25 ml volumetric flask and made up to the mark with distilled water(Aksoy and Sahin, 1998; Minganti et al., 2004). This was filtered with Whatmann No 1 filter paper to remove any remaining particulate and stored in a refrigerator for metal analysis using Inductively Coupled Plasma Mass Spectroscopy (ICPMS).

Table 6: Digestion parameters

STEP	1	2	3	4	5
POWER	250 W	0 W	250 W	400 W	600 W
TIME	1 min	2 min	5 min	5 min	5 min

3.1.2.2. METAL ANALYSIS BY INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY (ICPMS)

The metals were analysed using Inductively Coupled Plasma Mass Spectroscopy according to the procedure outlined by Zayed et al. (1998) and Wang et al. (1999). In this system samples are decomposed to neutral elements in high temperature argon plasma and analyzed based on their mass to charge ratios. There are four steps: (i) sample introduction; (ii) aerosol generation; (iii) ionization by an argon plasma source; and (iv) mass discrimination.

Results obtained from ICPMS Analysis were obtained mg/L and were converted to parts per million (ppm) using the following equation:

$$ppm = \frac{(mg/L \times V)}{m}$$

where V is the final volume that the sample was made up to after microwave digestion (25 ml) and m is the dry mass of sample that was digested with amount of heavy metals from the different portals.

3.1.3. DETERMINATION OF METAL CONCENTRATION IN SOILS FROM REGULARLY CULTIVATED AREA (RCA), LANDFILL SITE (LS) AND WASTE WATER TREATMENT SITE (WWTS)

The top 10 cm of soil (25 g) between plants from each of the three sites; RCA, LS and WWTS was collected, air dried for two days, sieved and stored in Schott bottles in a dark cupboard until analysis. Soil samples (1 g) were digested in 5 ml of 69.5% HNO_3 and 2 ml 30% H_2O_2 using microwave digestion. Once samples were cooled to room temperature the solutions were filtered and analysed for Cr, Hg, As, Pb, Cu and Ni (Duruibe et al., 2007). The levels of Cr, Hg, As, Pb, Cu and Ni were measured, in soil samples and the metal concentrations at each of the sites were compared. The concentration of metals found at each site was correlated using the students T-test with the recommended daily allowance (RDA) (Havel et al., 1989). As there are no South African guidelines, the maximum levels obtained were compared to acceptable standards that are stipulated by the Guidelines for Land Application and Storage in Nova Scotia (Environmental Monitoring and Compliance, 2004).

3.1.4. DETERMINATION OF METAL CONCENTRATIONS IN *A. dubius* PLANTS FROM REGULARLY CULTIVATED AREA (RCA), LANDFILL SITE (LS) AND WASTE WATER TREATMENT SITE (WWTS)

Plants were harvested without damaging the roots and rinsed in distilled water to remove dust, soil and mineral particles. These were then separated into roots, stems and leaves and dried at 60°C for 48 h in a convection oven.

Dried samples were milled to a fine powder using a Waring commercial laboratory blender, placed in aluminium covered 500 ml Schott bottles and stored. Chromium, Hg, As, Pb, Cu and Ni levels were measured in *A. dubius* plants harvested from RCA, LS and WWTS and the metal concentrations at each of the sites were compared. The levels from the edible portions were compared to RDA standards. Each of the metals were also measured in different portals of the plant i.e. leaves, stems and roots as well as total metal accumulated.

3.1.5. DETERMINATION OF THE MOVEMENT OF METALS FROM SOIL TO PLANT

The Bioconcentration Factor (BCF) of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005a) and is calculated using the formula:

$$\text{BCF} = \frac{\text{Metal concentration in plant tissue (whole plant/portal)}}{\text{Initial concentration of metal in substrate (soil)}}$$

The higher the BCF value the more suitable is the plant for phytoextraction (Blaylock et al., 1997). BCF Values > 2 were regarded as high values.

3.1.6. DETERMINATION OF THE MOVEMENT OF METALS FROM ROOTS TO PLANTS

To evaluate the potential of plants for phytoextraction the translocation factor (TF) was used. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol et al., 2004). It is represented by the ratio:

$$\text{TF} = \frac{\text{Metal concentration (stems + leaves)}}{\text{Metal concentration (roots)}}$$

Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1 with values > 1 indicating that the metals are stored in the stems and leaves.

3.2. METAL BIOSORPTION BY *Amaranthus dubius* GROWN *in-vitro*

3.2.1. GROWTH CONDITIONS

Wild source seeds of *A. dubius* were obtained from Reservoir Hills in the Durban area. Plant heads were collected and air dried in the laboratory for ± 2 days. The seeds were then removed from the flower head husks and stored in glass bottles in a refrigerator until germination. Seeds were allowed to germinate for one week (Figure 12) in potting soil obtained from the Randles Road Municipal Nursery. The soil collected had a loam texture together with the following parameters pH - 7.69; moisture content - 14%; potassium - 140 ppm; phosphorous - 77 ppm; calcium - 1850 ppm; magnesium - 520 ppm; sodium - 77 ppm. Twelve uniform seedlings all having reached a 5 leaf stage in growth (Figure 13) were planted at uniform distances apart in pots (volume = 1800 cm^3) and placed in a tunnel house for a further three weeks. Pot experiments were carried out in a tunnel house according to Marchiol (2004) so as to maintain an ambient temperature and prevent any rainfall from affecting the plants.

Figure 12: One week old germinated *A. dubius* seedlings.

Figure 13: Three week old seedlings of *A. dubius*.

Figure 14: Six week old *A. dubius* seedlings.

3.2.2. *In vitro* METAL EXPOSURE

Heavy metal salts for each metal were made up to their respective concentrations in distilled water. Concentrations of 25, 75 and 100 ppm of spiked soil was prepared for each metal respectively. Pot culture experiments were conducted using soil treated (spiked) with the following salts based on earlier research (Gardea-Torresdey et al., 2004; Odjegba and Fasidi, 2004; Ahalya et al., 2005), potassium dichromate [$K_2Cr_2O_7$], mercuric chloride [$HgCl_2$], arsenic trioxide [As_2O_3], lead nitrate [$Pb(NO_3)_2$], cupric nitrate[$Cu(NO_3)_2.3H_2O$] and nickel sulphate [$NiSO_4.6H_2O$] solutions corresponding to 25, 75 and 100 ppm of Cr, Hg, As, Pb, Cu and Ni respectively. Six week old seedlings (Figure 14) were transplanted into the pots, with 12 plants of uniform length (± 30 cm) placed a uniform distance apart in each pot. A separate pot with untreated soil was used to serve as a control. These were placed in trays so as to prevent any leachate from being lost (Giordani et al., 2005; Kos et al., 2003). The plants were watered daily with 500 ml of water per pot and all collected leachate returned to the respective experimental pot. Specimens were photographed at key stages of the experiment.

3.2.3. SAMPLING, EXTRACTION AND METAL ANALYSIS

Three plants from each treatment were harvested after 4 days without damaging the roots and rinsed in distilled water to remove dust and soil mineral particles. Plant samples were then separated into roots, stems and leaves and dried at 60°C in a convection oven for 48 h. The root lengths of the plants exposed to Cr, Hg, As, Pb, Cu and Ni at concentrations of 25, 75 and 100 ppm were recorded. The metals were extracted and analysed as in Section 3.1.2.

3.2.4. DETERMINATION OF THE EFFECT OF METAL ON GROWTH

3.2.4.1. EFFECT ON GROWTH

Morphological effects of the metals were studied by exposing plants to 25, 75 and 100 ppm of each metal and observing the effects on the growth and development of *A. dubius* plants on day four by measuring root length.

3.2.4.2. ULTRA-STRUCTURAL EFFECTS OF METALS ON *A. dubius*

Fresh root samples were viewed without any staining of the plant material for ultra-structural effects of Cr, Hg, As, Pb, Cu and Ni on *A. dubius*. Transverse portions of treated roots were fixed on aluminium stubs and viewed with a Phillips XL30 Environmental Scanning Electron Microscope (ESEM) was used.

3.2.4.3. EFFECT OF METAL CONCENTRATION ON *A. dubius*

The effect of the concentrations of the metals was analysed by measuring the amount of metals in roots, stems and leaves of *A. dubius* plants harvested after four days of exposure to 25, 75 and 100 ppm of each metal. Correlation analysis was used to describe the degree of strength by which one variable is linearly related to another (root length of the plant related to the heavy metal concentration). This measure is based on a scale between -1 and +1. If an inverse relationship exists, then Pearson's coefficient of correlation (r) will fall between 0 and -1. If there is a direct relationship, then r will fall between 0 and +1. If there is no relationship between the two variables, then r=0. A simple linear regression was also calculated with each of the doses and the part of the plant (root) as the independent variable.

The differences were considered to be significant at $p \leq 0.05$. If the regression coefficient was positive this meant that increasing doses of the metal lead to increased root growth. This is what is called a trend test, so there is a trend that a higher dose results in more root growth. This is often taken as proof of a relationship, because higher doses lead to more growth. It is therefore possible that this linear relationship only holds up to a certain dose and higher doses becomes toxic (Ghosh and Singh, 2005a).

3.2.4.4. EFFECT OF EXPOSURE TIME TO *A. dubius*

The effect of the exposure time of the metals was analysed by measuring the root length of *A. dubius* exposed to 25, 75 and 100 ppm over a sixteen day period. The dose for the control plants was taken as 0 and a simple linear regression between the change in root length from day 0 (taken as 8 cm) to day 16 was done. If the regression coefficient is positive this means that increasing exposure time of the metal led to increased root growth.

3.2.4.5. DETERMINATION OF PORTAL OF STORAGE

The amount of metals in the plant portals in 0.5 g of the roots, stems and leaves was calculated, to determine portal of storage for each metal.

3.2.4.6. DETERMINATION OF MOVEMENT OF METALS FROM SOIL TO PLANT

The BCF factor (described in Section 3.1.5.) was used to determine the movement of the metals from soil to aerial parts of the plant.

3.2.4.7. DETERMINATION OF MOVEMENT OF METALS FROM ROOTS TO AERIAL PARTS OF PLANT

The TF factor (described in Section 3.1.6.) was used to determine the movement of the metals from root to aerial parts of the plant.

3.3. MICROPROPOGATION OF *Amaranthus dubius*

3.3.1. PLANT MATERIAL

Aerial portions (10 g of stems and leaves) of *A. dubius* were collected from a cultivated site near the Umgeni River, in the suburb of Reservoir Hills, Durban. The plant material was thoroughly washed in tap water to remove impurities. The plants were subsequently washed three times in sterile distilled water.

3.3.2. CALLUS INDUCTION

3.3.2.1. SURFACE STERILIZATION OF EXPLANTS

Various concentrations of mercury chloride and sodium hypochlorite were investigated for optimum sterilization for callus initiation from the leaves of *A. dubius*. The leaves were disinfected either for 5 minutes in 0.1% mercury chloride and for 20 minutes in 40% sodium hypochlorite or for 5 minutes in 0.1% mercury chloride and for 15 minutes in 30% sodium hypochlorite or for 20 minutes in 30% sodium hypochlorite only. It was then thoroughly rinsed three times with distilled water under a laminar flow hood.

3.3.2.2. PREPARATION OF MEDIA FOR CALLUS INDUCTION

The MS medium (Murashige and Skoog, 1962) purchased from Sigma was used in this study. For callus initiation the basal MS media was manipulated with different auxin and cytokinin viz. benzylaminopurine (BAP) and 2,4 dicholoro-phenoxy acetic acid (2,4-D) in different concentrations. Combinations from 0.5 mg/L to 1 mg/L were used. The pH of the media was always verified to be 5.8. Cefotaxamine 25 mg/L and Fungizone 25 mg/L were the antibiotics used to prevent microbial contamination.

After sterilization the leaves were cut into square pieces (1 cm^3) using sterile scalpel blades and five squares placed on a Petri plate. The Petri plate contained MS medium (42.2 g/L) as well as different combinations of different concentrations of plant growth regulators which were 1 mg/L BAP and 1 mg/L 2,4 D.

All inoculations were performed under a laminar flow to maintain aseptic conditions. Instruments such as scalpels, blades and tweezers were sterilized in 70% ethanol and flamed. All cultures were maintained at 25°C in a 16 hour photoperiod in a plant growth chamber (Hotpack Illuminated Chamber). Callus tissue was then transferred to petri dishes containing basal salt media with different concentrations of Cr (VI) added as the salt potassium dichromate ($K_2Cr_2O_7$) for 4 days together with a control plate containing no $K_2Cr_2O_7$.

3.4. TRANSMISSION ELECTRON MICROSCOPY STUDIES OF CHROMIUM ABSORBTION BY *Amaranthus dubius*

3.4.1. SPECIMEN PROCESSING FOR ELECTRON MICROSCOPY

Callus cultures grown on MS media containing 10 ppb and 25 ppb of Cr (VI) for 4 days were immersed into Karnovsky's fixative [4% paraformaldehyde, 5% glutaraldehyde (Karnovsky, 1965)] for 60 min at 4°C. The specimen was then diced into 1 mm^3 cubes and re-immersed into fresh Karnovsky's fixative for a further 30 min. Thereafter the tissue was transferred in disposable baskets to an automated electron microscopy tissue processor (El-Reichert Lynx) in 0.2M sodium cacodylate, pH 7.2 maintained at 4°C. The machine was programmed to incubate the tissue in reagents as outlined below in Table 7. At the termination of the automated cycle, the tissue was removed, polymerised/embedded in Araldite [Electron Microscopy Services, USA; (Glauert and Glauert, 1958)] in polythene capsules (size 00, BEEM) for 48 h at 60°C.

3.4.2. ULTRAMICROTOMY

Semi-thin (1 μm) sections were cut with a Reichert Ultracut ultramicrotome using glass knives. Sections were collected onto glass slides, heat-fixed, stained with 1% toluidine blue and examined with a Nikon Optiphot microscope. Fields of interest were selected and located on the block face, and the block trimmed to produce a "mesa" with a trapezoidal shape. Ultrathin sections (50-60 nm) were cut, collected onto uncoated copper 200 mesh grids and double stained with uranyl acetate and Reynolds' lead citrate for 2 to 3 min respectively (Reynolds, 1963).

3.4.3. ELECTRONMICROGRAPHY

Sections were viewed on the Jeol 1011 Transmission Electron Microscopy and digitized.

Table 7: Standard processing protocol schedule for electron microscopy

STEP	PROCESS	SOLUTION	TEMPERATURE	TIME
1	Fixation	Karnovsky's fixative	4°C	1 h
2	Fixation	Karnovsky's fixative	4°C	30 min
3	Wash	0.2 M sodium cacodylate buffer (pH 7.2)	24°C	10 min
4	Wash	0.2 M sodium cacodylate buffer (pH 7.2)	24°C	10 min
5	Fixation/Contrast	1% osmium tetroxide (0.2M NaCacodylate buffer)	4°C	1 h
6	Wash	0.2 M sodium cacodylate buffer (pH 7.2)	24°C	10 min
7	Wash	0.2 M sodium cacodylate buffer (pH 7.2)	24°C	10 min
8	Dehydration	70% ethanol	24°C	30 min
9	Dehydration	90% ethanol	24°C	30 min
10	Dehydration	100% ethanol	24°C	30 min
11	Dehydration	100% ethanol	24°C	30 min
12	Intermediate	propylene oxide (1,2-epoxypropane)	24°C	30 min
13	Infiltration	propylene oxide : Spurr’s resin (1:1)	24°C	30 min
14	Infiltration	Spurr’s resin	60°C	1 h
15	Infiltration	Spurr’s resin	60°C	1 h
16	Polymerisation	Spurr’s resin	60°C	48 h

4. RESULTS

4.1. METAL CONCENTRATIONS IN SOIL SAMPLES FROM A REGULARLY CULTIVATED AREA (RCA), LANDFILL SITE (LS) AND WASTE WATER TREATMENT SITE (WWTS)

Concentrations of Cr, Hg, As, Pb, Cu and Ni in the soil from the RCA, LS and WWTS are shown in Figure 15. There is a difference in the concentration of Cr, Hg, As, Pb, Cu and Ni at the different sites. Chromium concentration ranged from 48 ppm - 1130 ppm; Hg concentration from 0 ppm - 2 ppm; As from concentration 4 ppm - 9 ppm; Pb concentration from 28 ppm - 52 ppm; Cu concentration from 18 ppm - 105 ppm and Ni concentration from 15 ppm - 65 ppm.

