CITRIC ACID INDUCED PHYTOEXTRACTION OF HEAVY METALS FROM URANIUM CONTAMINATED SOILS

OCKERT FOURIE SCHOLTZ

CITRIC ACID INDUCED PHYTOEXTRACTION OF HEAVY METALS FROM URANIUM CONTAMINATED SOILS

By

OCKERT FOURIE SCHOLTZ

A thesis submitted in fulfillment of the requirements for the degree

MAGISTER SCIENTIAE

in the
Faculty of Natural and Agricultural Sciences
Department of Plant Sciences
University of the Free State

November 2006

Supervisor: Dr. G.P. Potgieter
Co-supervisor: Mr. N. Scholtz

TABLE OF CONTENTS

CHAPTER 3: URANIUM, MOLYBDENUM AND ARSENIC IN THE ENVIRONMENT

CHAPTER 4: MATERIALS AND METHODS

CHAPTER 5: RESULTS

CHAPTER 6: DISCUSSION

CHAPTER 7: CONCLUSIONS

ACKNOWLEDGEMENTS

I wish to express gratitude to the following persons and institutions for allowing me to complete this study.

My sincere appreciation goes to my supervisors, Dr. G.P. Potgieter from the Department of Plant Sciences and Mr. N. Scholtz from the Geology Department, University of the Free State, for their guidance, support, and encouragement during the course of this study.

The National Research Foundation (NRF) for funding this research project.

The Department of Plant Sciences at the University of the Free State for the use of their laboratory facilities and greenhouse.

The Geology Department, University of the Free State for financial support and use of their laboratory facilities.

Mr. Christi Mocke of Rietkuil 307 for allowing me to collect soil samples from his farm.

Dr. H. Vandenhove from the Belgian Nuclear Research Centre and Dr. Johan van der Waals from the University of Pretoria for their knowledge and time in assisting me with data interpretation.

The staff at the Centre for Environmental Management, University of the Free State, for their moral support throughout the course of this study.

My parents for enabling me to study and their moral support.

My wife Amaré for her continuous support, patience and assisting in the finalization of this thesis.

LIST OF FIGURES

LIST OF TABLES

SUMMARY

Induced phytoextraction is an emerging soil remediation technology that makes use of soil amendments and high biomass crop species to accumulate and remove heavy metals from soils.

The failure to rehabilitate an uranium trial mine on Rietkuil 307 in the Karoo Uranium Province of South Africa, has led to above normal concentrations of uranium (U), molybdenum (Mo) and arsenic (As) in topsoil in the vicinity of U ore stockpiles. A greenhouse evaluation was executed to assess the potential of citric acid to induce metal uptake in the shoots of Indian mustard [*Brassica juncea* (L.) Czern, cultivars: 211000 and 426308] and sunflower (*Helianthus annuus* L.) to decontaminate the low-level U (28 mg U kg^{-1} soil), Mo (4.1 mg Mo kg^{-1} soil) and As (8.9 mg As kg^{-1} soil) contaminated soil to background concentrations. A further greenhouse study was performed in a soil with high levels of metal concentrations; U (165 mg U kg^{-1} soil), Mo (125 mg Mo kg^{-1} soil) and As (49 mg As kg^{-1} soil), to assess the feasibility of phytoextraction on a high-level metal contaminated soil.

Citric acid proved to be effective in enhancing shoot-U, -Mo and -As concentrations and the highest concentrations were observed in *B. juncea* 211000 (1788 mg U kg^{-1}; 467 mg Mo kg^{-1}; and 24 mg As kg^{-1}) grown in the low-level contaminated soil. With a biomass yield of 5.51 tonnes per year for *B. juncea* 211000, it would take 9 to 14 years to decontaminate Mo and U on Rietkuil 307 soil to background concentrations. As a result of the low As solubility in soil and subsequent low shoot concentrations of the plant species, it is suggested that other measures for As remediation be investigated.

Furthermore, plants grown in the high-level contaminated soil achieved considerably lower biomass yields and shoot-metal concentrations than in the low-level soil due to metal toxicity. Phytoextraction will, therefore, not be a feasible remediation technology on high-level U, Mo and As contaminated soils.

These results suggest that citric acid induced phytoextraction may provide an environmentally friendly alternative for the decontamination of low-level U and Mo contaminated soils.

OPSOMMING

Geïndusseerde fito-ekstraksie is ‘n belowende tegnologie wat gebruik maak van grond byvoegmiddels en hoë-biomassa gewasse wat swaarmetale akkumuleer en dit sodoende uit gekontamineerde grond verwyder.

Die gebrek aan rehabilitasie van ‘n uraan proefmyn op Rietkuil 307 in die Karoo Uraan Provinsie, Suid-Afrika, het tot gevolg gehad dat bogrond in die omtrek van U erts-hope, bo normale konsentrasies van uraan (U), molibdeen (Mo) en arseen (As) bevat. ’n Glashuisstudie is uitgevoer om die vermoë van sitroensuur as keleringsagent te bepaal om swaarmetaalopname in Indiese mosterd (*Brassica juncea*, kultivars: 211000 en 426308) en sonneblom (*Helianthus annuus*) te verhoog om die vlakke van U (28 mg kg^{-1}), Mo (4.1 mg kg^{-1}) en As (8.9 mg kg^{-1}) in die grond na agtergrond konsentrasies te verlaag. ’n Verdere glashuisstudie is uitgevoer in grond wat hoë U (165 mg kg^{-1}), Mo (125 mg kg^{-1}) en As (49 mg kg^{-1}) vlakke bevat, om die uitvoerbaarheid van fito-ekstraksie in swaar gekontamineerde gronde te bepaal.

Sitroensuur was doeltreffend om die metaalkonsentrasies in bogrondse plantdele te verhoog. Die hoogste konsentrasies is in *B. juncea* 211000 (1788 mg U kg^{-1} grond; 467 mg Mo kg^{-1} grond; and 24 mg As kg^{-1} grond), wat in die grond met lae metaalvlakke gegroei is, gevind. Met ’n beraamde biomassa van 5.51 ton per jaar vir *B. juncea* 211000, sal dit 9 tot 14 jaar vir die plant neem om die Mo en U in die bogrond van Rietkuil 307 te verminder na agtergrondvlakke. As gevolg van die lae oplosbaarheid van As in die grond en die gevolglike lae konsentrasies in die bogrondse plantdele, word dit aanbeveel dat ander remediërings metodes vir As dekontaminasie in die grond ondersoek word.

Plante wat in die hoë-vlak gekontamineerde grond gegroei is, het aansienlik minder biomassa behaal in vergelyking met die plante in die lae-vlak

gekontamineerde grond. As gevolg hiervan word fito-ekstraksie nie aanbeveel as ’n remediëringstegnologie vir hoë vlak U, Mo en As gekontamineerde grond nie.

Die resultate verkry in hierdie studie stel voor dat sitroensuur geïndusseerde fito-ekstraksie, ’n omgewingsvriendelike alternatief vir die dekontaminasie van lae U- en Mo- vlakke in die grond kan inhou.

KEYWORDS

Karoo Uranium Province, remediation, uranium, molybdenum, arsenic, phytoextraction, citric acid, Indian mustard, sunflower, low-level- and high-level contaminated soil

LIST OF ABBREVIATIONS

Elements/Metals/Isotopes

Al	aluminum
As	arsenic
Au	gold
C	carbon
Cd	cadmium
Cl	chlorine
Co	cobalt
Cr	chromium
Cu	copper
Fe	iron
Ga	gallium
Hg	mercury
Mg	magnesium
Mn	manganese
Mo	molybdenum
Na	sodium
Nb	niobium
Ni	nickel
P	phosphorous
Pb	lead
Pd	palladium
Pt	platinum
S	sulfur
Sb	antimony
Se	selenium
Te	tellurium
Th	thorium
Tl	thallium
U	uranium

V	vanadium
Y	yttrium
Zn	zinc
Zr	zirconium
^{137}Cs	cesium 137 isotope
^{90}Sr	strontium 90 isotope
^{239}Pu	plutonium 239 isotope
^{234}U	uranium 234 isotope
^{235}U	uranium 235 isotope
^{238}U	uranium 238 isotope
^{65}Zn	zinc 65 isotope

Other

Bq	Becquerel
$CaCO_3$	calcium carbonate
CEC	cation exchange capacity
cf.	refer to
CO_2	carbon dioxide
COOH	carboxyl group
C_{soil}	soil concentration
$CuSO_4$	copper sulfate
DMS	Dimercaptosuccinate
DTPA	diethylenetrinitrilotetraacetic acid
DW	dry weight
EC	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol bis-2-aminoethyl ether-n,n,n’,n'-tetraacetic acid
H_2O	Water
H_3BO_3	boric acid
HCl	hydrochloric acid
HEDTA	hydroxyethylenediaminetriacetic acid
HM	heavy metal

HNO_3	nitric acid
ICP-OES	inductively coupled plasma optical emission spectrometer
IOT	index of tolerance
KCl	potassium chloride
KH_2PO_4	mono potassium phosphate
KNO_3	potassium nitrate
KOH	potassium hydroxide
LLD	lowest limit of detection
LMWOA	low molecular weight organic acid
LOI	loss on ignition
$MgSO_4$	magnesium sulfate
MoO_3	molybdenum trioxide
MoO_4^{2-}	Molybdenite
NaOH	sodium hydroxide
NH_4OH	ammonium acetate
nm	Nanometer
OH^-	hydroxyl ion
PC	Phytochelatin
PNEC	predicted no effect concentration
ppm	parts per million
SSP	site specific phytoextraction
TF	transfer factor
t	Tones
µm	Micrometer
U_3O_8	triuranium octaoxide (yellowcake)
UO_2^{2+}	uranyl ion
$ZnSO_4$	zinc sulfate

CHAPTER 1

INTRODUCTION

1.1 Overview

Uranium (U) is a naturally occurring radioactive element that is present in soil (Francis *et al*., 1999; U.S. Department of Health, 1999), water (Spalding and Druliner, 1981; Skwarzec *et al*., 2004; Hirose and Sugimura, 2003) and air (Hirose and Sugimura, 1981; Bliss and Meyerhof, 1987). The mining and milling of U have, however, resulted in localised enrichment of U in the atmosphere, hydrosphere, lithosphere and even the biosphere (U.S. Department of Health, 1999; Liator, 1995; Jones and Serne, 1995). Uranium mill tailings are often associated with elevated concentrations of a variety of heavy metals and the erosion of these tailings may result in elevated metal levels within aquifers (Pilon-Smits, 2005; Waggitt, 2004; Rahn *et al*., 1996).

Uranium poses potential radiation toxicity towards humans (US Department of Health, 1999; Howard *et al*., 1991; Ragnarsdottir and Charlett, 2000; Athar and Vohora, 1995), fauna (Ammerman *et al*., 1980; Sheppard *et al*., 1992), flora (Sheppard *et al*., 1985) and soil microbes (Meyer *et al*., 1998; Norberg and Molin, 1983). Chemically, U is potentially carcinogenic and responsible for renal failure in humans (Howard *et al*., 1991; Athar and Vohora, 1995; Hossner *et al*., 1998; US Department of Health, 1999). Radionuclide and heavy metal pollution in soil, water (*cf.* Appendix A1 for Target Water Quality Ranges in South Africa) and food resources may, as a result, be a threat to the environment and human health through accumulation or biomagnification.

Nuclear energy, with U as its source of energy, has been proposed as an energy-producing alternative to fossil fuels (Schrattenholzer, 2004; Sims *et al*., 2003; Percebois, 2003; Duffey, 2005). One gram of enriched U has the energy equivalent of two thousand litres of petrol or three tonnes of coal (Persebois, 2003). In addition, compared to the burning of fossil fuels, it provides safer (Percebois, 2003), more reliable and cheaper power (Sims *et al*., 2003; Schrattenholzer, 2004) and produces less CO_2 emissions, when coupled with effective and secure waste disposal (Duffey, 2005). As a result, the global demand for nuclear energy is increasing (Hertsgaard, 2004) and the rise in the

market price of yellowcake (U_3O_8) since July 2003 (Ux Consulting Company, 2006)[1] has led to an increased interest in U mining and exploration.

During 1970, up to fifteen companies were actively involved in U exploration in the Karoo Basin in South Africa (van der Merwe, 1990; Le Roux, 1994). Various mining methods and techniques were tested in the Karoo Uranium Province (Figure 1.1) which included the excavation of a trial mining open pit on Rietkuil 307.

On March 28, 1979, a nuclear reactor on Three Mile Island near Middletown Pennsylvania in the United States of America suffered a core meltdown. The accident had serious economic consequences and furthered a public popularity decline towards U and nuclear power. Environmental and human safety issues regarding nuclear power resulted in a decrease in U demand and price. Uranium exploration decreased and companies were forced not only to cease exploration activities but also to discontinue small-scale mining operations (Hertsgaard, 2004). Exploration in the Karoo Uranium Province was forced to cease and companies withdrew without proper rehabilitation of the mining sites. When U mines are left unrehabilitated it could pose potential ecological hazards to the immediate environment (Lozano *et al*., 2000; Scholtz, 2003; Schneider *et al*., 2001; Scholtz *et al*., 2005) and an immediate rehabilitation strategy should precede mine closure. The South African Minerals Act no 50 of 1991 states: “If the Director: Mineral Development is of the opinion that having regard to the known and disclosed mineral reserves of any mine, that mine is likely to cease mining operations within a period of five years, he shall in writing give notice accordingly to the owner of that mine and such owner shall not dispose of any of his assets in relation to that mine without a certificate furnished by the Director: Mineral Development to the effect that the necessary steps have been taken or adequate provision has been made for the rehabilitation of the mining area concerned.”

[1] The Ux Consulting Company, LL (UxC), 700 Twelfth ST, NW, Suite 700, Washington DC 20005

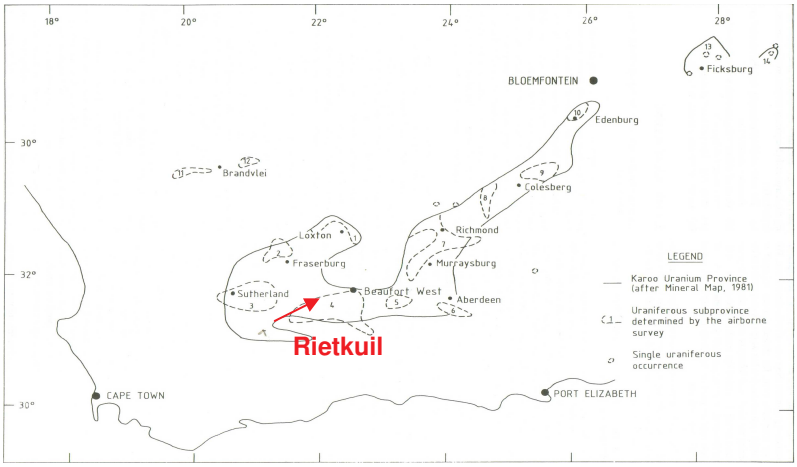

Figure 1.1 The Karoo Uranium Province with the arrow indicating the location of Rietkuil 307 (After Cole *et al*., 1991).

Scholtz (2003) reported that an estimated 6100 tonnes of stockpiled U ore (uraniferous sandstone; Turner, 1979) is present on Rietkuil 307 in the Karoo Uranium Province (Figures 1.2, 1.3 and 1.4). Uranium is associated with molybdenum (Mo), arsenic (As) and copper (Cu) in the U-Mo-As-Cu-Pb association within the Karoo Uranium Province (Le Roux and Brynard, 1994). Scholtz (2003) postulates that due to lack of rehabilitation the leaching of these elements into the topsoil is eminent and documented elevated concentrations of U, Mo and As in the soil within 500 m of the stockpiles on Rietkuil 307.

Scholtz *et al*. (2005) furthermore revealed that the Karoo plant species Kriedoring (*Lycium cinereum*) and Thimble grass (*Fingerhuthia africana*), growing in close vicinity of the stockpiles, accumulated U, Mo and As to levels exceeding those concentrations found in their background counterparts. They studied the potential environmental impact resulting from inadequate rehabilitation of U trial mining on Rietkuil 307 and found that the above-mentioned plants accumulated high levels of U, Mo and As in their roots and shoots grown in close vicinity (1 to 10 m radius) of the ore stockpiles. Potential biomagnification of radionuclides and heavy metals in animals and eventually humans should be cause for concern.

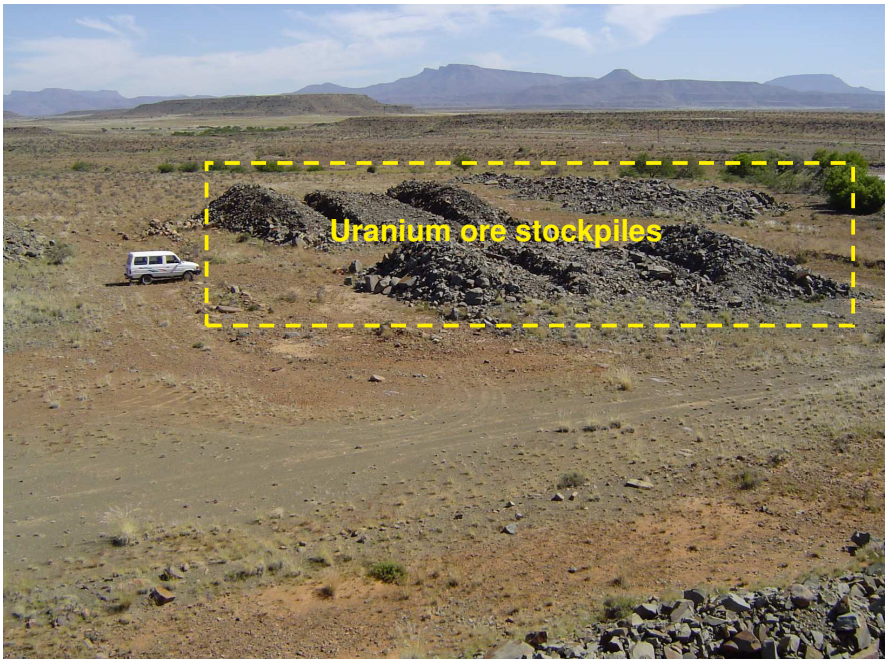

Figure 1.2 Uranium ore stockpiles on Rietkuil 307.

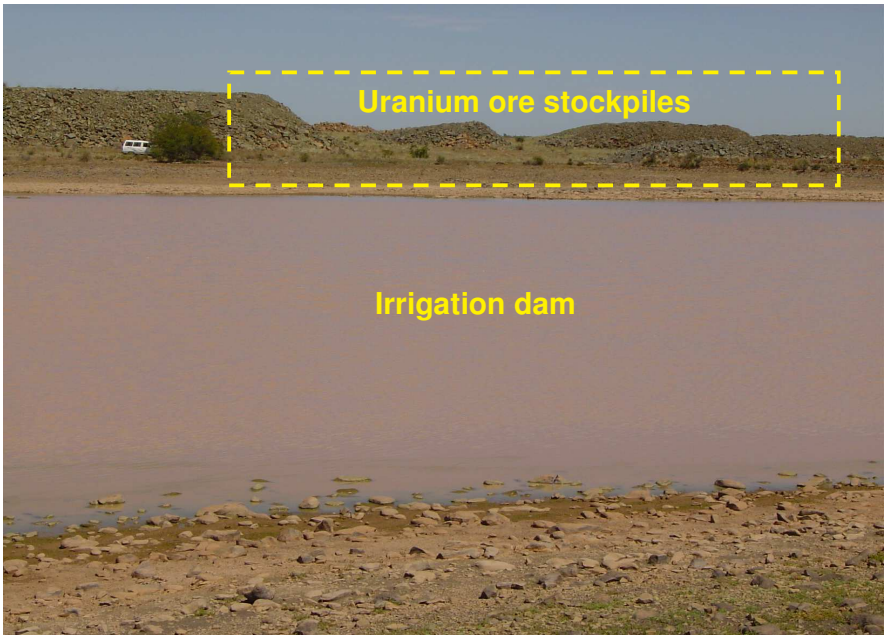

Figure 1.3 Uranium ore stockpiles and uncontaminated irrigation dam (Scholtz, 2003) on Rietkuil 307.