The heavy metal concentrations from the RCA, LS and WWTS were compared in Figure 16. The RCA site was characterized by the highest levels of Cr (1130 ppm) and Ni (30 ppm) (Figure 16 A). The LS was characterized by high levels of Cr (48 ppm) and Pb (48 ppm) (Figure 16 B). The WWTS was characterized by high levels of Cr (236 ppm), As (9 ppm), Pb (52 ppm), Cu (105 ppm) and Ni (65 ppm) (Figure 16 C).

Chromium levels were the highest, and were found in the RCA (Figure 16 A). Hg levels were the highest in the LS (Figure 16 B). Arsenic was highest in the WWTS (Figure 15 C). Pb was high in both LS and WWTS (Figure 16 B and C). Cu and Ni were highest in WWTS (Figure 16 C).

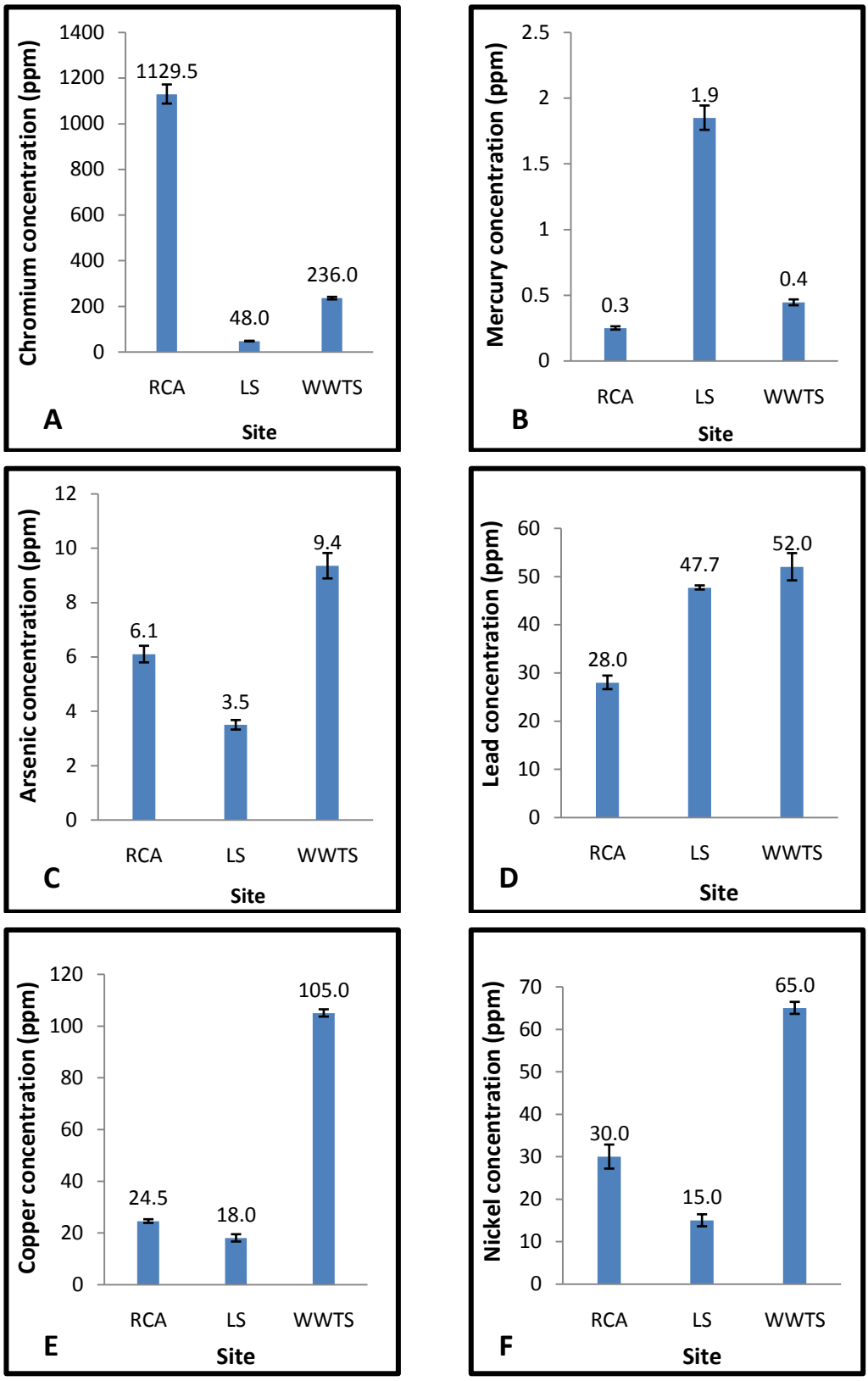

Figure 15: Concentration of metals in soil samples from a Regular Cultivated Area-(RCA); Landfill Site-(LS) and Waste Water Treatment Site-(WWTS). [Bars denote mean ± standard deviation (*n*=3)]

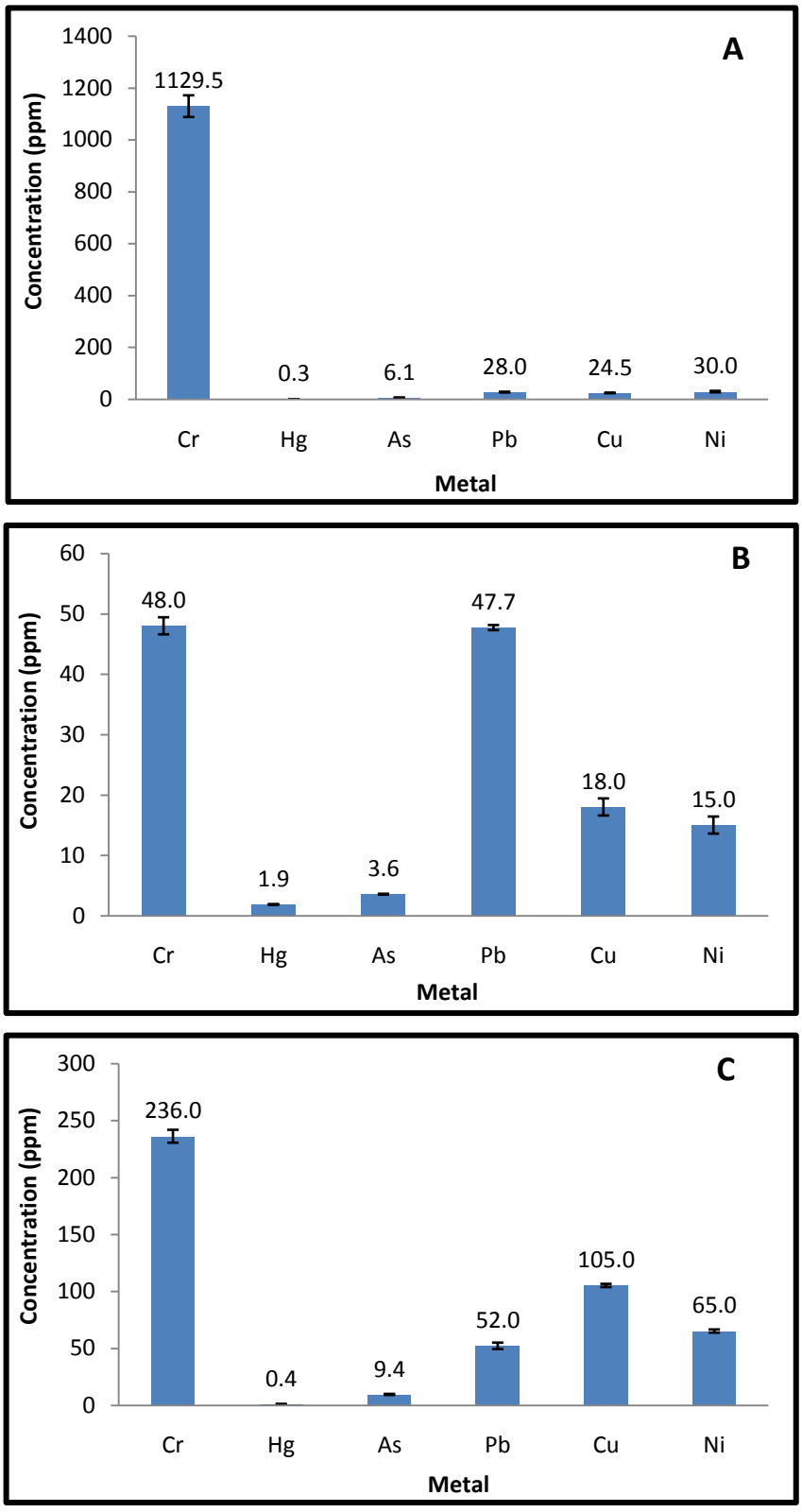

Figure 16: Comparison of metal content in soil from the three respective sites [Regular Cultivated Area-RCA (A), Landfill Site-LS (B) and Waste Water Treatment Site-WWTS (C)]. [Bars denote mean ± standard deviation (*n*=3)]

Comparison of the max levels of the various heavy metals in the soils from RCA, LS and WWTS to acceptable standards is shown in Table 8. Chromium, Hg, Cu and Ni were above the stipulated standards. Lead and As were the only metals within an acceptable range.

Table 8: Guidelines for Land Application and Storage in Nova Scotia maximum contamination levels for soil compared to maximum concentration levels found at the Regularly Cultivated Area (RCA), Landfill Site (LS) And Waste Water Treatment Site (WWTS)

Heavy Metal	Maximum concentration (ppm)	Maximum acceptable concentration for soil*
Cr	**1130**	64
Hg	**2**	1
As	9	12
Pb	52	60
Cu	**105**	63
Ni	**65**	32

***As per Guidelines for Land Application and Storage in Nova Scotia**

4.2. METAL CONCENTRATIONS IN PLANT SAMPLES FROM A REGULARLY CULTIVATED AREA (RCA), LANDFILL SITE (LS) AND WASTE WATER TREATMENT SITE (WWTS)

Amaranthus dubius from the RCA showed Cr to be the predominant metal species present with 59 ppm -roots, 16 ppm - stems and 31 ppm - leaves (Figure 17 A). The plants from this site were also characterized by high levels of Pb and Cu. In the plants from the LS the predominant metal accumulated was Cu (39 ppm - roots, 8 ppm - stems and 15 ppm - leaves) (Figure 17 B). The accumulation of metals in the plants from the WWTS varied with Cr ranging from 45 ppm - roots, 4 ppm - stems and 5 ppm - leaves, Hg ranging from 1 ppm - roots, 3 ppm - stems and 4 ppm - leaves, Cu ranging from 40 ppm - roots, 9 ppm - stems and 16 ppm - leaves and Ni ranging from 20 ppm - roots, 3 ppm - stems and 5 ppm - leaves (Figure 17 C).

A comparison of the maximum levels in the leaves (edible portion of *A. dubius*) compared to RDA standards showed that all the metals, Cr, Hg, As, Pb, Cu and Ni in the leaves were above the stipulated standards (Havel et al., 1989).

The concentration of heavy metals in the different portals of the plants harvested from the three sites show a high level of metal accumulation in the roots except for Hg which was higher in the leaves of plants from the RCA and WWTS, and Pb which was higher in the leaves from the RCA and stems in the LS (Figure 18).

When considering whole plants (roots, stems and leaves) the highest amount of Cr (107 ppm) was found in the RCA (Figure 18 A), the highest amount of Hg (8 ppm) was found in the LS (Figure 18 B), the highest amount of As (22 ppm) was found in the LS (Figure 18 C) and the highest level of Pb (55 ppm) was also present in the LS (Figure 18 D). The highest amount of Cu (70 ppm) and Ni (27 ppm) were found in plants harvested from the LS and WWTS respectively (Figure 18 E and F).

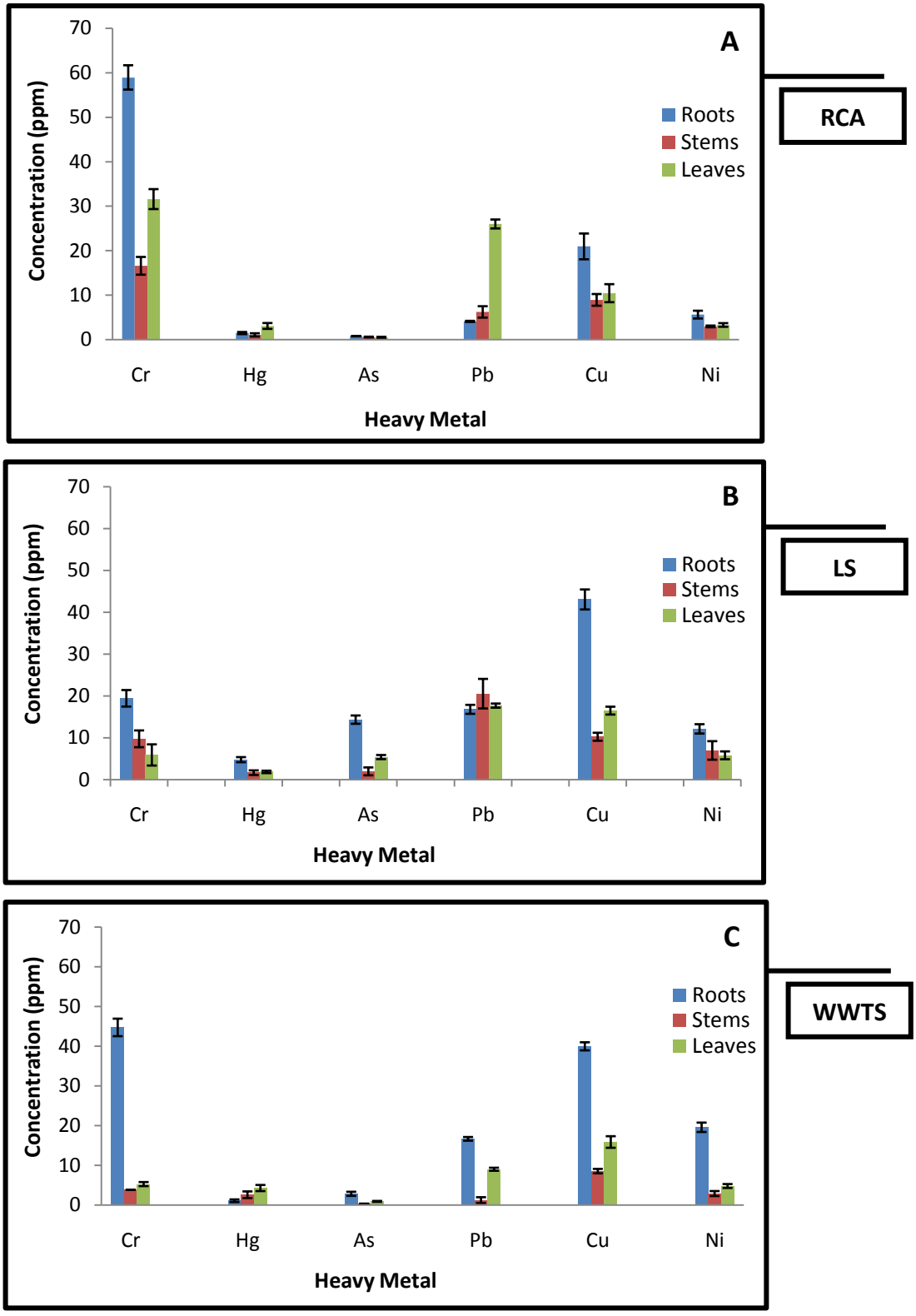

Figure 17: Heavy metal content (Cr, Hg, As, Pb, Cu and Ni) of *A. dubius* at each of the three respective sites under investigation: A-Regular Cultivated Area; B-Landfill Site; C-Waste Water Treatment Site. [Bars denote mean ± standard deviation (*n*=3)]