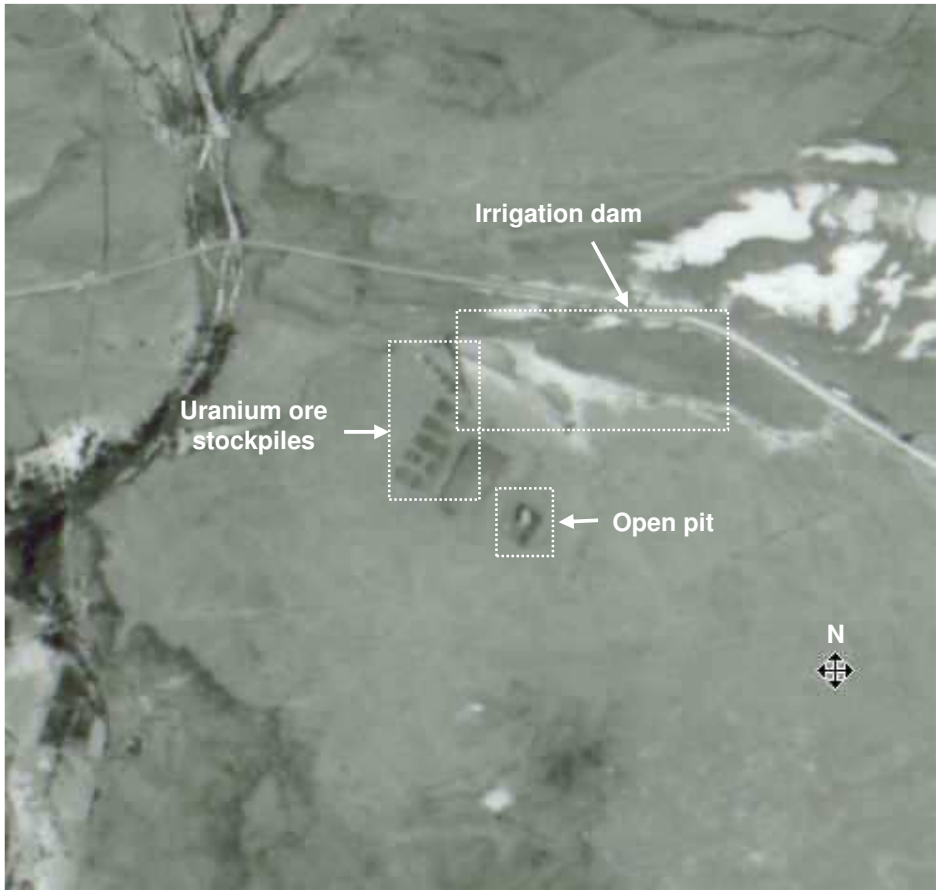

Figure 1.4 An aerial photograph of the unrehabilitated U trial mine and the irrigation dam on Rietkuil 307.

1.2 Soil metal remediation technologies

Soil remediation is defined by Allen (1988) as the return of soil to a condition of ecological stability together with the establishment of plant communities it supports or supported to conditions prior to disturbance. Conventional technologies involve the removal of metals from polluted soils by transportation to laboratories, soil washing with chemicals to remove metals, and finally replacing the soil at its original location or disposing of it as hazardous waste (Francis *et al*., 1999). This decontamination strategy is an *ex situ* approach and can be very expensive and damaging to the soil structure and ecology (Salt *et al*., 1995a; Mason *et al*., 1997; Huang *et al*., 1998).

Immobilization of heavy metals through the addition of lime (Lothenbach *et al.*, 1997; Krebs *et al.*, 1999), phosphate (Ebbs *et al.*, 1998; Cooper *et al.*, 1999), calcium carbonate ($CaCO_3$) (Chen *et al.*, 2000) and clay (Wenger, 2000) have been suggested as remediation techniques. These remediation technologies have the advantage of immediately reducing the risk factors arising from metal contamination, but may only be considered temporary alternatives because the metals have not been removed from the soil environment. A potential environmental friendly technique for the purpose of permanent removal of radionuclides and heavy metals from soil is phytoextraction (Huang *et al.*, 1998).

1.3 Phytoextraction of metals from soils

Phytoextraction is an environmentally friendly and cost effective technique that has been proposed by researchers to extract U and various other inorganic contaminants from soils (Salt *et al.*, 1995a; Cunningham *et al.*, 1995; Dushenkov *et al.*, 1997; Ebbs *et al.*, 1998; Huang *et al.*, 1998). This technology involves the extraction of metals by plant roots and the translocation thereof to shoots. The shoots are subsequently harvested to remove the contaminants from the soil. Salt *et al.* (1995a) reported that the costs involved in phytoextraction would be more than ten times less per hectare compared to conventional soil remediation techniques. Phytoextraction also has environmental benefits because it is considered a low impact technology. Furthermore, during the phytoextraction procedure, plants cover the soil and erosion and leaching will thus be reduced. With successive cropping and harvesting, the levels of contaminants in the soil can be reduced (Vandenhove *et al.*, 2001). Harvested biomass can be incinerated to reduce volume (Chaney *et al.*, 1997) and stored as hazardous waste or the metals can be recycled and sold (termed phytomining; Anderson *et al.*, 1999). To remove sufficient amounts of heavy metals with this technique, plants have to be highly efficient in metal uptake and translocation into their aboveground vegetative parts. The phytoextraction process is, however, limited because of low bioavailability of metals in soils.

Natural hyperaccumulating plants such as *Thlapsi caerulescens*, a Ni hyperaccumulator, possess specialized physiological abilities that allow them to accumulate large amounts of metals during their life cycle (Brooks *et al*., 1977; Salt *et al*., 1995a). This type of metal-uptake is typical of plants that grow on soils rich in various metals, but are usually small in biomass and slow growing.

Induced phytoextraction (Figure 1.5) involves the use of metal-chelates, which when applied to contaminated soils, induces the phytoaccumulation of the pollutant metal of interest (Ebbs *et al*., 1998; Huang *et al*., 1998; Shahandeh and Hossner, 2002a). This soil remediation technique makes use of fast growing high biomass crop plants, which differ from natural hyperaccumulating plants in that they are not capable of accumulating and translocating sufficient amounts of metals without the addition of chelates (Blaylock *et al*., 1997; Huang *et al*., 1998; Vandenhove *et al*., 2001). Chelates bind metals in the soil and/or acidify the soil solution, which increases bioavailability and aid in the translocation of metals from root to shoot (Blaylock *et al*. 1997). For instance, Huang *et al*. (1998) increased U concentrations in the soil solution 200-fold and more than a 1000-fold in *Brassica juncea* shoots following citric acid additions. Similary, Blaylock *et al*. (1997) increased the Pb concentration in *B. juncea* shoots 16-fold following the addition of EDTA (ethylenediaminetetraacetic acid). Several researchers (Huang *et al*., 1998; Qualls and Haines, 1992; Jones *et al*., 1996; Jones and Darrah, 1994) have reported that citric acid is a more environmentally friendly chelate to use in phytoextraction due to the rapid degradation rate of citric acid. Huang *et al*. (1998) reported that *Brassica juncea* achieved maximum shoot-U concentrations after three days of citric acid addition whereafter the concentration curve reached a steady state. This *in situ* decontamination strategy is also more environmentally friendly and cost effective than the conventional soil remediation techniques (Cunningham *et al*., 1995), which include soil excavation and metal leaching.

A further limitation of phytoextraction is that it can only be applied to decontaminate low to medium levels of soil contamination since high-levels of metal concentrations severely inhibits plant growth due to toxicity (Wenger, 2000; Shahandeh and Hossner, 2002a and b; Vandenhove *et al*., 2001).

Chelate induced phytoextraction also poses the potential risk of soluble metals migrating down the soil profile into groundwater.

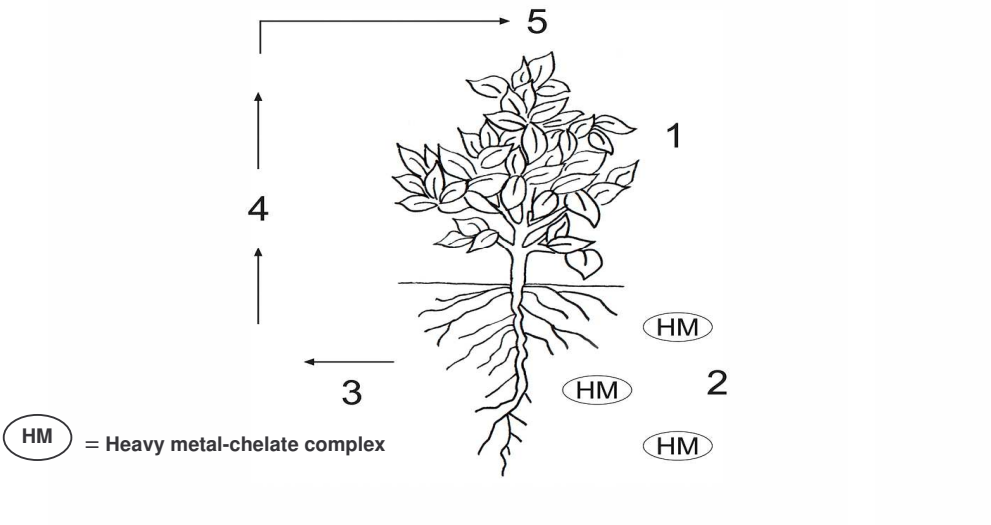

Figure 1.5 The success of induced phytoextraction depends on: 1) fast growing high biomass plant species; 2) the addition of chelates to increase metal solubility in the soil solution; 3) plant uptake through roots; 4) root to shoot transport; and 5) plant-metal tolerance.

1.4 Study objectives

The main objective of this greenhouse study was to investigate the feasibility of citric acid induced phytoextraction to remediate low-level U, Mo and As contaminated soil (soil-1), resulting from failure to rehabilitate a former U trial mine on Rietkuil 307 in the Karoo Uranium Province, South Africa. Citric acid was chosen as metal chelate because of its high binding capacity for U as well as its rapid degradation rate in soils. Based on reports from previous studies, the plants selected for the greenhouse trials were the crop species Indian mustard [*Brassica juncea* (cultivars: 211000 and 426308)] and sunflower (*Helianthus annuus*). The Site-Specific Phytoextraction Potentials (SPP) of these species were estimated to predict the annual U, Mo and As removal from one hectare of soil. Soil with higher metal concentrations (soil-2; created in the laboratory) was also used in the greenhouse trials to determine how effective phytoextraction would be if the metal concentrations in the soil were to increase

as a result of long term failure to remove the U ore stockpiles. Because of the rapid degradation rate of citric acid, a further objective was to investigate whether successive citric acid applications, compared to a single application method, could enhance metal concentrations in the shoots of selected plants.

CHAPTER 2

LITERATURE STUDY

2.1 Metal contamination in soils

Topsoil serves as a sink for metal contaminants (Shimwell and Laurie, 1972). Some metals occur in the soil environment as radioactive isotopes (^{238}U, ^{235}U, ^{234}U, ^{137}Cs, ^{239}Pu, ^{90}Sr, etc.) posing radioactive threats to plants, animals and humans (Zhu and Shaw, 2000). It is, however, not enough to predict a toxicity threshold concentration in soil based on the total concentration level since high concentrations of these metals in soils do not automatically imply their release and secondary incorporation by organisms (Elliot and Shields, 1988).

Metals in the soil distribute amongst various soil components, which in turn determine their mobility and bioavailability (Elliot and Shields, 1988). The nature of this association has often been described as speciation (Ramos *et al.*, 1994). Metals in the soil environment can exist as: (i) water-soluble free metal ions; (ii) carbonate complexes; (iii) metal ions occupying ion exchangeable sites and is specifically adsorbed onto inorganic soil constituents; (iv) organically bound metals; (v) compounds of oxides and hydroxides; and (vi) metals in the structure of silicate minerals (Tessier *et al.,* 1979; Ahumuda *et al.*, 1999). A drawback concerning phytoextraction is that only fraction (i), (ii) and possibly some components of fraction (iii), are readily bioavailable (Tessier *et al.,* 1979).

2.2 Plant-metal interactions

All plants have the ability to accumulate “essential” metals (Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, V and Zn) from the soil solution. Plants need different concentrations for growth and development. This ability also allows plants to accumulate other “non-essential” metals (Al, As, Au, Cd, Cr, Hg, Pb, Pd, Pt, Sb, Te, Tl and U) which have no known biological function (Djingova and Kuleff, 2000). Moreover, metals cannot be broken down and when concentrations inside the plant cells accumulate above threshold or optimal levels, it can cause direct toxicity by damaging cell structure (due to oxidative stress caused by reactive oxygen species) and inhibit a number of cytoplasmic enzymes (Assche and Clijsters, 1990). In addition, it can cause indirect toxic effects by replacing

essential nutrients at cation exchange sites in plants (Taiz and Zeiger, 2002). Baker (1981) proposed, however, that some plants have evolved to tolerate the presence of large amounts of metals in their environment by the following three ways:

1. Exclusion, whereby transport of metals is restricted and constant metal concentrations are maintained in the shoot over a wide range of soil levels.
2. Indication, whereby shoot metal concentrations reflect those in the soil solution in a linear relationship.
3. Bioccumulation, whereby metals are accumulated in the roots and upper plant parts at both high and low soil concentrations.

2.3 Phytoremediation

Phytoremediation is defined as an *in situ* remediation strategy which makes use of green plants to remove pollutants from the environment or to render these pollutants harmless (Baker and Brooks, 1989). The science of phytoremediation is based on earlier biogeochemical prospecting. Studies showed that chemical analysis of vegetation provided alternative means for detecting subsurface mineralization (Brooks, 1972; Cannon, 1971). Phytoremediation includes several subsets (Baker and Brooks, 1989):

- phytoextraction, where plants take up metals from polluted soils and translocate them from root to shoot. Plant shoots are then harvested using conventional agricultural methods. The harvested biomass is incinerated to reduce volume and stored as hazardous waste or the metals can be recycled;
- rhizofiltration, whereby plant roots are used to precipitate and concentrate metals from polluted waters;
- phytovolatilization, whereby plants extract volatile metals from soil and volatilize them from the foliage and;

- phytostabilization, in which plants stabilize metals in soils by controlling erosion and migration into the soil profile thus rendering them harmless.

Of the above-mentioned strategies, only phytoextraction can permanently remove metals from contaminated soils. A factor which limits phytoextraction is the availability of metals within the soil solution. In general, crop species are preferred for metal phytoextraction because of their fast growth rate and high biomass production. Many studies have demonstrated that the crop species, Indian mustard (*Brassica juncea*) and sunflower (*Helianthus annuus*), show potential for U phytoextraction from contaminated soils (Huang *et al*., 1998; Ebbs *et al*., 1998; Vandenhove *et al*., 2001; Shahandeh and Hossner, 2002a; Dushenkov *et al*., 1997). These authors suggested, however, that the bioavailability of U in soil would have to be artificially manipulated if these plants were to accumulate sufficient amounts of U in shoots for phytoextraction to be viable. The manipulation of the soil environment to increase metal bioavailability is discussed in 2.5.1.

2.4 Metal hyperaccumulating plants

In contrast to crop species, metal hyperaccumulating plants accumulate high concentrations of heavy metals in their shoots. Hyperaccumulators were originally defined by Brooks *et al.* (1977) as plants containing >1000 mg Ni kg^{-1} dry shoot biomass. This concentration was selected on the basis of it being 100 times the Ni concentration of non-accumulator plants, even when growing in Ni-rich soils and can be applied for other metals as well. An extreme example of a hyperaccumulating plant is the New Caledonian Tree (*Sebertna acuminata*), which can contain up to 25% Ni (250 000 mg kg^{-1}) in dry weight (Jaffré *et al*., 1976). Chinese brake fern (*Pteris vittata*) was the first terrestrial plant known to hyperaccumulate As (Ma *et al*., 2001). Several of these remarkable metal hyperaccumulators have been discovered for a variety of metals and include more than 400 taxa from 80 families (Baker *et al*., 2000).

A hyperaccumulator will concentrate more than 10 mg Hg-; 100 mg Cd-; 1000 mg Co-, Cr-, Cu-, Pb-, Ni-; and 10 000 mg Zn-, Mn per kilogram of dry shoot

biomass (Baker *et al.* 2000). It is believed that these plants have evolved over metal rich soils to deter herbivores, insects and/or pathogens (Baker and Brooks, 1989). However, Cunningham *et al.* (1995) reported that hyperaccumulators are of small height and biomass and are only found on certain metalliferous soils. Thus, metal hyperaccumulating plants may be able to accumulate and tolerate high concentrations of certain metals in its tissues, but their abilities as phytoextractors of metal contaminated soils are in doubt. So far only one hyperaccumulator species, the Ni hyperaccumulator *Alyssum bertolonii* has been successfully applied for phytoextraction purposes in the field (Chaney *et al.,* 2000; Li *et al.,* 2003).

A comparative study conducted by Salt *et al.* (1995a) on the removal of metals from contaminated sites by the hyperaccumulator Alpine pennycress (*Thlapsi caerulescens*) and the high biomass non-hyperaccumulator, Indian mustard (*Brassica juncea*), showed that *B. juncea*, can bioaccumulate certain metals in its shoots and produce more than 20 times the biomass of *T. caerulescens* with the subsequent addition of chelates to the soil. This would give it the potential to remove higher percentages of metals from contaminated soils in a single cropping, given that the initial soil-metal concentration is not too high to cause substantial toxic effects to the non-hyperaccumulating seedling.

2.5 The potential of phytoextraction

Phytoextraction is the most acceptable and applied phytoremediation technique that can successfully remove metals from soils (Cunningham *et al*., 1995; Dushenkov *et al*., 1997; Ebbs *et al*., 1998; Huang *et al*., 1998). The potential of non-hyperaccumulators to be successful metal phytoextractors depends on several factors, including increased metal availability in the soil solution, plant-metal uptake, root to shoot transport, plant-metal tolerance and high biomass production.

2.5.1 Availability of metals in the soil solution

For plant material to accumulate a metal it must be available in the soluble mineral phase of the soil (Vandenhove *et al*., 2001). Consequently, the metal concentration inside the plant is proportional to the metal concentration in the soil solution (Vandenhove *et al*., 2001). Therefore, manipulation of the soil environment to enhance the availability of metals is vital for effective phytoextraction.

Soil pH is an important factor controlling the solubility of metals in soils. At neutral soil pH, heavy metal cations are strongly bound to soil minerals and are not bioavailable. Numerous studies (Ebbs *et al.,* 1998; Huang *et al.,* 1998; Shahandeh and Hossner, 2002b) have shown that lowering the soil pH will decrease the adsorption of heavy metals resulting in an increased concentration in the soil solution. Therefore, metal toxicities are often observed in plants growing in extremely acidic soils, due to high metal bioavailability (Salt *et al.,* 1995a).

Because some metals (anions and cations) in soils are bound to or adsorbed on oxides there is potential for enhancing solubility through dissolution of Fe-, Mn- and Al-oxides at a low soil pH (Salt *et al*., 1995b; Huang *et al*., 1998) which will simultaneously release bound or adsorbed metals into the soil solution. Huang *et al*. (1998) found a positive correlation between U and Fe or U and Al in the soil solutions after lowering the pH with citric acid addition.

The physical and chemical characteristics of the soil system will influence the transformation, retention, and movement of pollutants through the soil. Clay content, organic matter content, texture and cation exchange capacity (CEC) will influence the rate of migration and the form of the chemical species found in leachate migrating from the waste (Mason, 1992). These physical and chemical characteristics are the main parameters that influence a soil's buffer capacity which is defined as the ability of soil to resist acidification.

Moreover, natural chelators that are released by plants and bacteria may enhance bioavailability of metals in soils. Grasses (Poaceae) are known for excreting phytosiderophores (chelating agents) that bind metals into metal-chelate complexes, making them more available for plant uptake (Ma and Nomoto, 1996). Some bacteria secrete biosurfactants, such as rhamnolipids, that enhance metal bioavailability (Volkering *et al.,* 1998).

The bioavailability of metals in soils can also be successfully enhanced by adding chelates to the soil. Synthetic chelates and low molecular weight organic acids (LMWOA) are the most common chelates used in phytoextraction. For example, citric acid for U (Huang *et al*., 1998), EDTA (ethylendiamintetraacetic acid) for Pb, EGTA (ethylene glycol bis-2-aminoethyl ether-n,n,n',n'-tetraacetic acid) for Cd (Blaylock *et al.,* 1997) and ammonium thiocynate for gold (Anderson *et al*., 1998) have been used. Chelates are capable of forming soluble metal-chelate complexes, thereby increasing the bioavailability of metals in the soil. Metal solubility can also be increased through acidification of the soil solution by adding synthetic and LMWOA's.

Many researchers have reported that citric acid has a high binding capacity for U (Huang *et al*., 1998; Vandenhove *et al*., 2001; Shahandeh and Hossner, 2002a and b) and together with its rapid degradation rate (Huang *et al*., 1998; Qualls and Haines, 1992; Jones *et al*., 1996; Jones and Darrah, 1994), makes it an ideal chelate to use for U phytoextraction from contaminated soils.

2.5.2 Minimizing the leaching risk of soluble metal chelate complexes

According to Blaylock *et al*. (1997), EDTA is probably the most efficient chelate used for phytoextraction purposes. It is capable of solubilizing a wide variety of metals (Pb, Cr, Cu, U) followed by root uptake and translocation to shoots. The very slow degradation rate of EDTA (Kari and Giger, 1996), however, increases the metal leaching risk into the soil profile and eventually into groundwater. The rapid biodegradation rate of low molecular weight organic acids (LMWOA's) make them safer chelates to use in the field compared to EDTA (Wu *et al*.,

2004). Dodge and Francis (1994) reported that citric acid easily biodegrades to carbon dioxide and water. They also found that upon exposure to visible light U-citrate complexes photodegrade to acetic acid and carbon dioxide. This rapid degradation of the U-citrate complex reduces the risk of soluble U-complexes migrating further into the soil profile and groundwater.