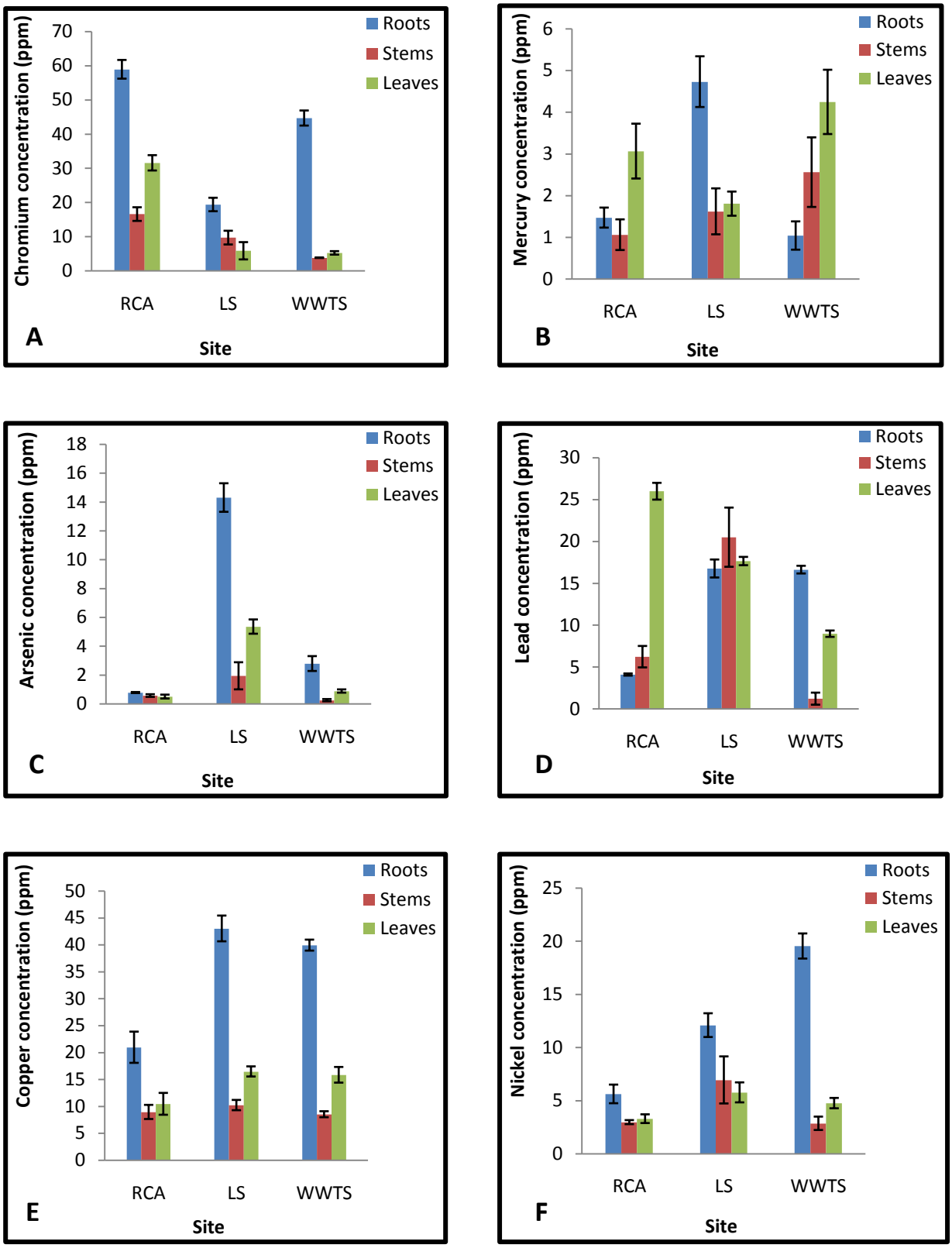

Figure 18: Individual metal distribution in the different portals of *A. dubius* from the three respective sites (Regular Cultivated Area-RCA, Landfill Site-LS and Waste Water Treatment Site-WWTS): A-Cr; B-Hg; C-As; D-Pb; E-Cu; F-Ni. [Bars denote mean ± standard deviation (*n*=3)]

4.3. COMPARISON OF METAL CONCENTRATION IN THE SOIL AND PLANTS FROM THREE SITES

A comparison of the predominant metals in soil and plants is shown in Table 9. In the RCA there is a very high concentration of Cr in both the soil and plants harvested. In the LS high levels of Pb were found in soil and plants. In the WWTS high levels of trace metals Ni and Cu were found. Mercury and Arsenic levels showed no consistency with regards to their presence in soil and the plant.

Table 9: Relative metal accumulation in soil and plant samples

	Cr		Hg		As		Pb		Cu		Ni	
	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant
RCA	**1129.5**	**107.1**	0.25	5.6	6.1	1.8	28.0	36.3	24.5	40.3	30.0	11.9
LS	**48.0**	34.9	1.9	**8.2**	3.5	**21.6**	**47.7**	**54.9**	18	69.7	15.0	24.8
WWTS	**236.0**	53.7	0.4	7.8	9.4	3.9	52.0	26.8	**105.0**	**64.3**	**65.0**	**27.1**

X = high concentrations of metal (ppm)

4.4. BIOCONCENTRATION FACTOR

The Bioconcentration Factor (BCF) (Table 10) represents the ability of *A. dubius* to extract heavy metals from the soil. BCF values of zero indicate limited movement from the soil to the plant. The BCF index at the RCA was highest for Hg followed by Cu, Pb, Ni, As and Cr. The BCF index of the metals at the LS was the highest for As followed by Hg, Cu, Ni, Pb and Cr. At the WWTS the highest BCF index was for Hg followed by Cu, Pb, As, Ni and Cr. The highest BCF value was found for Hg followed by As.

Table 10: Bioconcentration Factor (BCF) movement from soil to plant of each metal at the Regularly Cultivated Area (RCA), Landfill Site (LS) And Waste Water Treatment Site (WWTS)

	Bioconcentration Factor (BCF)		
	Regular Cultivated Area (RCA) [Merebank]	**Landfill Site (LS) [Springfield Landfill Site]**	**Waste Water Treatment Site (WWTS) [Northern Waste Water Works]**
Cr	0	1	0
Hg	**22***	**4***	**18***
As	0	**6***	0
Pb	1	1	1
Cu	2	**4***	1
Ni	0	2	0

*** - high BCF value (values > 2)**

4.5. TRANSLOCATION FACTOR

Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1. Values greater than one indicate translocation to the aerial parts of the plant. These are represented in Table 11. TF values > 1 were found for Hg, As, Pb and Ni at the RCA. TF values > 1 were also found for Pb and Ni from LS, and TF values > 1 for Hg from WWTS. All other TF values were < 1.

Table 11: Translocation Factor (TF) of each metal under investigation at the three respective sites to determine the indirect movement of each metal from the roots to the aerial parts of the plant

	Translocation Factor (*TF*)		
	Regular Cultivated Area (Merebank)	**Landfill Site (Springfield Landfill Site)**	**Waste Water Treatment Site (Northern Waste Water Works)**
Cr	0.8	0.8	0.2
Hg	**2.8***	0.7	**6.5***
As	**1.4***	0.5	0.4
Pb	**7.9***	**2.3***	0.6
Cu	0.9	0.6	0.6
Ni	**1.1***	**1.1***	0.4

***Values > 1 are regarded as high**

4.6. EFFECT OF METALS ON *Amaranthus dubius*

4.6.1. CHROMIUM (Cr)

Effect on growth and development

The effect of Cr on the growth and development of *A. dubius* plants after four days showed that in the soil treated with 25 ppm Cr, the plant samples showed a higher rate of growth in comparison to 75 and 100 ppm. Plants exposed to 75 ppm showed signs of chlorosis, which is the yellow, pale yellow or white in leaves as a result of the inability of the leaves to produce chlorophyll and those exposed to 100 ppm also showed signs of wilting (Figure 19).

Figure 19: Plants of *A. dubius* 4 days after being spiked with Cr.

Ultra structural effect on *A. dubius*

Scanning electron microscopy studies of the root section of the Control plant (Figure 20) showed signs of dehydration mainly from the parenchymatous cells. This resulted in the vascular bundles appearing as distinct units. The central stele (CS) was obscurely pentarch and the anomalous growth in thickness resulted in collateral vascular bundles (CV) which arise from rings of secondary meristematic tissue in the pericycle. Plants exposed to Cr showed a narrow cortical area and damage throughout the root section, with large apertures (AP) in the central stele region followed by some cellular damage (CD) towards the central region of the bundles with anomalous growth.

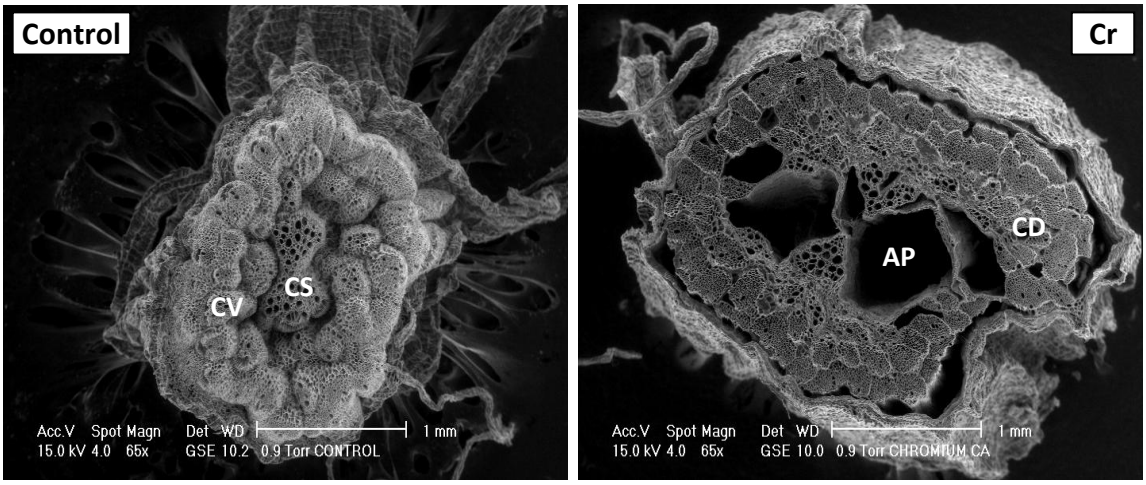

Figure 20: SEM of fresh root sections of *A. dubius* exposed to Cr for 24 h. [central stele (CS); collateral vascular bundles (CV); apertures (AP); cellular damage (CD)]

Effect of concentration and time

The effect of increasing Cr concentrations at day 16 shows that at 25 and 75 ppm the plants can tolerate Cr, but at 100 ppm they absorb less Cr than the unexposed plants with a decrease in the root length (Figure 21).

Portal of storage

The concentration of Cr accumulated ranged from 17 ppm to 311 ppm with the root system accumulating the highest concentration of Cr. The levels of Cr absorbed in the different plant portals was significantly different (p value = 0.0046), meaning that the Cr absorbed by the different portals of the plant were different. Most of the Cr was stored in the root system (least square analysis: roots = 166.0; stems = 59.1; leaves = 51.1), where at an increasing Cr concentration (25, 75 and 100 ppm) plants absorbed more Cr (Figure 22).

Movement of Cr in the plant

The Bioconcentration Factor (BCF) indicates the ability of the plant to absorb chromium from soil containing 25, 75 and 100 ppm. In this study Cr concentrations > 2 were regarded as high. Values > 2 were found for all three concentrations tested. The movement of Cr from roots to aerial parts as indicated by the Translocation Factor (TF) shows that Cr is translocated at concentrations of 25 ppm (TF > 1). At concentrations of 75 and 100 ppm the TF value is less than one (Table 12).

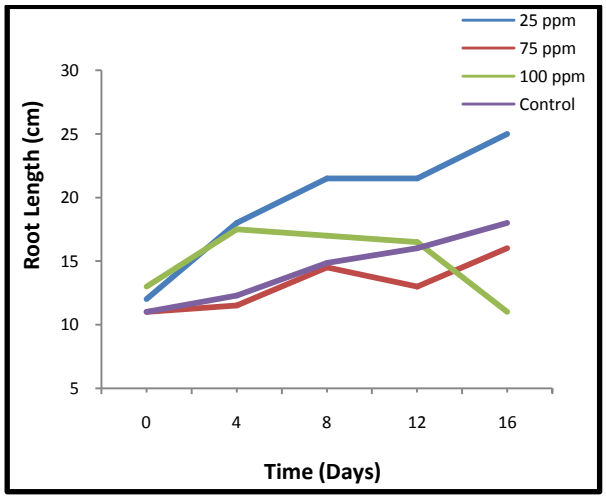

Figure 21: Effect of Cr concentrations at 25 ppm, 75 ppm and 100 ppm on the root growth of *A. dubius* over a 16 day period.

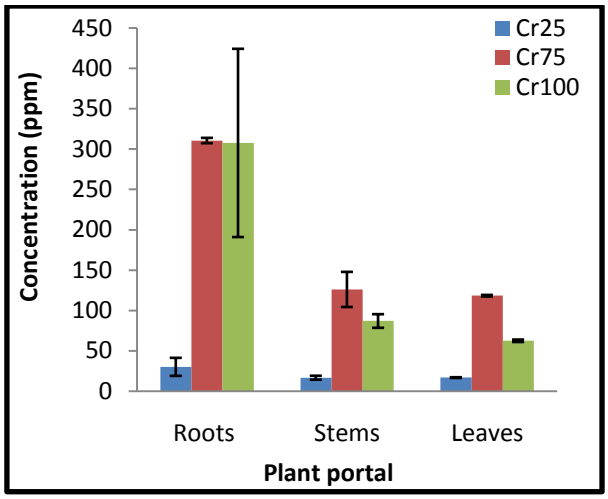

Figure 22: Cr levels in the roots, stems and leaves of *A. dubius* at 25 ppm, 75 ppm and 100 ppm in soil after 4 d. [Bars denote mean ± Standard deviation (*n*=3)]

Table 12: Concentration of Cr accumulated in the roots, stems and leaves of *A. dubius* grown for four days and the Translocation Factor (TF) and Bioconcentration Factor (BCF) (n=3)

Metal	Concentration in soil (ppm)	Concentration in roots (ppm)	Concentration in stems (ppm)	Concentration in leaves (ppm)	TF	BCF
Cr	25	30 ± 11	17 ± 2	17 ± 1	1.1	2.6
	75	311 ± 3	126 ± 22	118 ± 1	0.8	7.4
	100	308 ± 117	87 ± 9	63 ± 2	0.5	4.6

Ability of callus cultures to take up Cr

Further experiments were carried out to see if callus cultures from *A. dubius* were capable of accumulating Cr. As the concentration of chromium in callus was low it could not be determined using Atomic Absorption Spectroscopy (AA), the bio-accumulation was visualized by Transmission Electron Microscopy (TEM) as per Figure 43.