Huang *et al*. (1998) performed a time-dependant U accumulation study in shoots of *B. juncea* by assaying shoot concentrations at various times after applying citric acid. Shoot-U concentrations reached the highest levels after three days, whereafter it reached a steady state until harvesting on day seven, suggesting U unavailability following citric acid degradation. Qualls and Haines (1992) and Jones *et al*. (1996) observed similar rapid biodegradation patterns of citric acid. Jones and Darrah (1994) estimated that the half-life of citric acid in soils is 12 hours. This degradation makes citric acid an environmentally friendly soil amendment for U phytoextraction but may also decrease metal bioavailability and subsequent plant accumulation.

To maintain the metal bioavailability for phytoextraction purposes, however, it might be necessary to add citric acid several times at low dosages (Ebbs *et al*., 1998; Wenger, 2000). The optimum time span between two treatments depends on the rate of citric acid degradation (Wenger, 2000). In this approach, citric acid applications could be optimized to meet plant water requirements and also metal accumulation. In so doing, the amount of citric acid and soluble metals in soil will be rapidly reduced, minimizing its leaching risk towards groundwater.

2.5.3 Plant-metal uptake

Plants extract and accumulate metals from the soil solution. Before the metal can move from the soil solution into the plant, it must pass the surface of the root. This can either be a passive process, with metal ions moving through the porous cell wall of the root cells, or an active process by which metal ions move symplastically through the cells of the root. This latter process requires that the metal ions traverse the plasmalemma, a selectively permeable barrier that

surrounds cells (Pilon-Smits, 2005). Special plant membrane proteins recognize the chemical structure of essential metals; these proteins bind the metals and are then ready for uptake and transport. Numerous protein transporters exist in plants. For example, the model plant thale cress (*Arabidopsis thaliana*) contains 150 different cation transporters (Axelsen and Palmgren, 2001) and even more than one transporter for some metals (Hawkesford, 2003). Some of the essential, non-essential and toxic metals, however, are analogous in chemical structure so that these proteins regard them as the same. For example arsenate is taken up by P transporters. Abedin *et al.* (2002) studied the uptake kinetics of As species, arsenite and arsenate, in rice plants and found that arsenate uptake was strongly suppressed in the presence of P. Meharg and Macnair (1990) investigated the mechanism of P and arsenate uptake in arsenate-tolerant and non-tolerant plants and found that arsenate and P were taken up by the roots via the same system, a high-affinity P transporter. Similarly, Reuveny (1977) reported that S and Mo are analogous in their chemical structure. Clarkson and Luttge (1989) reported that Cu and Zn, Ni and Cd compete for the same membrane carriers. No analogue for U has yet been reported.

2.5.4 Root to shoot transport

For phytoextraction to be a viable technology, it is essential that the extracted metal accumulate in a harvestable portion of the plant. Phytoextraction is based on vascular plants' natural ability to take up a variety of chemical elements through the rooting system. These elements are transported via the vascular system to the above-soil biomass (shoots). The shoots are harvested, incinerated to reduce volume, disposed of as hazardous waste, or precious metals can be recycled (phytomining). Different chelators may be involved in the translocation of metal cations through the xylem, such as organic acid chelators (malate, citrate, histidine: Salt *et al*., 1995b; von Wiren *et al*., 1999), or nicotianamine (Stephen *et al*., 1996; von Wiren *et al*., 1999). Since the metal is complexed within a chelate it can be translocated upwards in the xylem without being adsorbed by the high cation exchange capacity of the xylem (von Wiren *et al*., 1999).

Huang *et al*. (1998) increased U accumulation in *B. juncea* shoots more than a 1000-fold from less than 5 mg U kg^{-1} dry biomass to more than 5000 mg U kg^{-1} dry biomass following the addition of 20 mmol citric acid kg^{-1} soil. They evaluated 30 plant species, including *B. juncea* (cultivars 211000 and 426308) and *H. annuus*, for U accumulation when grown on U contaminated soil (750 mg U kg^{-1} soil). They found that the two cultivars of *B. juncea* and *H. annuus* accumulated considerable amounts of U with citric acid additions.

Vandenhove *et al*. (2001) carried out a feasibility study on the phytoextraction of low-level U contaminated soils. They found that the addition of 25 mmol citric acid kg^{-1} soil, one week before harvest, increased the radionuclide ^{238}U accumulation in *B. juncea* shoots by more than 500-fold (317 Bq ^{238}U kg^{-1} soil).

Shahandeh and Hossner (2002a) increased U accumulation in *B. juncea* 426308 by more than 150-fold to 1400 mg U kg^{-1} dry biomass with the addition of 20 mmol citric acid kg^{-1} soil (600 mg U kg^{-1} soil). They concluded that the high shoot-U concentrations could have been caused as a result of a low soil pH induced by citric acid addition that damaged the root cell wall enhancing U transfer and accumulation in shoots by mass flow. Huang *et al.* (1998), however, performed a comparative study on the role of various soil amendments in triggering U bioaccumulation in plants and found that citric acid increased U desorption more than the use of nitric and sulfuric acids at the same pH values. Their results indicate that the reduction in soil pH contributed to only part of the enhanced soil desorption and plant accumulation. They suggested that the main driving force behind the excessive bioaccumulation was a result of the chelation between U and citric acid and the subsequent plant accumulation of the soluble U-citrate complexes. Several studies have shown that once chelates are incorporated into roots, they are translocated almost entirely to the shoots (Hamon *et al*., 1995; Vassil *et al*., 1998).

Metal transporters and metal-binding proteins are involved in the sequestration of the metals to their final destination. It is thought that one class of metal

chelating molecules may play a role in sequestration, the metallothioneins. Metallothioneins are synthesized under conditions of high metal availability. These gene encoded peptide ligands were first identified as Cd binding proteins in mammalian tissues (Goldsbrough, 2000; Cobbett and Goldsbrough, 2000). Metallothioneins may likely play a role in homeostasis of essential metals and in tolerance to non-essential metals. Toxic metals are usually sequestered in the cell's storage organ, the vacuole, where metals can do the least harm to vital cellular processes. For storage inside the vacuole, certain metals may also be complexed by phytochelatins (PC's) which are small cysteine-rich, enzyme induced metal binding peptides, that occur in most plants (Zenk, 1996).

2.5.5 Metal tolerance

Assche and Clijsters (1990) and Reichman (2002) reported that high soil-metal concentrations inhibited a number of cytoplasmic enzymes and proteins which suppressed the ability to translocate metals. Reichman (2002) also found that at high soil-metal concentrations, photosynthesis was reduced, enzyme and protein production was affected and nutrient transport was altered.

High metal exposure to plants can also inhibit root and shoot growth (Fargasova, 1994; Liu *et al*., 1994), cause oxidative damage in shoots (Mylona *et al*., 1998) and decrease chlorophyll content in leaves (Mascher *et al*., 2002).

If high biomass plant species are to accumulate high concentrations of metals in their shoots and simultaneously maintain high growth rates, it is important that they are capable of tolerating the toxic effects of the metal. Tolerance to heavy metals in plants may be defined as the ability to survive in a soil that is toxic to other plants (Macnair *et al.,* 2000).

Some plants possess a range of potential cellular mechanisms that may be involved in the detoxification of toxic metals and thus tolerance to metal stress. Following metal uptake, plants need to store excess essential metals and non-essential metals in localities where they can do the least possible harm. Even

the essential plant metals can become toxic when excessive levels accumulate within plant tissue (Assche and Clijsters, 1990). Ernst *et al.* (1992) showed that the vacuole is the site of sequestration for a number of metals including Zn and Cd. Brune *et al*. (1994) traced the isotope ^{65}Zn into barley leaves and suggested that Zn is rapidly accumulated in the vacuole, away from sensitive organelles functioning in cell growth. Sequestration into the vacuole seems a likely mechanism for dealing with high levels of metals in plants.

2.6 Drought and heat tolerance of *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus*

Gunasekera (2003) did an assessment based on the adaptation of *B. juncea* to dry land conditions in Western Australia, which is characterized by a low to medium annual rainfall (318 mm rainfall per year) and characterized by long dry summers (25 to 40 ℃) and short wet winters (0 to 7 ℃). In one experiment, 10 week old *B. juncea* plants were subjected to severe water stress by erecting a rain shelter covering 1 m^2 of plants grown 18 cm apart. Compared to irrigated plants, shoot biomass production decreased after 3 weeks of growth, but the shoots, nevertheless, doubled in biomass before a severe water stress was experienced. This indicates that *B. juncea* is very well adapted to dry and warm climates. Kimber and McGregor (1995) reported that *B. juncea* can also survive and thrive in low temperatures and is one of the few crops that can be cultivated as a winter crop.

Oram and Kirk (1992) reported that *B. juncea* germinates well in dry soils and the higher concentrations of mucilage in the testa were suggested to contribute to its good ability to germinate in soils with sub-optimum moisture content.

Johnston *et al*. (2002) summarized research from the Canadian prairie and adjacent border states of the United States of America and concluded that *B. juncea* and *H. annuus* were well adapted to areas with warm temperatures and long growing seasons.

Based on these studies, *B. juncea* and *H. annuus* are potential phytoextractors which can be used to remediate heavy metal contaminated soils in areas with warm dry climates.

CHAPTER 3

URANIUM, MOLYBDENUM AND ARSENIC IN THE ENVIRONMENT

3.1 Uranium

3.1.1 Uranium properties and toxicity

Uranium is a naturally occurring element in the earth's crust with an average content of 2 to 3 mg kg^{-1} (U.S. Department of Health, 1999). Uranium is the third element in the actinide series of the periodic table; it has an atomic number of 92, an atomic weight of 238.04 and valences of 3, 4 and 6. Natural U is a mixture of three radioisotopes; ^{234}U (0.01%), ^{235}U (0.72%), and ^{238}U (99.27%). When ^{238}U decays, it changes through a series of different isotopes, including ^{234}U until a stable non-radioactive element has formed, namely ^{206}Pb (U.S. Department of Health, 1999). The half-lives of U isotopes are $2.4x10^5$ for ^{234}U; $7.1x10^7$ years for ^{235}U; and $4.5x10^9$ years for ^{238}U (U.S. Department of Health, 1999). The shorter half-life makes ^{234}U the most radioactive, but because of its low natural abundance, it does not pose any great threat to the environment. The longer half-life makes ^{238}U the least radioactive and poses little radio-toxicity to biological organisms. Acute cell damage by radiation has, however, been observed after exposure to very large doses of radioactivity (US Department of Health, 1999). It is the chemical toxicity of U, however, that poses a great threat to the environment and organisms (Sheppard *et al*., 2005). Uranium compounds can produce cellular injury and tubular necrosis in a variety of mammalian organ systems, including kidney, liver, lung, cardiovascular and central nervous system (Diamond *et al*., 1989).

Kataba-Pendias and Pendias (1984) reported that normal concentrations of U in soil, depending on soil characterstics, are 0.7 to 9 mg U kg^{-1} soil. Bowen (1979) reported, however, that concentrations above 1 mg kg^{-1} soil could already be potentially toxic to biological organisms.

Shahandeh and Hossner (2002a and b) observed significant correlations between shoot-U concentrations of *H. annuus* and indices of tolerance when grown on calcareous soil. With a concentration of 50 mg U kg^{-1} soil, an index of tolerance of 50% was calculated for *H. annuus* shoots. Gulati *et al*. (1980)

observed a decreased dry matter yield in wheat (*Triticum aestivum*) at 6 mg U kg^{-1}soil.

3.1.2 Uranium in soils and plant uptake

Under oxidative soil conditions, U occurs primarily as the uranyl (UO_2^{2+}) cation. Chemically, U behaves similarly to other metal cations in the soil. Ebbs *et al*. (1998) showed that at soil pH of 5.0 to 5.5, the uranyl cation predominates and is the form most readily accumulated by plants. Under calcareous soil conditions, however, U can also be highly mobile and bioavailable due to the formation of soluble carbonate complexes in water (Mason *et al*., 1997; Shahandeh and Hossner, 2002a and b).

Although U and its decaying isotopes have not been shown to be essential or beneficial to either plants or animals, many plant species will absorb U and incorporate it into their biomass along with other heavy metals (Sheppard *et al*., 1989). Laroche *et al*. (2005) studied the uptake and translocation of U in the Common Bean (*Phaseolus vulgaris*) using hydroponic solutions at pH 4.9, 5.8 and 7.0 and found that the dominant species were uranyl ions, hydroxyl complexes and carbonates. The free uranyl ion was the main U-species that accumulated at pH 5.8, whilst hydroxyl- and carbonate complexes did not accumulate readily. Significant U accumulation variance does, however, exist between plant species. Huang *et al*. (1998) examined U shoot translocation in 30 plant species, which included agronomic crops and weeds. Of the 30 plant species tested, only four species (*Brassica juncea*, *B. chinensis*, *B. narinosa* and *Amarantus cruentus*) demonstrated significant potential in U shoot accumulation. The highest shoot and total U accumulation was achieved by *B. juncea*. As a result, further assessments were performed on four cultivars of *B. juncea* (cv. 531268, 18293, 211000 and 426308) to test variance within a species. These cultivars were chosen because of their higher Pb accumulation potential compared to other *B. juncea* cultivars. Cultivar 426308 showed the highest Pb accumulation potential (Kumar *et al*., 1995) while Huang *et al*. (1998) found that cultivar 426308 also displayed the highest shoot U

accumulation. These findings illustrate that *B. juncea* is a promising candidate for U phytoextraction.

3.1.3 The potential of chelate assisted phytoaccumulation of uranium

Chaney *et al*. (1997) and Huang *et al*. (1998) investigated the effects of various soil amendments (synthetic chelates, inorganic and organic acids, sodium- and potassium bicarbonates) to trigger U hyperaccumulation in plants. The organic acid, citrate, proved to be the most effective amendment in enhancing U desorption from soil to soil solution, as well as enhancing U accumulation in plants. They found that the driving force for this enhancement was mainly due to the chelation between U and citric acid and to a lesser extent reduced soil pH. Shahandeh and Hossner (2002a and b) evaluated practical ways to induce U phytoaccumulation in crop plants by chelation, soil acidification and complexation by using DTPA (diethylenetrinitrilotetraacetic acid), HEDTA (hydroxyethylethylenediaminetriacetic acid) and citric acid. The addition of citric acid increased shoot-U accumulation significantly and out of the 34 plant species tested, *Brassica juncea* and *H. annuus* accumulated more U than any other species tested. Ebbs *et al*. (1998) investigated the extent to which HEDTA and citric acid were capable of acidifying U contaminated soil, increasing U solubility and enhancing U uptake by *Beta vulgaris*. Both these amendments decreased the soil pH to 5.5 or less, increasing the solubility of the uranyl cation considerably. Out of these two amendments, citric acid proved to be the most effective chelate in increasing U accumulation. Vandenhove *et al*. (2001) performed a feasibility study regarding the potential of phytoextraction to clean up low-level U contaminated soils. They found that the addition of citric acid, 1 week before harvest, increased U accumulation in *Brassica juncea* 500-fold. In addition, Chen *et al*. (2006) reported that *Pteris vittata*, in association with different mycorrhizal fungi, can increase root-U concentrations significantly.

3.2 Molybdenum

3.2.1 Molybdenum properties and toxicity

Molybdenum (Mo) has an atomic number of 42 and its atomic mass is 95.94. Molybdenum is considered an essential trace element in plants, animals and humans, functioning as a cofactor for various enzymes including xanthine oxidase, an enzyme involved in the breakdown of purines to uric acid in humans. Limited literature regarding Mo toxicity in humans is available, but one of the effects of prolonged Mo exposure can be an increase in uric acid and subsequent development of gout-like diseases and other bone/joint disorders (Wennig and Kirsch, 1988). An outbreak of genu valgum (knock-knees) in India was attributed to an increase in Mo levels in sorghum, the main staple food of the region (Jarrell *et al*., 1980). Ruminants are very susceptible to Mo toxicity. Albasel and Pratt (1989) discovered that a narrow line exists between Mo nutrition in plants (0.5 mg Mo kg^{-1} dry shoot biomass) and potential toxicity in ruminants (10 mg Mo kg^{-1} dry shoot biomass). Mullen *et al*. (2005) found that forages containing excessive amounts of Mo (>10 mg Mo kg^{-1}) could cause a Cu deficiency in cattle and sheep known as molybdenosis. This toxicity is explained as a Cu deficiency, since Mo and S are involved in the formation of thiomolybdates, which bind Cu and render it unavailable to the animal. Neunhäuserer *et al*. (2001) also documented that molybdenosis occurs among cattle feeding on forage with Mo concentrations in excess of 10 mg Mo kg^{-1} DW or a Cu:Mo ratio less than 2. Anderson (1956) recommended a content of <1.5 mg Mo kg^{-1} for safe feed production on alkaline soils.

More literature dealing with the toxic effects of Mo on animals is available compared to the toxicity of Mo on plants. Kataba-Pendias and Pendias (1984), however, reported that a concentration of >2 mg Mo kg^{-1} soil, is a critical concentration at which plant toxicity is considered possible.

3.2.2 Molybdenum in soils and plant uptake

A normal range for Mo in soils is 0.1 to 40 mg Mo kg^{-1} soil (Bowen, 1979). Molybdenum bioavailability is strongly affected by pH; bioavailibility increases with an increasing soil pH (Karimian and Cox, 1978; Foth, 1990; Neunhäuserer *et al*., 2001). Molybdenum in the soil solution at pH >5.5 exists primarily as the anion MoO_4^{2-} and is greatly affected by soil pH. At high pH, Mo is thought to be associated with Ca and as a result readily available for plant uptake. Oxides of Fe and Al also provide positively charged adsorption sites for Mo at low soil pH (Reisenauer *et al*., 1962). The amount of Mo adsorbed has also shown to be closely related to soil organic matter content (Karimian and Cox, 1978).

3.2.3 The potential of chelate assisted phytoaccumulation of molybdenum

Williams and Thornton (1973) used soil amendments, which included the metal chelate EDTA (ethylenediaminetetraacetic acid), and NH_4OH (ammonium hydroxide) to estimate plant-available Mo in potentially toxic soils. Ammonium hydroxide resulted in a higher concentration of Mo being desorbed than using EDTA. They argued that the EDTA extractable Mo was loosely bound in organic complexes, which were chelated by EDTA. The higher NH_4OH extractable Mo was attributable to higher pH and the fact that OH^- groups competed with the Mo on anion exchange sites which increased the Mo bioavailability. They also found that Mo in solution increased with increased organic carbon (C) content.

Neunhäuserer *et al*. (2001) increased Mo bioavailability in Mo contaminated soil (2.5 to 11 mg Mo kg^{-1} soil) by adding phosphate fertilizer, ammonium sulfate, vermiculite, humic acid and sewage sludge. They concluded that the most successful amendment, however, was P fertilizer.

3.3 Arsenic

3.3.1 Arsenic properties and toxicity

Arsenic (As) is a carcinogen, and is associated with animal and human skin, lung and bladder cancers (Ng *et al*., 2003; ATSDR, 2005). Arsenic, often described as a metalloid, has an atomic number of 33 and its atomic mass is 74.92. In soils, arsenate and arsenite are the main forms of As (Harper and Haswell, 1988). During the mining and smelting of metals such as Cu (copper) and Pb (lead), As may enter the environment and cannot be destroyed or broken down. It can only change form or become attached or separated from soil particles (U.S. Department of Health, 2005). Sheppard *et al*. (1992) mentioned that As has a low to moderate mobility in soils and that excavation is probably the best remediation option available. Arsenic has been recognized as a toxin and can be carcinogenic depending on its chemical and physical forms, concentration and duration of exposure (Sheppard *et al*., 1992; U.S. Department of Health, 2005).

Kataba-Pendias and Pendias (1984) reported that a As concentration of 20 mg As kg^{-1} soil is considered to be toxic to organisms. Deuel and Swoboda (1972) reported a 55% decrease in *Glycine max* (soybean) shoot biomass when grown in 12.5 mg As kg^{-1} soil. Leaf wilting, violet coloration (increased anthocyanin), root discoloration and cell plasmolysis are the main As toxicity symptoms. However, the most common symptom is growth reduction (Kitagishi and Yamane, 1981). Speer (1973) reported that As reacts with sulfhydryl groups of proteins causing disruption of root functions and also acts as a decoupler of phosphorylation in mitochondria (Terwelle and Slater, 1967). Mylona *et al*. (1998) also reported increased oxidative stress in maize plants exposed to 0.01 to 0.1 mM As (0.74 to 7.4 mg As l^{-1}).

3.3.2 Arsenic in soils and plant uptake

Normal ranges for As in soils is 0.1 to 40 mg As kg^{-1} soil. Arsenic is predominantly present as an anion in soils (Sadiq *et al*., 1983). In general, arsenate compounds predominate in aerobic soils, whereas arsenite compounds predominate in slightly reduced soils. Arsenate adsorbs more strongly to soil particles than arsenite and is thus the less mobile form (Pierce and Moore, 1982). Arsenate is chemically analogous to P and plant root uptake takes place through the same plasmamembrane proteins (Barrow, 1974). These proteins, however, have a greater affinity for P than As (Meharg and Macnair, 1990). Arsenic is a nonessential element for plants, and inorganic As species are generally highly phytotoxic (Smith *et al*., 1998). Arsenic can disrupt phosphate metabolism and inhibit plant cellular function leading to plant death (Meharg and Hartley-Whitaker, 2002).