4.6.2. MERCURY (Hg)

Effect on growth and development

Amaranthus dubius exposed to Hg show no visible phenotypical changes, with samples exposed to different concentrations of Hg (25, 75 and 100 ppm) showing a uniform growth rate. The plants grown in the soil containing 25 ppm Hg showed a higher biomass in comparison to the plants grown in soil containing 75 and 100 ppm Hg (Figure 23).

Figure 23: Plants of *A. dubius* 4 days after being spiked with Hg.

Ultra structural effect on *A. dubius*

The effect of mercury is shown by SEM of the root section (Figure 24). There is widespread damage present in the cortical region (CR) with damage of parenchymatous cells around the central stele (CS) and other bundles being pronounced.

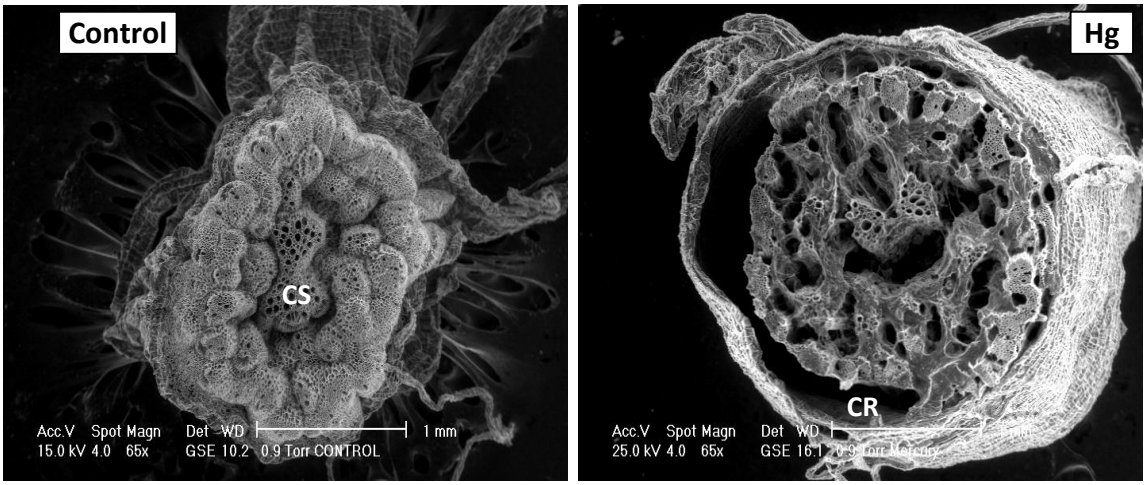

Figure 24: SEM of fresh root sections of *A. dubius* exposed to Hg for 24 h. [cortical region (CR); central stele (CS)]

Effect of concentration and time

The effect of exposing the plants to Hg over a 16 day period was analysed by measuring the root length, results are shown in Figure 29. Simple linear regression analysis between the change in root length from Day 0 (taken as 8 cm) to Day 16 shows that the relationship between dose and root growth is not linear. For doses 25 and 75 the root length increases, but for 100 it decreases. The graph shows this pattern (Figure 25). Hg could be absorbed by the plant only for doses up to 75 ppm, and at 100 ppm it retards growth.

Portal of storage

The uptake of Hg ranged from 3 ppm to 666 ppm with the root system accumulating the highest concentration of Hg. There was a significant difference ($p = 0.0048$) in Hg absorption at 25, 75 and 100 ppm. The higher the dose the greater the amount of Hg was absorbed. The absorption of Hg by the different plant portals was also statistically significant, meaning that the level of Hg absorbed in the different parts of the plant was different. Least square means for different parts of the plant are as follows: roots: 292.8 (95% CI: 181.0 to 404.6); leaves: 9.3 (95% CI: 0 to 121.2) and stems: 4.1 (95% CI: 0 to 123.6). This indicates that higher levels of Hg are accumulated in the roots than in the leaves or stems (Figure 26).

Movement of Hg in the plant

The Bioconcentration Factor (BCF) which indicates the ability of the plant to absorb Hg was determined for *A. dubius* grown in the different soils containing Hg (in our study a BCF value > 2 was regarded as high). The BCF for Hg at 25, 75 and 100 ppm was greater than two. The TF value was greater than 1 only at 25 ppm while at 75 and 100 ppm the TF was less than one (Table 13).

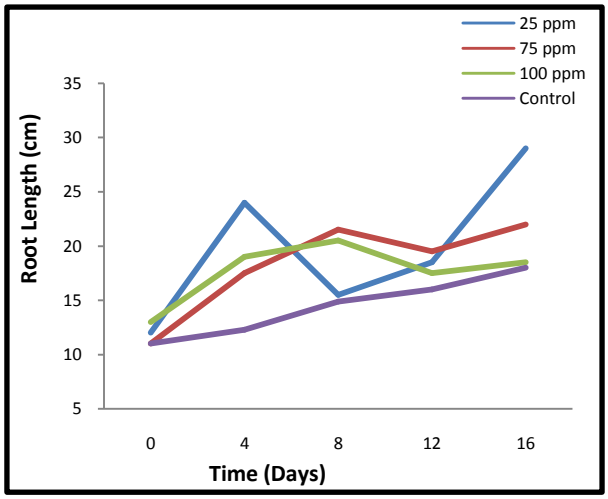

Figure 25: Effect of Hg concentrations at 25 ppm, 75 ppm and 100 ppm on the root growth of *A. dubius* over a 16 day period.

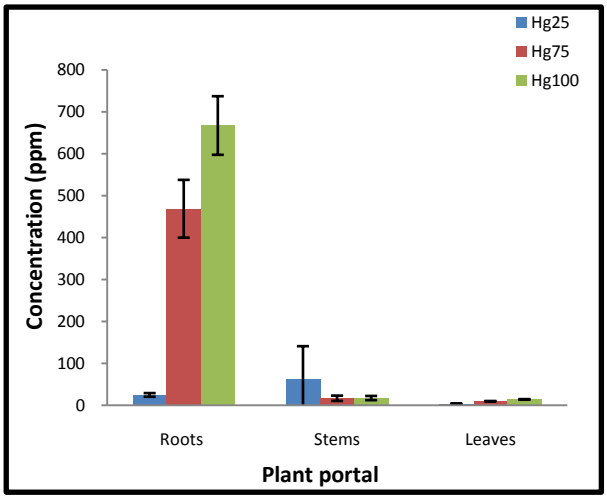

Figure 26: Hg levels in the roots, stems and leaves of *A. dubius* at 25 ppm, 75 ppm and 100 ppm in soil after 4 d. [Bars denote mean ± Standard deviation (*n*=3)]

Table 13: Concentration of Hg accumulated in the roots, stems and leaves of *A. dubius* grown for four days and the Translocation Factor (TF) and Bioconcentration Factor (BCF) (n=3)

Metal	Concentration in soil (ppm)	Concentration in roots (ppm)	Concentration in stems (ppm)	Concentration in leaves (ppm)	TF	BCF
Hg	25	24 ± 4	61 ± 80	3 ± 0	2.6	3.5
	75	468 ± 69	16 ± 6	9 ± 0	0.1	6.6
	100	667 ± 70	17 ± 5	14 ± 1	0.1	7.0

4.6.3. ARSENIC (As)

Effect on growth and development

In the soil containing As the pattern was similar to that of the plants grown in the soil containing Cr. The plant samples in the soil containing 25 ppm of As showed no phenotypical changes however the samples exposed to 75 and 100 ppm showed both chlorosis and severe wilt (Figure 27).

Figure 27: Plants of *A. dubius* 4 days after being spiked with As.

Ultra structural effect on *A. dubius*

The effect of As on the roots of *A. dubius* is shown by SEM. The cortical region of the arsenic root section (Figure 28) is more or less intact. Parenchymatous cells around the central stele and inner bundles showed damage and large apertures.

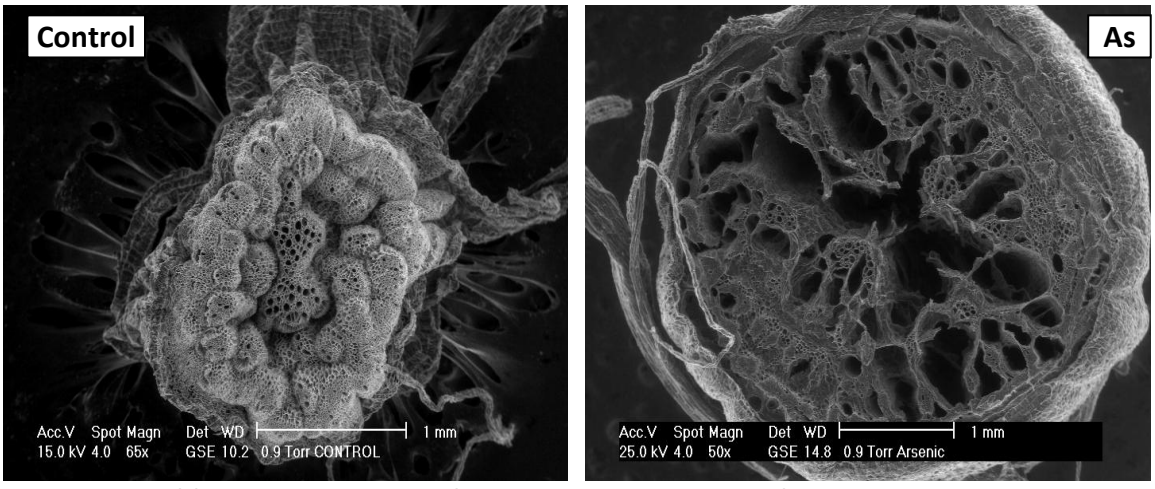

Figure 28: SEM of fresh root sections of *A. dubius* exposed to As for 24 h.

Effect of concentration and time

The effect of exposing the plants to As over a 16 day period was analysed by measuring the root length, results are shown in Figure 29. Simple linear regression analysis between the change in root length from Day 0 (taken as 8 cm) to Day 16 show that at 25 ppm there is an increase in root length, at 75 ppm the root length increases till day 12 and then decreases and at 100 ppm after day four.

Portal of storage

The concentration of As accumulated ranged from 4 ppm to 200 ppm. Plants exposed to 25 ppm, 75 ppm and 100 ppm of As showed a significant difference with a p value < 0.0001. More As was absorbed by the plant at higher doses. The concentration of As in the roots, stems and leaves showed no statistically significant difference (p = 0), with no relationship between concentration of metal accumulated by the roots, stems and leaves. The least square means for different parts of the plant are as follows: roots: 62.2 (95% CI: 39.1 to 85.4); stems: 94.1 (95% CI: 71.0 to 117.3) and leaves: 77.6 (95% CI: 54.4 to 100.7). Therefore As levels were higher in the stem than in the leaves or roots. The accumulation of As was different in comparison to the other modes of accumulation thus far with the lowest rate of accumulation in the roots (Figure 30).

Movement of As in the plant

The Bioconcentration Factor (BCF) which indicates the ability of the plant to absorb As was determined for *A. dubius* grown in the different soils containing As (in our study a BCF value > 2 was regarded as high). The BCF for As at 75 and 100 ppm was greater that two. The movement of As from roots to aerial parts as indicated by the Translocation Factor (TF) shows that As is translocated at concentrations of 25, 75 and 100 ppm (TF > 1) (Table 14).

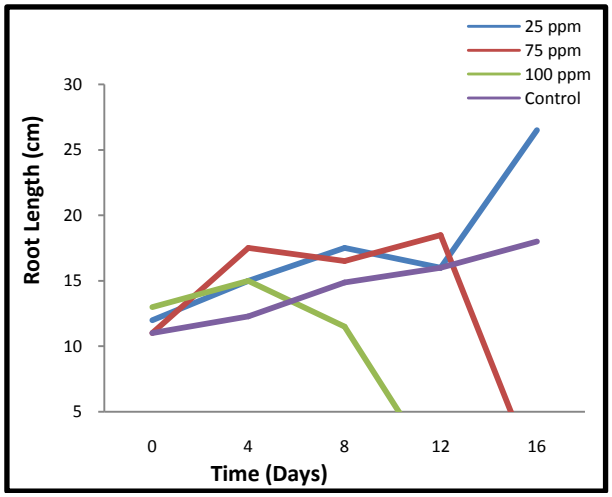

Figure 29: Effect of As concentrations at 25 ppm, 75 ppm and 100 ppm) on the root growth of *A. dubius* over a 16 day period.

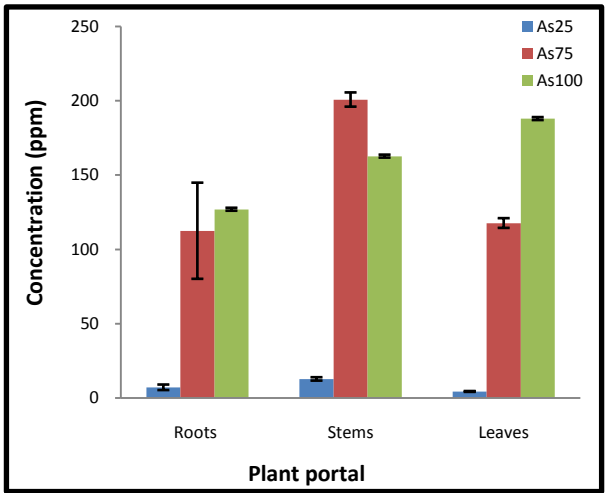

Figure 30: As levels in the roots, stems and leaves of *A. dubius* at 25 ppm, 75 ppm and 100 ppm in soil after 4 d. [Bars denote mean ± Standard deviation (*n*=3)]

Table 14: Concentration of As accumulated in the roots, stems and leaves of *A. dubius* grown for four days and the Translocation Factor (TF) and Bioconcentration Factor (BCF) (n=3)

Metal	Concentration in soil (ppm)	Concentration in roots (ppm)	Concentration in stems (ppm)	Concentration in leaves (ppm)	TF	BCF
As	25	7 ± 2	13 ± 1	4 ± 0	2.4	1.0
	75	112 ± 32	201 ± 5	118 ± 3	2.8	5.7
	100	127 ± 32	163 ± 17	188 ± 4	2.8	4.8

4.6.4. LEAD (Pb)

Effect on growth and development

The plant samples exposed to Pb showed chlorosis at all three concentrations (25, 75 and 100 ppm) with a uniform growth rate at the 25 ppm and 75 ppm Pb concentrations while the 100 ppm showed a slightly slower growth rate, but the leaves were larger in comparison to the plants exposed Cr, Hg, As, Cu and Ni (Figure 31).

Figure 31: Plants of *A. dubius* 4 days after being spiked with Pb.

Ultra-structural effect on *A. dubius*

In the lead root section (Figure 32) the cortical area is damaged in parts with apertures widespread throughout the stelar and vascular regions.