Smith *et al*. (1998) and Jain *et al*. (1999) investigated the adsorption properties of As in soil and found that As was strongly adsorbed onto soil particles, was highly correlated with Fe-oxide and relatively immobile in soils. Wang *et al*. (2002) also reported that the transfer of As from soil to plant is low as a result of: i) the restricted uptake by plant roots due to low As bioavailability; ii) the limited translocation of As from root to shoot; iii) and As phytotoxicity even at low concentrations in plant tissues.

Jones *et al*. (1997), Darland and Inskeep (1997) and Sadiq *et al*. (1983) increased As bioavailability by increasing soil pH with the addition of lime. They reported that because As is mostly present as an anion in soils, its bioavailability increases with increasing pH. Jang *et al*. (2005) investigated alkaline and acid washing effluents to determine optimum parameters for remediation of As contaminated soils by alkaline washing. Sodium hydroxide (NaOH) was 10 to 20 times more effective than citric- or hydrochloric acid (HCl). They reported that at a high pH, OH^- ions displace As from anion exchange sites into the soil solution.

3.3.3 The potential of chelate assisted phytoaccumulation of arsenic

Limited literature regarding chelate assisted phytoaccumulation of As is available. The addition of the dithiol As chelator dimercaptosuccinate (DMS) to As contaminated soils has been suggested to promote As accumulation in plant shoots (Pickering *et al*., 2000).

The fern *Pteris vittata* (Chinese brake fern), capable of hyperaccumulating As without added chelates, was recently discovered showing exceptional accumulation, translocation and tolerance of As. It is up to now the only plant that can successfully be utilized as an As phytoextractor (Ma *et al*., 2001; Chen *et al*., 2002; Doucleff and Terry, 2002; Salido *et al*., 2003). Doucleff and Terry (2002) reported that this primitive plant thrives on As. They reported that this fern's biomass increased considerably when subjected to 100 mg As kg^{-1} soil. This fern's remarkable translocation of As from roots to fronds makes it an ideal plant to use for the phytoextraction of As from contaminated soils (Doucleff and Terry, 2002).

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

Indian mustard (*Brassica juncea* cv. 211000 and 426308, supplied by North Central Regional Plant Introduction Station[2], Iowa State University, Ames, USA) and sunflower (*Helianthus annuus* cv. Pannar 7351; supplied by Pannar[3], South Africa) were used in the greenhouse and laboratory investigations.

All chemicals used were purchased from Merck South Africa[4] and was of the highest analytical grade.

4.2 Methods

4.2.1 Glassware and instrumentation

All glassware and instrumentation used in this study were pre-washed with 14% HNO_3 and rinsed with deionized water according to the method of Tessier *et al*. (1979).

4.2.2 Soil Sampling

Soil samples were collected during May 2005 from an abandoned U-trial mining site on the farm Rietkuil 307 in the Beaufort West district, Karoo Uranium Province (*cf.* Figure 1.1). Soil was sampled 1 to 3 m from the ore stockpiles at a depth of 20 cm using a stainless steel scoop (Boulding, 1994). Soil samples were also collected in random sample plots four kilometers upwind from the ore stockpiles to be used as background soil in greenhouse and laboratory investigations. Sampled soil was allowed to air dry for two weeks. This was followed by sieving the soil through a 2-mm stainless steel mesh to remove large stones and debris to obtain soil samples with particles <2mm. The soil was thoroughly mixed in order to obtain a representative sample (soil-1) and

[2] North Central Regional Plant Introduction Station, Iowa State University, Ames IA 50011-1170, United States of America.
[3] Pannar Seed (Pty) Ltd, P.O. Box 19, Greytown, 3250, South Africa.
[4] Merck (Pty) Ltd, South Africa, www.merck.co.za

was stored at room temperature (22 °C) in the laboratory until greenhouse pot trials and further analyses.

4.2.3 Artificial contamination of soil

Heavy metal concentrations of the original soil (soil-1) were artificially enhanced by mixing pulverized U ore, collected from the ore stockpiles on Rietkuil farm 307, to some of the sampled soil at a rate of 100 g ore/900 g soil. This supplied a high-level metal concentrated soil (soil-2), which was used in the metal phytoextraction assessments. This was done to determine the effectiveness of phytoextraction if the metal concentrations in the soil were to increase as a result of long term failure to remove the U ore stockpiles. This soil-2 mixture was also stored at room temperature (22 °C) until pot trials and further analyses.

4.2.4 Physical and chemical properties of soils

Organic-, inorganic- and total carbon (C) content and CEC (cation exchange capacity) of soils were measured by the Small Grain Institute in Bethlehem[5], South Africa. Particle size distribution was measured by Glen Agriculture College, Bloemfontein[6], South Africa.

The carbon content of soils was measured by total combustion using a Skalar Primacs Carbon Analyzer. For total carbon, the samples were heated to 1200 ˚C in the carbon analyzer and the excited C measured by a non-dispersive infrared detector. For inorganic carbon, phosphoric acid was added to the sample and the excited C measured by a non-dispersive infrared detector. Organic carbon was calculated from the difference between total C and inorganic C.

Cation exchange capacity (CEC) was determined by extracting 3 g air dried soil with two successive 60 ml aliquots of 1 *M* NH_4OAc (ammonium acetate) at pH 7

[5] Small Grain Institute, Private Bag X29, Bethlehem, 9700, South Africa.
[6] Glen Agriculture College, Private Bag X01, Bloemfontein, 9360, South Africa.

and the resulting leachate was analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The CEC was then calculated from the sum of the exchangeable cations (Page *et al.*, 1982).

Particle size distribution was measured by the hydrometer method. This method quantitatively determines the physical proportions of three sizes of primary soil particles (sand ranges from 2000 – 50 µm; silt from 50 – 2.0 µm; and clay <2.0 µm) as determined by their settling rates in an aqueous solution using a hydrometer according to the methods of Gee and Bauder (1986).

Soil pH (H_2O) was measured in a 1:1 soil:water ratio mixture (USDA, 2004). Ten grams of soil were shaken in 25 ml of distilled water for 10 minutes. The suspension was allowed to settle for 30 minutes followed by pH analysis of the cleared suspension using a PHM 85 Precision pH meter.

Electrical conductivity of the soils was measured according to the ISO 11 265 (1994) method. Distilled water in the ratio 5:1 (water:dry soil) was added to soils, mechanically shaken for 30 minutes, filtered and the EC measured in the extract.

4.2.5 Analysis of total trace metals in soil samples

Soil samples were analysed for trace element concentrations on an Axios X-ray spectrometer (XRF) at the Geology Department, University of the Free State. Trace element analyses were performed on pressed powder briquettes. Loss of water was determined at 110 °C and is reported as H_2O-. Loss on ignition (LOI) was determined at 1000 °C. The results obtained correspond to the overall/total trace elemental content of the soil (Pfeifer *et al.*, 2000).

4.2.6 Water soluble metal fractions of soils

The water soluble metal fraction of each soil was determined by adding 25 ml of deionized water to a 2 g sample of each soil. The solutions were stirred for 16 h

on a magnetic stirrer and then centrifuged at 4000g for 20 minutes. The pH of the desorbed solutions was measured by placing the pH electrode in the supernatant. The supernatants were then filtered through Whatman's no. 1 filter paper and stored in glass bottles until further analysis (Huang *et al.,* 1998; Shahandeh and Hossner, 2002a). The desorbed solutions were analyzed for its U, Mo and As content using ICP-OES.

4.2.7 Acid digestion of heavy metals from soil samples

The potential bioavailable uranium (U), molybdenum (Mo) and arsenic (As) in soils were extracted by acid digestion using aqua regia [hydrochloric acid (HCl):nitric acid (HNO_3); 3:1] according to method 3050B (USEPA, 1996). Digestion with aqua regia is a strong acid digestion technique that will digest almost all elements that could become environmentally available. Elements bound in silicate structures are not normally digested by this procedure as they are not usually mobile in the environment (Pfeifer *et al*., 2000; USEPA, 1996; Chen and Ma, 1998). The sampled soil (soil-1) and soil-ore mixture (soil-2) were weighed to 0.5 g and placed in glass digestion tubes. Nine mililiters of aqua regia was added to the tubes and the contents digested on a Labotec Heat-O-Mat hotplate (Figure 4.1) at 90 ℃ for 3 hours. Following cooling, 6 ml of 65% HNO_3 and 3 ml of 30% H_2O_2 were slowly added to the tubes to prevent foaming and were subsequently replaced at 90 ℃ for 13 h. After digestion, solutions were allowed to cool followed by filtration through Whatman's No.1 filtration paper. The filtered solutions were diluted to 100 ml with deionized water. The heavy metal content in these solutions were analysed using ICP-OES. The acid digested soil-metal analyses were performed in triplicate.

4.2.8 Measuring metals in liquid solutions using Inductively Coupled Plasma Optical Emission Spectrometry

ICP-OES is a spectrometer used for measuring trace amounts of metals in liquid solutions (sensitivity <1 ppm). A liquid sample of both soil and plant extracts were sprayed into argon plasma where the metals were atomized at

8000 °C and excited to emit characteristic ultraviolet and visible radiation. Radiation wavelength (U – 385.958 nm; Mo – 202.032 nm; As – 188.985 nm) is used to identify the element and radiation intensity is used to determine its concentration. All metal analyses were performed by the Institute of Groundwater Studies[7], University of the Free State.

4.2.9 Heavy metal soil desorption

Desorption procedures were carried out to test citric acid's chelating ability of U, Mo and As. The citric acid concentrations used were based on the concentrations of Huang *et al.* (1998) and Shahandeh and Hossner (2002a) namely: 0.8 mM, 1.2 mM, 1.6 mM and 2.0 mM citric acid stock solutions.

Twenty five (25) milliliters of each solution was added to 2 g soil samples (soil-1 and soil-2), delivering final concentrations of 10-, 15-, 20-, and 25 mmol citric acid kg^{-1} soil, respectively. Samples were prepared the same way as the water soluble metal measurements and soil pH analysis (*cf.* 4.2.6).

4.2.10 Germination of seeds

Seeds of *Brassica juncea* (21000 and 426308) and *Helianthus annuus* were placed on moistened filter paper in 9 cm Petri dishes and incubated at 27 °C for three days. Germinated seeds were transplanted to seedling trays containing a commercial seedling growth medium. Seedlings were moistened when required by spraying the seed trays with distilled water. Seedlings were allowed to establish for 2 weeks before transplantation into pots for the greenhouse trials.