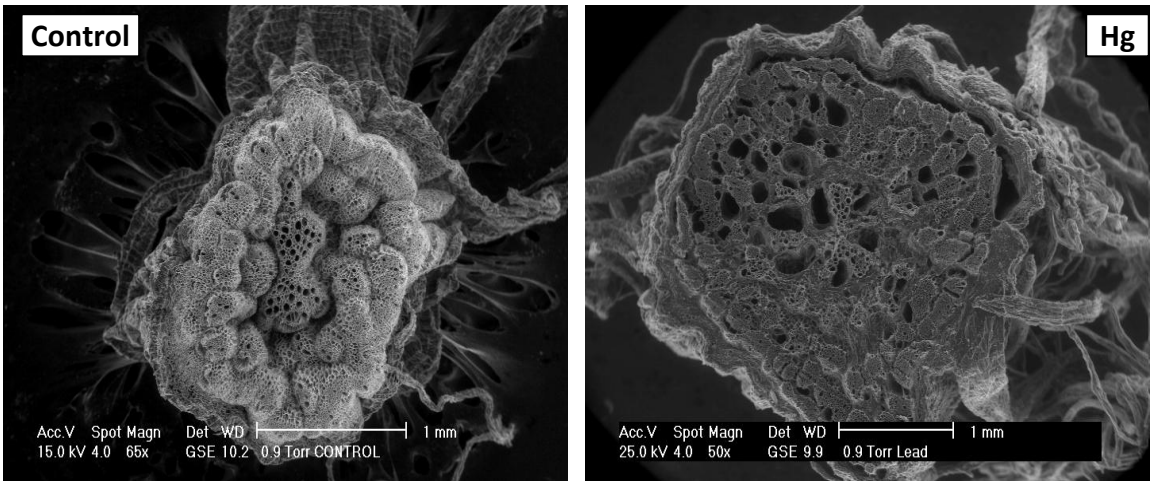

Figure 32: SEM of fresh root sections of *A. dubius* exposed to Pb for 24 h.

Effect of concentration and time

At doses of 25 ppm and 75 ppm the root length increases, but at 100 ppm it decreases (Figure 33). For smaller doses root length increases and then decreases at larger doses.

Portal of storage

The concentration of Pb accumulated ranged from 2 ppm to 138 ppm with the highest concentration accumulated by the roots (Figure 34). There was a significant difference between the 25, 75 and 100 ppm ($p = 0.0245$). More Pb was absorbed at higher doses. The organ of the plant was also statistically significant, meaning that the level of Pb absorbed in the different organs of the plant was different. The least square means for different parts of the plant are as follows: roots: 51.6 (95% CI: 29.4 to 73.8); stems: 10.7 (95% CI: 0 to 32.9) and leaves: 4.7 (95% CI: 0 to 26.9). Therefore much higher levels of Pb were found in the roots compared to the leaves or stems.

Movement of Pb in plant

The Bioconcentration Factor (BCF) which indicates the ability of the plant to absorb Pb was determined for *A. dubius* grown in the different soils containing Pb (in our study a BCF value > 2 was regarded as high). The BCF for Pb at 75 ppm was greater than two. The TF value was greater than one only at 25 ppm while at 75 and100 ppm the TF value less than one (Table 15).

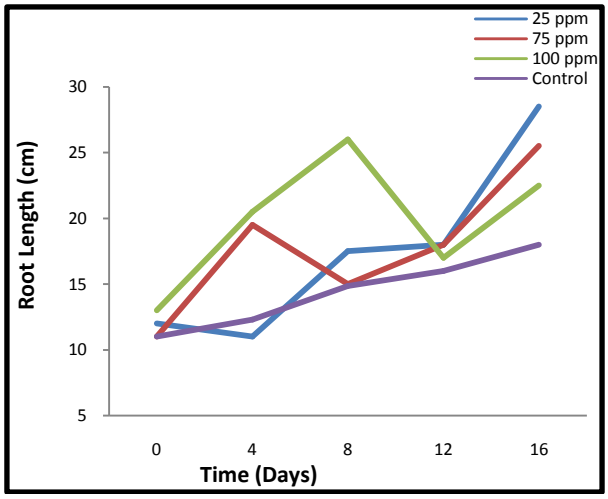

Figure 33: Effect of Pb concentrations at 25 ppm, 75 ppm and 100 ppm) on the root growth of *A. dubius* over a 16 day period.

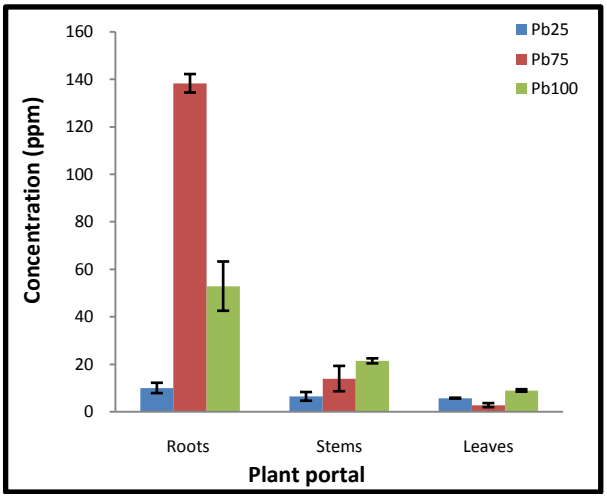

Figure 34: Pb levels in the roots, stems and leaves of *A. dubius* at 25 ppm, 75 ppm and 100 ppm in soil after 4 d. [Bars denote mean ± Standard deviation (*n*=3)]

Table 15: Concentration of Pb accumulated in the roots, stems and leaves of *A. dubius* grown for four days and the Translocation Factor (TF) and Bioconcentration Factor (BCF) (n=3)

Metal	Concentration in soil (ppm)	Concentration in roots (ppm)	Concentration in stems (ppm)	Concentration in leaves (ppm)	TF	BCF
Pb	25	10 ± 2	6 ± 2	6 ± 0	1.2	0.9
	75	138 ± 4	14 ± 5	3 ± 1	0.1	2.1
	100	53 ± 10	21 ± 1	9 ± 1	0.6	0.8

4.6.5. COPPER (Cu)

Effect on growth and development

In the plants grown in the soil containing Cu there were no visible phenotypical changes between the three concentrations. Plants exposed to 25 ppm showed a slower rate of growth in comparison to those grown in 75 ppm and 100 ppm (Figure 35).

Figure 35: Plants of *A. dubius* 4 days after being spiked with Cu.

Effect of concentration and time

For doses for 25 ppm, 75 ppm the root length gradually increases over the sixteen day period, but plants exposed to the 100 ppm dose show an increase in root length up to day four after which there is a decrease as shown in Figure 36. Cu could be a good medium, only for doses up to 75 ppm.

Portal of storage

The concentration of Cu accumulated ranged from 20 ppm to 172 ppm with the highest concentration being accumulated in the roots (Figure 37). Exposure of plants to 25, 75 and 100 ppm of copper showed no specific pattern with regard to metal accumulation in the roots, stems and leaves was not significant ($P = 0.7378$). However, the amount of copper in the different plants was significantly different ($P < 0.0001$).

Least square means for different parts of the plant are as follows: roots: 139.3 (95% CI: 116.2 to 162.5); stems: 43.1 (95% CI: 19.9 to 66.3) and leaves: 33.8 (95% CI: 10.6 to 57.0). Therefore there were much higher Cu levels in roots than in the stems or leaves. TEM analysis for Cu was not done as there was no evidence of toxicity

Movement of Cu in plant

The Bioconcentration Factor (BCF) which indicates the ability of the plant to absorb Cu was determined for *A. dubius* grown in the different soils containing Cu (in our study a BCF value > 2 were regarded as high). The BCF value for Cu at 25 ppm, 75 ppm and 100 ppm were greater than two. The TF value was less than one at 25 ppm, 75 ppm and 100 ppm (Table 17).

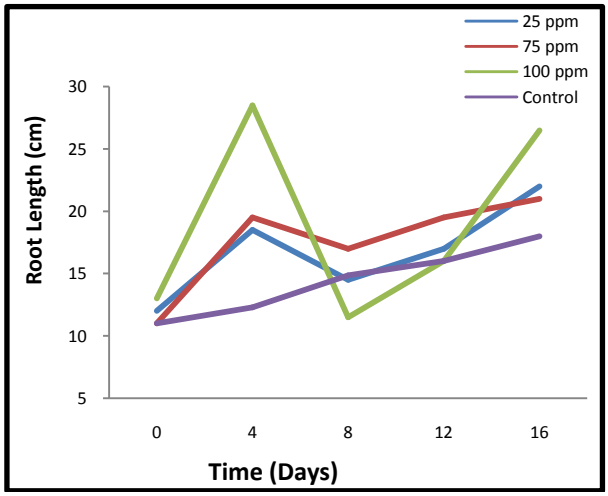

Figure 36: Effect of Cu concentrations at 25 ppm, 75 ppm and 100 ppm) on the root growth of *A. dubius* over a 16 day period.

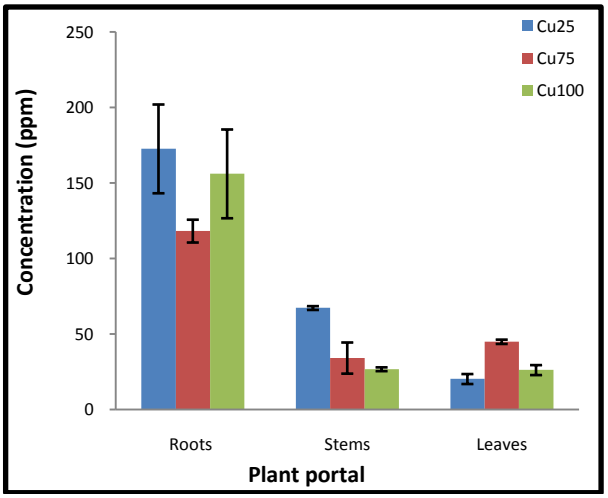

Figure 37: Cu levels in the roots, stems and leaves of *A. dubius* at 25 ppm, 75 ppm and 100 ppm in soil after 4 d. [Bars denote mean ± Standard deviation (*n*=3)]

Table 16: Concentration of Cu accumulated in the roots, stems and leaves of *A. dubius* grown for four days and the Translocation Factor (TF) and Bioconcentration Factor (BCF) (n=3)

Metal	Concentration in soil (ppm)	Concentration in roots (ppm)	Concentration in stems (ppm)	Concentration in leaves (ppm)	TF	BCF
Cu	25	173 ± 28	67 ± 18	20 ± 3	0.5	10.4
	75	118 ± 8	34 ± 10	45 ± 1	0.7	2.6
	100	156 ± 29	27 ± 1	26 ± 3	0.3	2.1

4.6.6. NICKEL (Ni)

Effect on growth and development

The plants grown in the soil containing Ni showed no phenotypical changes apart from the leaves appearing darker green in colour when compared to the plants exposed to the other metals (Figure 38).

Figure 38: Plants of *A. dubius* 4 days after being spiked with Ni.

Effect of concentration and time

For all doses (25, 75 and 100 ppm) the root length increasesup until day eight, after which there is a decrease for dose 25 and 75 ppm. This is shown in Figure 39, where root length increases but at prolonged exposure plants at lower doses (25 and 75 ppm) show a decrease in root length. Ni could be a good medium, only for doses of 100 ppm.

Portal of storage

Concentrations of Ni accumulated ranged from 8 ppm to 205 ppm with highest concentration being stored in the roots (Figure 40). There was a significant difference ($p = 0.0031$) in the amount of Ni absorbed at 25, 75 and 100 ppm. This means that the higher the dose the greater the amount absorbed. There was also a significant difference in the Ni absorbed by the different plant portals ($p = 0.0086$). Least square means for different parts of the plant are as follows: roots: 75.7 (95% CI: 46.5 to 104.9); stem: 15.7 (95% CI: 0 to 44.8) and leaves: 16.1 (95% CI: 0 to 45.3). Therefore much higher Ni levels were found in the roots rather than in the stems or leaves.

Movement of Ni in the plant

The Bioconcentration Factor was determined for *A. dubius* grown in the different soils containing Ni (in our study a BCF value > 2 was regarded as high). The BCF values for Ni at 25 and 100 ppm were greater than two. The TF value was less than one at 25 ppm, 75 ppm, and 100 ppm (Table 17).

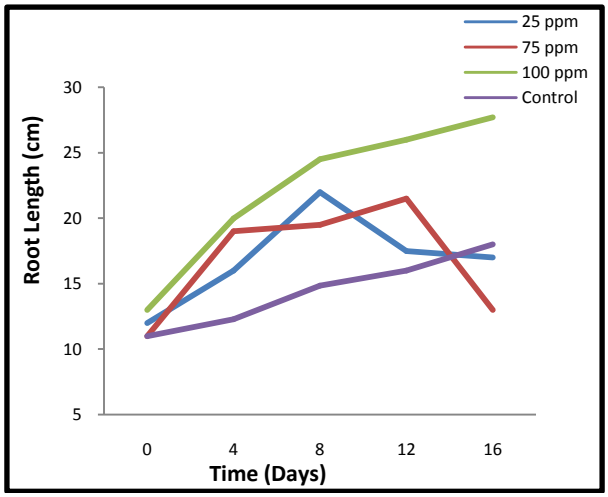

Figure 39: Effect of Ni concentrations at 25 ppm, 75 ppm and 100 ppm) on the root growth of *A. dubius* over a 16 day period.

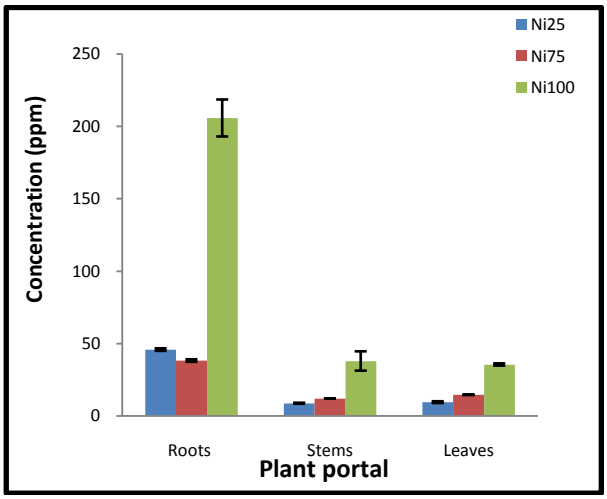

Figure 40: Ni levels in the roots, stems and leaves of *A. dubius* at 25 ppm, 75 ppm and 100 ppm in soil after 4 d. [Bars denote mean ± Standard deviation (*n*=3)]

Table 17: Concentration of Ni accumulated in the roots, stems and leaves of *A. dubius* grown for four days and the Translocation Factor (TF) and Bioconcentration Factor (BCF) (n=3)

Metal	Concentration in soil (ppm)	Concentration in roots (ppm)	Concentration in stems (ppm)	Concentration in leaves (ppm)	TF	BCF
Ni	25	46 ± 1	9 ± 0	9 ± 1	0.4	2.6
	75	38 ± 1	12 ± 0	15 ± 0	0.7	0.9
	100	206 ± 13	38 ± 7	36 ± 1	0.4	2.8

4.7. CHROMIUM EFFECT ON CALLUS TISSUE

There was little morphological variation between induced calluses exposed to Cr (VI), with all callus tissue being soft and white in colour with a smooth, wet looking surface and easy to break into small pieces when touched. New white calluses developed from the older (brown) callus mass (Figure 41 and Figure 42). Sterilization regime and hormone concentrations are described in Section 3.3.

Figure 41: Formation of root hair (RH) on leaf material callus tissue.

Figure 42: New growth (NG) on leaf material callus tissue.

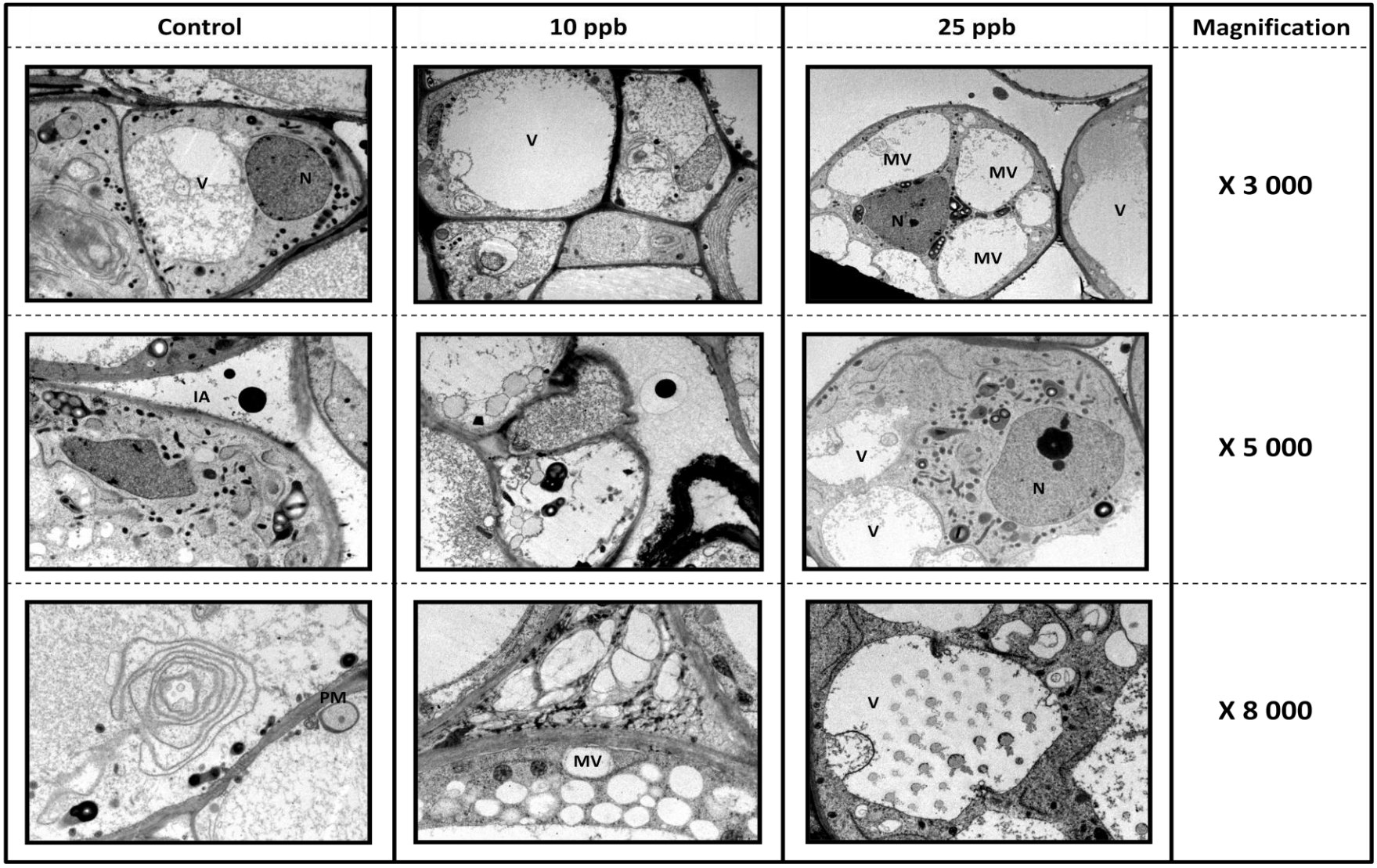

Figure 43: TEM scans of callus tissue exposed to different concentrations of Cr (10 ppb and 25 ppb). N: nucleus; V: vacuole; Ve: vessicles; IA: inter-cellular air space; PM: plasma membrane; MV: mini-vacoules

In the control sections the callus tissue appears to be composed of metabolically active, isodiametrically-shaped parenchyma cells. The latter are loosely arranged with large intercellular air spaces. The cytomatrix of the control cells appear dense due to the presence of large nuclei, abundant ribosomes, osmiophilic bodies, endoplasmic reticulum (ER) cisternae, dictyosomes, mitochondria and colourless plastids/leucoplasts (Figure 44). There appears to be no evidence of membrane organization in these plastids. The osmiophilic bodies/droplets appear to be restricted to the cell periphery. The cytoplasm appears highly granular which may be attributed to the fixation procedures carried out. The walls of the control cells appear normal. There are however unusually large dictyosome associated vesicles observed in close proximity to the cell walls (Figure 43).

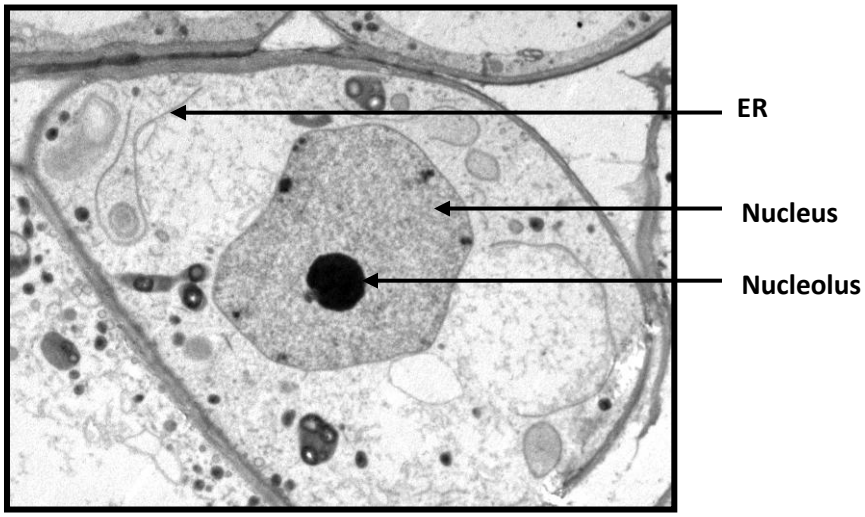

Figure 44: TEM of control callus tissue (magnification x 4000)

In the callus tissue exposed to 10 ppb of Cr some of the cells appear highly vacuolated whilst others contain numerous mini-vacuoles (small vacuoles). Abundant mitochondria appear to proliferate in these cells. The plasma membrane closely follows the contours of the cell wall in some cells while in some cells the membrane in indiscernible. Occasionally possible ER cisternae appear to surround plastids. An interesting feature is evident in Figure 45 where part of the cytoplasm appears to be engulfed by the vacuole, which is probably a necrotic cell. Some leucoplasts contain unusually large starch grains and appear highly disorganized.

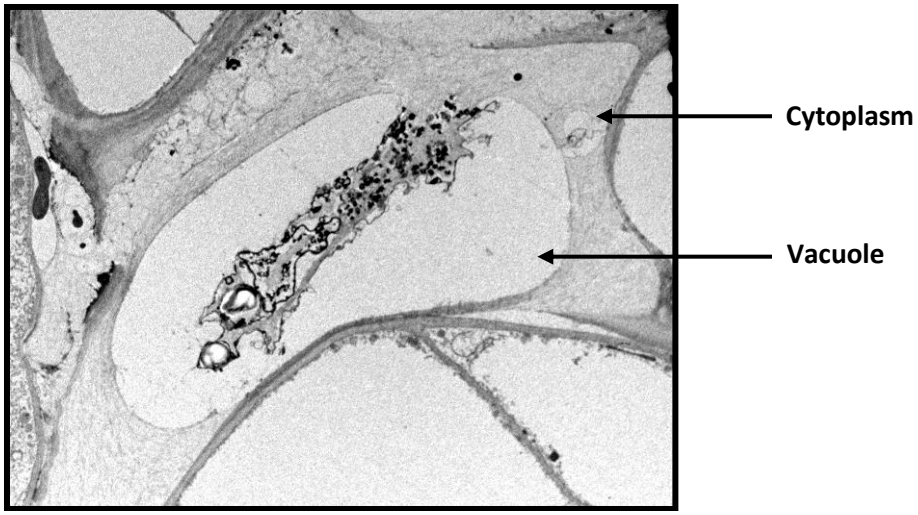

Figure 45: TEM of callus tissue exposed to 10 ppb Cr (magnification × 3 000)

In the callus tissue exposed to 25 ppb Cr the cells contain numerous small vacuoles, some of which contain little vesicles contaminated with stain, with the nuclei appearing lobed (Figure 46). Endoplasmic reticulum cisternae appear to be restricted to the cell periphery. The leucoplasts appear highly disorganized with no organization of the lamellae in the matrix of some cells. There appears to be some disintegration of the cell wall material in certain cells. There appears to be no uniformity in the cellulose microfibrillar arrangement of the walls of certain cells. There is great proliferation of mitochondria within these cells, however some mitochondria appear disorganized with dilated cristae (Figure 47). Finally some cells appear totally necrotic with sparsely distributed organelles in the cytomatrix.

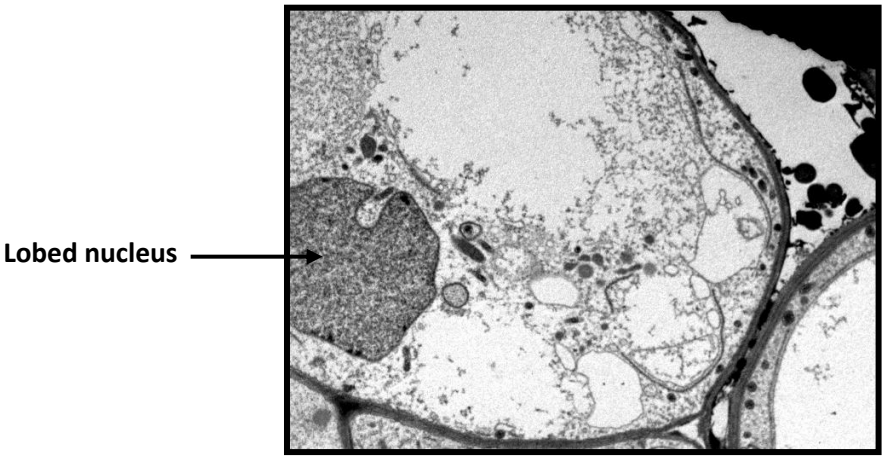

Figure 46: TEM of callus tissue exposed to 25 ppb Cr (magnification × 5 000)

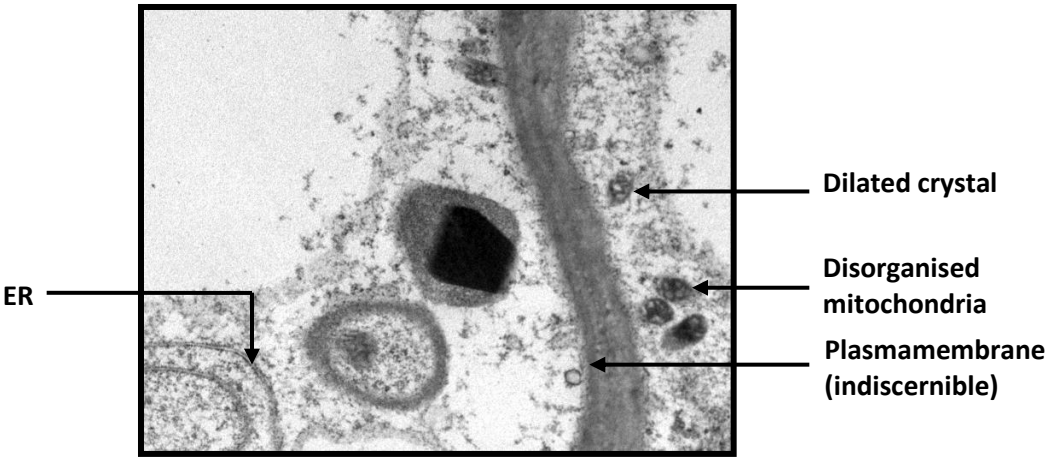

Figure 47: TEM of callus tissue exposed to 25 ppb Cr (magnification × 20 000)

5. DISCUSSION

5.1. METAL BIOSORPTION OF *Amaranthus dubius* GROWING IN A REGULARLY CULTIVATED AREA (RCA), LANDFILL SITE (LS) AND WASTE WATER TREATMENT SITE (WWTS)

5.1.1. CONCENTRATIONS FOUND AND HEALTH IMPLICATIONS

A survey of Cr, Hg, As, Pb, Cu and Ni in soils and naturally adapted *A. dubius* plants from a RCA, LS and WWTS indicate high levels of Cr, Hg and As. These levels were far above acceptable standards set for soils in the guidelines for Land Application and Storage in Nova Scotia (Table 7) and the levels in the plants were far above the standards set for the Recommended Dietary (Daily) Allowance (RDA). Although the methylated forms of Hg, As and Pb have no known biological significance consumption of these by humans at low concentrations is toxic (Duruibe et al., 2007).

In this study three different sites were chosen. The RCA was chosen because it is a site where the soil is frequently tilled, thus it would be expected to have little or no metal pollution. However the results of this study showed very high levels of Cr as high as 1130 ppm (Figure 15 A and Figure 16 A). This could have been due to the fact that this area is surrounded by a paper and pulp industry, petroleum industry and the Durban International Airport (Figure 10 i). The high Cr level in the soil from the RCA may be attributed to contaminants which may have leached into the cultivated land (Wilson-Jones, 2008). Heavy metal contamination may also be as a result of atmospheric pollution due to the proximity of the RCA to Durban International Airport (Pison and Menut, 2004, Singh et al., 1997).

The LS is a dumping ground for many industries and hence the high levels of Cr, Pb, Cu and Ni are as expected. Landfills hold waste containing a wide range of organic molecules of both natural and xenobiotic origin (Nagendran et al., 2006). The WWTS receives both domestic and industrial solid waste and had high levels of Cr, As, Pb, Cu and Ni in the soil possibly as a result of leaching. As the sludge is a concentrated medium the presence of these metals was as expected (Moodley et al., 2007).

The BCF index was > 2 for Hg at the RC, LS and WWTS site and the TF index >1. These results enable the conclusion that *A. dubius* can tolerate Hg and can also sequester Hg from the soil and translocate it to the shoots. The BCF and TF indices for Cr indicate that this metal is not sequestered by the plant and also not translocated from any of the sites. The BCF and TF indices for Pb, As, Ni, Cu indicate that these metals have some degree of transportability, but varies from site to site. This could be due to different forms in which these metal ions are available at these sites. These results are shown in Table 18.

Table 18: Composite Bioconcentration Factor (BCF) and Translocation Factor (TF) values for *Amaranthus dubius* from the Regularly Cultivated Area (RCA), Landfill Site (LS) And Waste Water Treatment Site (WWTS)

Metal	Regular Cultivated Area (RCA) [Merebank]		Landfill Site (LS) [Springfield Landfill Site]		Waste Water Treatment Site (WWTS) [Northern Waste Water Works]	
	BCF	TF	BCF	TF	BCF	TF
Cr	0.1	0.8	0.7	0.8	0.2	0.2
Hg	**22.4***	**2.8**	**4.4***	0.7	**17.6***	**6.5**
As	0.3	**1.4**	**6.2***	0.5	0.4	0.4
Pb	1.3	**7.9**	1.2	**2.3**	0.5	0.6
Cu	1.6	0.9	**3.9***	0.6	0.6	0.6
Ni	0.4	**1.1**	1.7	**1.1**	0.4	0.4

*** high BCF value (values > 2)**

5.1.2. RELATIONSHIP OF METAL LEVELS IN SOIL AND PLANT

Of the three sites tested, a correlation between the heavy metal concentration in the soil and plants could only be made for Cr and Cu as the TF values indicate that there was limited translocation of metal to the aerial parts of *A. dubius* harvested from these sites. The levels of As and Hg found in soil to the levels found in plants showed no correlation. The Translocation Factor (TF) of each of the metals Cr, Hg, As, Pb, Cu and Ni in the tunnel house studies at 25 ppm, 75 ppm and 100 ppm is illustrated in Table 11. *Amaranthus dubius* accumulates Cr, Hg and Pb at 25 ppm and translocates it to the shoots, but at higher concentrations of 75 ppm and 100 ppm the metal is stored in the roots.

In the case of As, accumulation occurs at all the concentrations and the metal is translocated to the shoots. For Cu and Ni the results indicate that the metal is stored mainly in the roots. The root length of the plants, indicate that there is a relationship between metal and dose on root growth. In this study it was found that for Pb, Cu and Ni there was an increase in the root length of the plant as the plant was exposed to higher concentrations of the metal. For Hg and As there was a decrease in root length with an increase in metal concentration indicating that at higher concentrations these metals may becomes toxic to the plant. For Cr there was no visible relationship between root length and concentration (Figure 29).

Table 19: Comparison of metal phytoremediation from Regularly Cultivated Area (RCA), Landfill Site (LS) And Waste Water Treatment Site (WWTS) to Tunnel House studies

	Soil (ppm)	Plants (ppm)	Plants Part	TF	BCF
RCA	Cr – 1130	Cr – 107	Cr – Roots Hg – Minumum uptake As – No uptake Pb – Small amounts Cu – All organs Ni – All organs	Pb>Hg>As>Ni>Cu>Cr	Hg>Cu>Pb>Ni>As>Cr
LS	Cr – ±50 Pb – ±50	Pb – > 50	Cr – Roots Hg – Minumum uptake As – Roots Pb – All organs Cu – All organs Ni – All organs	Pb>Ni>Cr>Hg>Cu>As	As>Hg>Cu>Ni>Pb>Cr
WWTS	Cr – 250 Pb – >50 Ni – >50 Cu – >100	Pb – > 50 Ni – > 50 Cu – > 50	Cr – Roots Hg – Minumum uptake As – Minimum uptake Pb – Roots Cu – All organs Ni – All organs	Hg>Pb>Cu>As >Ni>Cr	Hg>Cu>Pb>As>Ni>Cr
Tunnel House	Cr – 75 Hg – 100 As – 100 Pb – 75 Cu – 25 Ni – 100	Cr – 555 Hg – 697 As – 478 Pb – 155 Cu – 260 Ni – 279	Cr – Roots Hg – Roots As – Leaves Pb – Roots Cu – Roots Ni – Roots	As>Cr>Cu>Ni>Pb >Hg	Cu>Hg>As>Ni>Cr>Pb

5.2. PHYTOREMEDIATION POTENTIAL OF *Amaranthus dubius*

5.2.1. CHROMIUM

According to Chaney et al. 1997; Barazani et al. 2004; Marchiol et al. 2004 for a plant to be considered for phytoremediation should have a few of the following traits to make its use feasible:

- Ability to extract, degrade or stabilize the contaminant
- Tolerance to high levels/concentrations of the contaminant
- Rapid growth rate and high biomass production
- Cosmopolitan growth and ease for harvesting

The study shows that at 25 ppm there is no toxic effect but at higher concentrations such as 75 and 100 ppm the plants show signs that affect the growth and development. At low concentrations the chromium is found most in the vacuoles and according to the studies of Davies et al. (2002) this renders it to have less toxicity. This may be due to the fact that the metal although toxic is stable in the vacuole.

The observation of the growth of the plant over a sixteen day period indicates that *A. dubius* can tolerate high Cr concentrations as indicated by the high BCF index (Table 12). However, the ability of *A. dubius* to be considered for phytoextration has to be viewed with caution, as the TF index indicates that only when the Cr concentration is 25 ppm is Cr being translocated to the aerial parts of the plant. Findings by Gardea-Torresdey et al. (2004) support Cr being concentrated in the roots and not translocated to the aerial parts of the plant by determining the uptake and accumulation of Cr by *Convolvulus arvensis* L.

Amaranthus dubius clearly demonstrates that it can tolerate difficult soil conditions as the field experiment samples were procured from waste dumps and a sewage site [Figure 9 and Figure 11]. Furthermore, the plants collected had well-developed rooting structures. The impact of Cr contamination in the physiology of plants depends on the metal speciation, which is responsible for its mobilization, subsequent uptake and resultant toxicity in the plant system.

Chromium (VI) is considered the most toxic form of Cr and chromium toxicity results in reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and causes mutagenesis. Although *A. dubius* is a cosmopolitan weed that has a rapid growth rate and is easy to harvest it cannot be considered for phytoremediation of Cr, as this metal is toxic to plants at high concentrations. Chromium is sequestered from soil, but is not translocated to the aerial parts of the plant.

5.2.2. MERCURY

Exposure of *A. dubius* to doses of up to 100 ppm showed no visible phenotypical changes, and uniform growth rate (Figure 22). The lowest uptake of Hg, 3 ppm was found to be in the leaves whilst the roots were found to have 666 ppm. There are several other studies that also show that plant roots accumulate Hg when they were exposed to Hg-contaminated soils (Kalac and Svoboda, 2000). Laboratory studies showed that plant roots absorbed Hg from solution and roots accumulated much greater amount of Hg than shoots (Godbold and Hütterman, 1988). Some field and laboratory studies have demonstrated that plants accumulate more Hg when it is introduced in organic form than in an inorganic form (Ribeyre and Boudou, 1984). Our studies did not investigate the absorbtion of the gaseous Hg via the stomata of leaves, however studies of Cavallini et al. (1999) reported that uptake of Hg0 by the leaf increased with increasing Hg vapour concentration, temperature, and illumination. Leaves can also absorb Hg after deposition of particulate Hg on the leaf surface and release gaseous Hg into the atmosphere. Furthermore, Hanson et al. (1995) reported that at low external Hg concentrations in the air, the release of Hg from leaf to air was higher than the leaf Hg absorption from the air in the tree species *Picea abies L. Liriodendron tulipifera L., Quercus alba L., and Acer rubrum L.* Similar results were also found by Ericksen and Gustin (2004). This evidence suggests that foliage can manage both uptake and volatization of gaseous Hg (Figure 48).

In the study a measurement of the root length over a sixteen day period showed that the plant can tolerate doses of 75 ppm, but at 100 ppm there is a decrease in the root length (Figure 25). This is further manifested by the toxic changes that are seen in SEM (Figure 24) section of the root showing the changes in cortical section of the roots.

All physiological and biochemical processes in plants may be negatively affected by Hg when plants are exposed to Hg-contaminated soil, water or air (Patra and Sharma, 2000). The ability of the plant to absorb Hg from the soil is well demonstrated by the high BCF index, however a TF of 2.6 is only found at 25 ppm (Table 13). Thus it is evident from our results that *A. dubius* can be used for phytoremediation, when the concentrations of Hg are low. Our results also show that the translocation of Hg from the roots to the shoots is consistent with previous studies on *Pisum sativum L., Mentha spicata L., and Picea abies (L.) Karst* (Godbold and Hütterman, 1988). The low translocation of Hg to the shoots is probably due to the high affinity of the roots of *A. dubius* for Hg.

No high Hg-accumulators were found among the species growing naturally at the Hg-contaminated sites. Although the roots efficiently take up Hg from solution, the translocation to the shoots, i.e. the harvestable parts is low. Nonetheless, as plant roots are able to efficiently take up Hg from the available Hg pool in the soil and to accumulate Hg in roots, phytostabilization might be a promising approach to remediate aged Hg-contaminated soils (Figure 26). In this process, the massive plant root system traps the bioavailable Hg and reduces the leakage of Hg from contaminated soil.

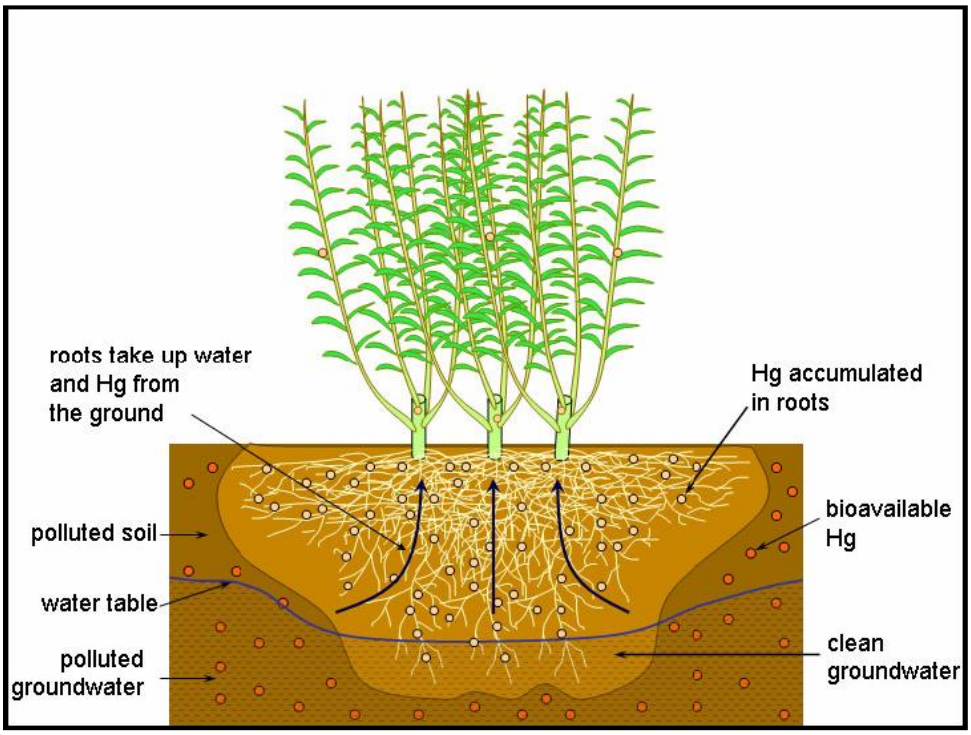

Figure 48: Phytoremediation of Hg (Wang, 2004)

5.2.3. ARSENIC

Plant samples in the soil containing 25 ppm of As showed no phenotypical changes, however, the samples exposed to 75 and 100 ppm showed changes (Figure 27). The accumulation of As was different in comparison to Cr and Hg in that the lowest was found in the roots and the highest levels were found in the leaves (Figure 30). The root length was found to increase for 25 ppm over a sixteen day period, but at 75 ppm this increase was only observed to day 12, thereafter there was a decrease in root length (Figure 29). This is also observed in the SEM section of As (Figure 28). The absorption of As from the soil by the roots is lower than that of leaves and shoots. Further evidence indicating that As is translocated in the leaves and shoots is indicated by the TF and BCF values respectively (Table 14). As the objective is to evaluate *A. dubius* for the phytoremediation of As, this study shows that it can tolerate As levels of 75 ppm, and can also translocate most of the As to the aerial parts of the plant up to 100 ppm. The results of field test show that it is cosmopolitan plant. Thus one can conclude that *A. dubius* is a hyperaccumulator of As.

Previous work shows arsenic accumulation from mining and smelting waste sites, but does not provide any evidence that any of the plants are hyperaccumulators since in their studies the arsenic concentrations in the roots are greater than that in shoots of these plants (Porter and Peterson, 1975). Recently, the fern *Pteris vittata* (Chinese brake fern) was identified as an arsenic hyperaccumulator (Tu et al., 2002, Wei and Chen, 2006). Another fern, *Pityrogramma calomelanos* (silver fern) has also been discovered as an arsenic hyperaccumulator in Thailand, and showed great potential in phytoremediation of arsenic contaminated soils (Francesconi et al., 2002, Visoottiviseth et al., 2002). The Chinese brake has great arsenic tolerance and accumulating ability, it grew healthily both in tailings with as high as 23 400 mg $As.kg^{-1}$ in the field and in soils spiked with 1 500 mg $As.kg^{-1}$ in greenhouse conditions (Ma et al., 2001). Field tests have also shown that it has great potential in phytoremediation of arsenic contamination in soils (Salido et al., 2003). Besides, greenhouse experiments have also shown that *Pteris cretica* (Cretan brake fern) was an arsenic hyperaccumulator (Meharg, 2002, Zhao et al., 2002). Several studies have demonstrated that arsenic reduction from arsenate to arsenite is an important mechanism for arsenic tolerance and accumulation (Tu et al., 2002).

5.2.4. LEAD

Lead is a metal with limited availability for plant uptake due to complexation with solid soil fractions (Rieuwerts et al., 1998), sequential extractions, or a very small fraction of total soil Pb was present in a form (in soil solution or exchangeable from soil colloids) directly available to plants. In the study the effect of exposing *A. dubius* to Pb showed uniform growth rate at 25 and 75 ppm of Pb, and slightly lower growth rate at 100 ppm. A similar trend was observed with root length (Figure 33). Analysis of the Pb concentrations in the different portals showed that most of the Pb was in the roots (Figure 34). SEM images show some damage of the cortical area and apertures in the vascular regions (Figure 32). The ability of the plant to take up Pb from the soil is only evident at 75 ppm which has a BCF value of 2.1 (Table 15).

However, ability of the plants to move the lead to the aerial parts of the plant is limited, and only the plants exposed to 25 ppm showed a TF > 1. *Thlaspi rotundifolium* and *Thlaspi caerulescens* show similar results (Reeves and Brooks, 1983). A few plant species have been reported to accumulate lead to high concentrations in the above-ground parts and are called hyperaccumulators (Kumar et al., 1995, Brooks, 1998, Huang et al., 1997, Barlow et al., 2000, Jarvis and Leung, 2002). Results of the study s are not consistent and therefore it is difficult to conclude that *A. dubius* is a hyperaccumulator of Pb.

5.2.5. COPPER AND NICKEL

It is well known that elements such as Cu, Mo, Ni, Cr, and Zn, among others, are essential for plant growth in low concentrations (Taiz and Zeiger, 1998). Nevertheless, beyond certain threshold concentrations, these same elements become toxic for most plant species (Blaylock and Huang, 2000). Thus the effect of Cu and Ni was studied. The results for both metals showed that Cu and Ni do not show any visible damage to *A. dubius* and higher doses gave higher growth rates. Cu and Ni are both stored in the roots. The study also shows that most of the copper is stored in roots and very little is translocated to the aerial parts (Table 16 and Table 17). Studies by Peralta et al. (2001) on alfalfa plants found that they were able to accumulate 8 500 $mg.kg^{-1}$ of Ni and 12 000 $mg.kg^{-1}$ of Cu.

This is in keeping with the fact that Cu is an important constituent of several proteins and enzymes involved in photosynthesis and respiration, and only in excess can it cause chlorosis, inhibition of root growth and damage to plasma membrane permeability. In studies carried out by Giordani et al. (2005) with the herbaceous crops barley (*Hordeum vulgaris*), cabbage (*Brassica juncea*), Spinach (*Spinacia*), sorghum (*Sorgum vulgare*), bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*) and castor oil (*Ricinus communis*) they found that spinach was the most efficient, followed by bean, cabbage, sorghum, barley and tomato.

5.3. COMPARISON OF DIFFERENT METALS

There was a difference in the growth rates between the different concentrations with the plants exposed to 100 ppm being the highest followed by 25 ppm and 75 ppm (Figure 49). A comparison of the dose (25 ppm, 75 ppm and 100 ppm) is represented in Table 20. The t-test shows a significant difference between the dose for both Cr and As. A comparison of the different organs of the plant (roots, stems and leaves) for each metal (Cr, Hg, As, Pb, Ni and Cu) is also represented in Table 21 and this shows a significant difference between the parts of the plant for Hg and Cu.

In the case of As accumulation at all the concentrations, the metal is translocated to the shoots. The Cu and Ni results indicate that the metal is stored in the roots mainly. Some metals also show a relationship between root length and dose, with some metals showing an increase in root length with an increase in dose as can be seen in Figure 49 for Pb, Cu and Ni. However in contrast the Hg and As show a decrease in root length with an increase in metal concentration while the Cr root length shows no visible relationship between root length and concentration. In the Cr treated soil, plant samples in the soil containing 25 ppm Cr showed a higher rate of growth in comparison to the other two concentrations (75 ppm and 100 ppm) as can be seen in Figure 21. The plants in the 75 ppm and 100 ppm soil also showed signs of chlorosis, which is the yellow, pale yellow or white in leaves as a result of the inability of the leaves to produce chlorophyll. After the four days of exposure to the different concentrations of Cr the plants exposed to 25 ppm and 75 ppm Cr were still showing signs of growth however the plant exposed to 100 ppm started to show signs of wilting.

The uptake of Cr ranged from 16 ppm to 310 ppm, with the highest concentration of Cr being accumulated in the root system (Figure 22). In the samples exposed to Cr the highest concentration of Cr was accumulated by the plants grown in the soil containing 75 ppm. The plants exposed to Hg showed no visible phenotypical changes, with the samples exposed to each of the different Hg concentrations showing a uniform growth rate (Figure 25). The uptake of Hg ranged from 3 ppm to 666 ppm with the root system accumulating the highest concentration of Hg. In the soil containing As the pattern was similar to that of the plants grown in the soil containing Cr. The plant samples in the soil containing 25 ppm of As showed no phenotypical changes however the samples exposed to 75 and 100 ppm showed both chlorosis and severe wilt (Figure 27). The concentration of As accumulated ranged from 4 ppm to 200 ppm. The accumulation of As was different in comparison to the other modes of accumulation thus far with the lowest accumulation in the roots (Figure 30).

The plant samples exposed to Pb showed chlorosis among all three concentrations (25, 75 and 100 ppm) with a uniform growth rate between the 25 ppm and 75 ppm Pb concentrations, while the 100 ppm showed a slightly slower growth rate; leaves were however larger in comparison to plants exposed to other metals (Figure 33). The concentration of Pb accumulated ranged from 2 ppm to 138 ppm with the highest concentration accumulated by the roots (Figure 34).

In the plants grown in the soil containing Cu there were no visible phenotypical changes between the three concentrations, however the plant exposed to 25 ppm showed a slower rate of growth in comparison to those grown in 75 ppm and 100 ppm (Figure 36). The concentration of Cu accumulated ranged from 20 ppm to 172 ppm with the highest concentration being accumulated by the roots (Figure 37). The plants grown in the soil containing Ni showed no phenotypical changes apart from the leaves appearing darker green in colour when compared to the plants exposed to the other metals. Concentrations of Ni accumulated ranged from 8 ppm to 205 ppm with highest concentration being stored in the roots (Figure 40). There was a difference in the growth rates between the different concentrations with the plants exposed to 100 ppm being the highest followed by 25 ppm and 75 ppm (Figure 39).

The Translocation Factor (TF) of each metal under investigation (Cr, Hg, As, Pb, Cu and Ni) and each of the 3 different concentrations (25 ppm, 75 ppm and 100 ppm) for each metal respectively is illustrated in Table 12, 13, 14, 15, 16 and 17. From the results the closer to 0 the higher the concentration of the metal stored in the roots instead of being translocated to the shoots/aerial parts of the plant. From the results, *A. dubius* accumulates Cr, Hg and Pb at 25 ppm and translocates it to the shoots, but at higher concentrations (75 ppm and 100 ppm) the metal is stored in the roots. In the case of As accumulation at all the concentrations the metal is translocated to the shoots. The Cu and Ni results indicate that the metal is stored in the roots mainly.

Some metals also show a relationship between root length and dose, with some metals showing an increase in root length with an increase in dose as can be seen in Figure 49 for Pb, Cu and Ni. However in contrast the Hg and As show a decrease in root length with an increase in metal concentration while the Cr root length shows no visible relationship between root length and concentration.

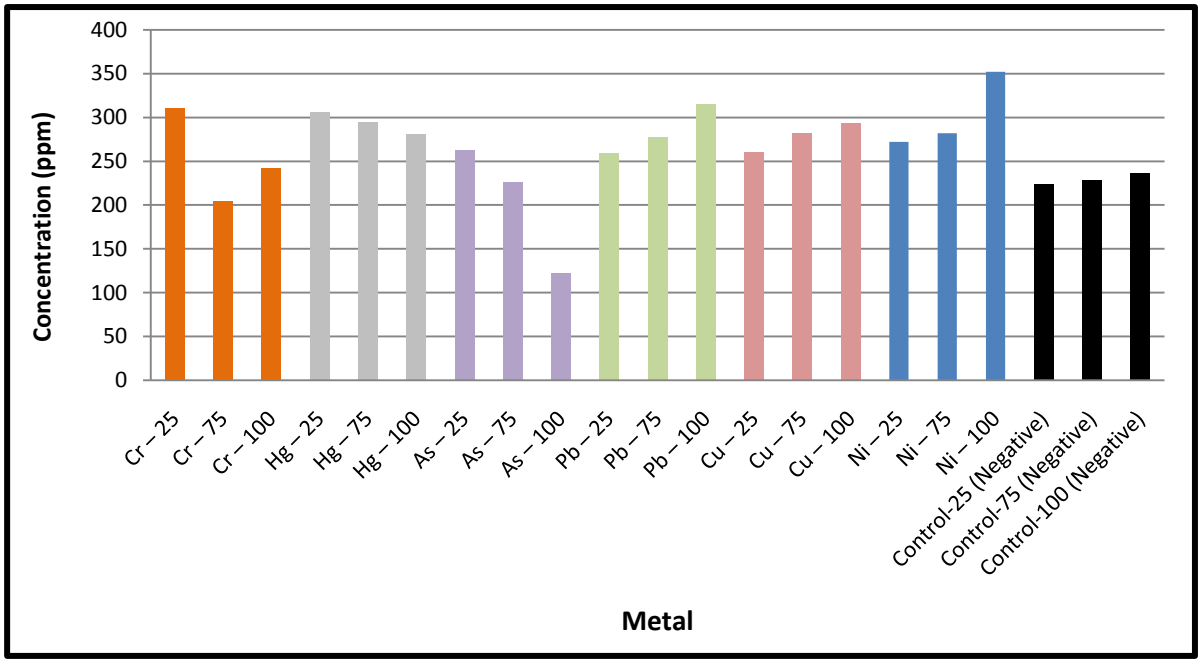

Figure 49: Comparison of root growth response of *A.dubius* of each metal (Cr, Hg, As, Pb, Ni and Cu) respectively.

Table 20: Comparison of metal (Cr, Hg, As, Pb, Cu and Ni) accumulation in the roots, stems and leaves of *Amaranthus dubius* grown for 4 d

					t – test	
Metal	**Concentration in soil (ppm)**	**Roots**	**Stems**	**Leaves**	**Dose**	**Part of plant**
Cr	25	30 ± 11	17 ± 2	17 ± 1	***	**
	75	311 ± 3	126 ± 22	118 ± 1		
	100	308 ± 117	87 ± 9	63 ± 2		
Hg	25	24 ± 4	61 ± 80	3 ± 0	**	***
	75	468 ± 69	16 ± 6	9 ± 0		
	100	667 ± 70	17 ± 5	14 ± 1		
As	25	7 ± 2	13 ± 1	4 ± 0	***	NSS
	75	112 ± 32	201 ± 5	118 ± 3		
	100	127 ± 32	163 ± 17	188 ± 4		
Pb	25	10 ± 2	6 ± 2	6 ± 0	*	*
	75	138 ± 4	14 ± 5	3 ± 1		
	100	53 ± 10	21 ± 1	9 ± 1		
Cu	25	173 ± 28	67 ± 18	20 ± 3	NSS	***
	75	118 ± 8	34 ± 10	45 ± 1		
	100	156 ± 29	27 ± 1	26 ± 3		
Ni	25	46 ± 1	9 ± 0	9 ± 1	**	**
	75	38 ± 1	12 ± 0	15 ± 0		
	100	206 ± 13	38 ± 7	36 ± 1		

Units in ppm ± standard deviation associated with the mean value (n = 2). Comparison of dose and part of plant (respectively) by trend test (t-test) are indicated [Key: *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.5$ significance and NSS (not statistically significant)].

6. CONCLUSION

All higher plants can accumulate heavy metals in different concentrations. However significant differences in metal accumulation exist between and within plant populations. In this study we chose *Amaranthus dubius* which is a popular nutritious leafy vegetable crop, rich in proteins, vitamins and minerals. The herb is consumed virtually on the whole continent of Africa, Asia and South America. Their quick growth and great biomass makes them some of the highest yielding leafy crops which could be beneficial for phytoremediation.

Initial survey showed that the RCA, LS and WWTS sites have higher than legislated levels of Cr, Hg, Cu and Ni. There was a significant correlation between the levels of Cr and Cu in the soil and plants from all three sites, but for Hg, Pb, Ni and As the levels in the soil were higher relative to their uptake, and hence the concentrations in plants were lower. Despite this, the BCF index which indicates the translocation from soil to aerial parts indicated that all of the metals tested (Cr, Hg, As, Pb, Cu and Ni) could be bioremediated.

All metals were found in the roots of *A. dubius* and for Pb, Cu and Ni there was an increase in root length with an increase in metal concentration. However, for Hg there was a decrease in root length with an increase in metal concentration and for Cr there was no visible relationship between root length and concentration. In field experiments it was found that the soil treated with Cr at 25 ppm showed a higher rate of growth in comparison to the other two concentrations, 75 ppm and 100 ppm. The exposure to higher concentrations also showed signs of chlorosis and wilting. The uptake of Cr ranged from 16 ppm to 310 ppm, with the highest concentration of Cr being accumulated in the root system. At the RCA site the soil concentration was high (1130 ppm) with approximately 1/10 taken up by the plant.

Tunnel house experiments with 100 ppm of Cr (VI) in the soil, indicate that at this concentration there is toxicity to the plant, or interference to absorption by other metals present in the soil. Cr is not translocated to the aerial parts of the plant however it is bioaccumulated from the soil to the roots. This indicates that *A. dubius* can accumulate and convert Cr (VI) to Cr (III) which is the less toxic form.

Plants exposed to Hg showed no visible phenotypical changes. The uptake of Hg ranged from 3 ppm to 666 ppm with the root system accumulating the highest concentration of Hg. Bioaccumulation of Hg can occur at high Hg soil concentrations as shown in the field studies. In the *in vitro* study, soil containing 100 ppm Hg, approximately 1160 ppm Hg was accumulated by the plant. From the three site experiments, the LS had the highest Hg soil concentration (1.9 ppm) together with the highest total plant Hg concentration (8 ppm). In both cases Hg was not translocated from the roots to the aerial parts of the plant, however at lower soil concentrations there is movement from the roots to the aerial parts. Bioaccumulation of Hg from the soil to the roots occurred at all soil concentrations. However, the translocation index for Hg was greater than one indicating that although Hg is phytoremediated, it is not sequestered to the leaves and thus phytovolatisation does not occur.

In the soil containing As the pattern was similar to that of the plants grown in the soil containing Cr. The plant samples in the soil containing 25 ppm of As showed no phenotypical changes, however the samples exposed to 75 and 100 ppm showed both chlorosis and severe wilt. The concentration of As accumulated, ranged from 4 ppm to 200 ppm. The accumulation of As was different in comparison to the other modes of accumulation thus far, with the lowest rate of accumulation in the roots. Maximum bioaccumulation of arsenic occurred in the tunnel house studies, with soil containing 100 ppm arsenic. Here approximately 477 ppm was accumulated by the plant. At this high soil concentration, arsenic was bioaccumulated from the soil to the roots as well as translocated from the roots to the aerial parts of the plant. Although these results indicate that *A. dubius* has all the characteristics required for use in bioremediation the findings of the initial survey are disturbing since the *A. dubius* from the LS, RCA and WWTS are consumed by the local communities.

The plant samples exposed to Pb showed chlorosis among all three concentrations (25, 75 and 100 ppm) with a uniform growth rate between the 25 ppm and 75 ppm Pb concentrations, while the 100 ppm showed a slightly slower growth rate. Leaves were however larger in comparison to the plants exposed to other metals.

The concentration of Pb accumulated ranged from 2 ppm to 138 ppm, with the highest concentration accumulated by the roots. In the plants grown in the soil containing Cu there were no visible phenotypical changes between the three concentrations. However the plant exposed to 25 ppm showed a slower rate of growth in comparison to those grown in 75 ppm and 100ppm. The concentration of Cu accumulated ranged from 20 ppm to 172 ppm, with the highest concentration being accumulated by the roots.

The plants grown in the soil containing Ni showed no phenotypical changes apart from the leaves appearing darker in colour when compared to the plants exposed to the other metals. Concentrations of Ni accumulated ranged from 8 ppm to 205 ppm, with highest concentration being stored in the roots. Bioaccumulation of Pb and Ni showed the highest accumulation in the *in vitro* studies with soil containing 75 ppm and 100 ppm respectively. Both metals showed similar results with translocation of the metals from the roots to the aerial parts of the plant at the low concentrations in soil (which were found in the RCA site [28 ppm (Pb) and 30 ppm (Ni)] and LS site [47 ppm (Pb) and 15 ppm (Ni)]. Bioaccumulation of both metals from the soil to the roots only occurred in the tunnel house studies

The translocation Factor (TF) of each metal under investigation (Cr, Hg, As, Pb, Cu and Ni) and each of the 3 different concentrations (25 ppm, 75 ppm and 100 ppm) for each metal show that *A. dubius* accumulates Cr, Hg and Pb at 25 ppm and translocates it to the shoots, but at higher concentrations (75 ppm and 100 ppm) the metal is only stored in the roots. In the case of As accumulation at all the concentrations, the metal is translocated to the shoots. The Cu and Ni results indicate that the metal is stored in the roots mainly.

This study shows that *A. dubius* can be defined as a hyperaccumulator of As since it has the ability to extract As and can tolerate high levels of this metal. *A. dubius* is a cosmopolitan plant with a rapid growth rate, yields a high biomass and is easy to harvest and the metal is sequestered from the soil and transported to the aerial parts. This phytoremediation of As by *A. dubius* can be exploited for commercialization since the study also shows that it is viable to culture *A. dubius* in tissue culture systems.

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