4.2.11 Procedure for pot trials

One kilogram of low-level- (soil-1), high-level contaminated soil (soil-2) and background soil was saturated to field capacity (150 ml kg^{-1}) with full strength of

[7] Institute of groundwater studies, University of the Free State, Bloemfontein, 9300, South Africa.

modified Johnson's nutrient solution (Johnson *et al*., 1957), which excluded Mo due to a possible interference with shoot-metal concentrations, as outlined in Table 4.1. Seedlings were transplanted into soil-1, soil-2 and background soil and kept moistened at field capacity with modified Johnson's nutrient solution. Leachate (if any) was collected in the collection pans underneath each pot and immediately reapplied to the soil surface. Established plants were grown for an experimental period of 42 days (six weeks) before harvesting (Huang *et al*., 1998; Wenger, 2000).

Table 4.1 Modified Johnson's nutrient solution.

Compound	Stock concentration	Volume/ℓ final solution, (ml/ℓ)		Element	Final concentration (ppm)
Macronutrients[1]					
KNO_3	1 M	6		N; K	224; 235
$Ca(NO_3)$	1 M	4		Ca	160
$MgSO_4 \cdot 7H_2O$	1 M	1		Mg; S	24; 32
Micronutrients[2]					
KCl	50 mM	1		Cl	1.77
H_3BO_3	25 mM	1		B	0.27
$MnSO_4 \cdot H_2O$	2 mM	1		Mn	0.11
$ZnSO_4 \cdot 7H_2O$	2 mM	1		Zn	0.131
$CuSO_4 \cdot 5H_2O$	0.5 mM	1		Cu	0.032

[1] No P (phosphorous) was added to the nutrient solution to avoid the formation of phosphate-metal complexes that would consequently be unavailable for plant uptake (Ebbs *et al*., 1998; Cooper *et al*., 1999). To prevent P deficiency, however, above soil plant parts were sprayed with 10 mM KH_2PO_4 (pH 6) twice a week until harvesting (Ebbs *et al*., 1998). Care was taken to prevent the foliar-P spray running down the stem onto the soil.

[2] No molybdenum (Mo) was added in the micronutrient stock solution.

[2] Made up into a single stock solution.

4.2.11.1 Citric acid additions to pot trials

Citric acid was chosen as metal chelating agent for this study. Based on previous studies (Shahandeh and Hossner, 2002a; Vandenhove *et al*., 2001;

Huang *et al*., 1998; Ebbs *et al*., 1998) plants from each species grown in soil-1 and soil-2 were randomly selected one week before harvest for one of the following three treatments: 1) a single addition of 50 ml of 125 mM citric acid stock solution (Treatment A: 25 mmol citric acid kg^{-1} soil); 2) successive additions of 50 ml of 62.5 mM citric acid stock solution three days apart (Treatment B: 2 x 12.5 mmol citric acid kg^{-1} soil); and 3) 50 ml of distilled water which was regarded as a control treatment. Each treatment was replicated three times per species (equaling 9 plants per species). A total of 5.25 g citric acid kg^{-1} soil was added with both of the citric acid treatments. Plants were harvested 1 week after the different treatments. A summarization of the experimental setup is shown in Figure 4.1.

4.2.12 Soil pH measurements following plant growth

At the time of harvesting plant shoots, soil samples from the pots treated with citric acid (treatments A and B) were taken for pH measurements in a 1:1 soil:water ratio (*cf.* 4.2.5).

4.2.13 Harvesting of plant material

After applicable treatments the plants were cut 1 cm above the soil surface. Shoots were brush-washed with distilled water to remove externally adhered metals. The harvested shoots were air dried in brown paper bags at room temperature (27 ˚C) for three weeks until constant dry weights (DW) were obtained.

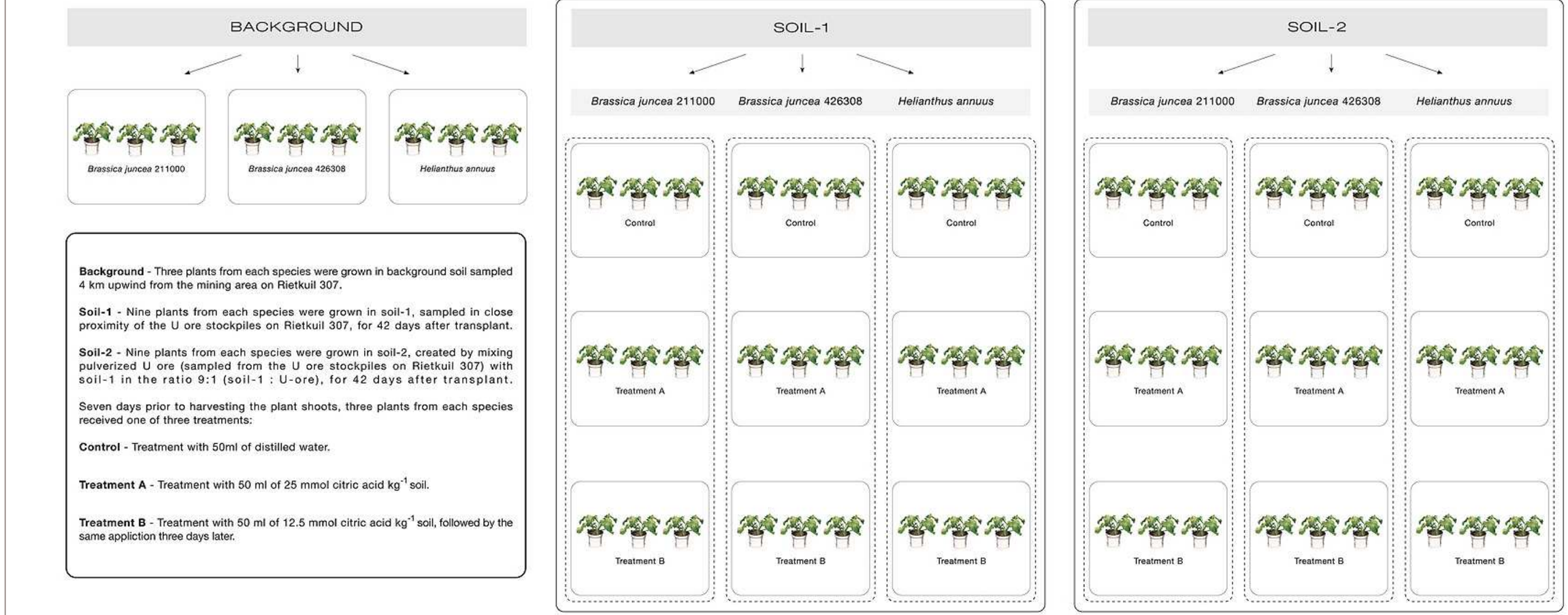

Figure 4.1 Experimental setup of the citric acid induced U, Mo and As phytoextraction pot trials for *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus*.

4.2.14 Acid digestion of heavy metals from plant tissue

Because phytoextraction involves the harvesting of plant shoots for metal removal, only plant shoots were harvested and analyzed for metal content (Vandenhove *et al*., 2001; Huang *et al*., 1998; Ebbs *et al*., 1998). A modified acid digestion technique of Lamas *et al.* (2002), where aqua regia (*cf.* 4.2.7) replaced Nitric acid, was used to extract heavy metals from plant tissue. Following air drying, shoot material was powdered in liquid nitrogen and 0.2 g of this tissue was placed in glass digestion tubes. Nine mililitres of aqua regia was added to the vessels and digested on a Labotec Heat-O-Mat hotplate (Figure 4.2) at 90 ˚C for 8 hours. After cooling, the samples were filtered using Whatman's no. 1 filter paper and the filtrate was made up to 50 ml with deionized water. Samples were stored in sterilized glass bottles until further analysis by ICP-OES.

Figure 4.2 Acid digestion of metals in soils and plant shoots in the Labotec Heat-O-Mat hotplate at 90 $^{\circ}$C.

4.2.15 The Site-Specific Phytoextraction Potential of *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus* from low- and high-level metal contaminated soils

The potential of phytoextraction depends on the amount of U extracted and amount of biomass produced. The Site-Specific Phytoextraction Potential (SPP) gives an estimate of a plant's potential to remove a metal contaminant (kg) from one hectare of soil after one year (Hinton *et al*., 2005) and can be calculated using equation 1.

Eq (1):

$$\mathbf{SPP} = \frac{\mathbf{[DW\ yield (kg\,ha^{-1})\ x\ IOT\ (\%)]\ x\ TF\ x\ C_{soil}\ (mg\,kg^{-1})}}{\mathbf{CF}}$$

$$= \mathbf{kg\,ha^{-1}}$$

where the **DW yield (kg ha^{-1})** represents a projected shoot dry weight (DW) yield in kg ha^{-1}. This was estimated using DW results obtained from the greenhouse pot trials; **IOT %** is the index of tolerance (*biomass of a species grown in the metal concentrated soil* divided by the *biomass of the plant species grown in the background soil x 100*); **TF** is the transfer factor (*mg kg^{-1} contaminant concentration in dry shoot biomass / mg kg^{-1} contaminant concentration in dry soil*); **C$_{soil}$ (mg kg^{-1})** represents the metal concentration in the soil; and **CF** the mg to kg conversion factor of 10^6.

The number of years required to phytoextract metals (U, Mo and As) from soil-1 and soil-2 to background soil metal concentrations can be predicted by using equation 2.

Eq (2):

$$\textbf{Number of years} = \frac{\textbf{[Contaminated soil metal] (kg ha}^{-1}\textbf{) - [Background soil metal] (kg ha}^{-1}\textbf{)}}{\textbf{SPP}}$$

where the **[Contaminated soil metal] (kg ha^{-1})** and the **[Background soil metal] (kg ha^{-1})** represents the acid digested concentration of U, Mo or As, predicted per hectare of soil, which were calculated based on the initial metal concentrations measured (mg kg^{-1}), at a soil depth of 20 cm and a soil density of 1.30 g cm^{-3}. The **SSP** represents the Site-Specific Phytoextraction Potential.

CHAPTER 5

RESULTS

5.1 Soil characteristics

The low-level metal containing soil (soil-1) was sampled near an abandoned U-trial mine in the Beaufort West district of the Karoo Uranium Province (*cf.* Figure 1.1). A high-level metal containing soil (soil-2) was prepared by mixing pulverized U-ore, from the stockpiles (*cf.* Figure 1.2 and Figure 1.3), with soil-1 in the ratio of 1:9 (*cf.* 4.2.3). A background soil was sampled upwind from the trial mining area.

Soil-1 and the background soil were similar in their physical and chemical properties. The addition of pulverized U-ore to soil-1 (soil-2), however, resulted in a 46% increase in clay content, 93% in organic- and 100% in inorganic carbon (C) content. Increases in the electrical conductivity (EC) of 600% and the cation exchange capacity (CEC) of 100%, also occurred when soil-2 was prepared. The soil pH and the bulk densities, however, remained approximately the same between the different soils (Table 5.1).

Table 5.1 Physical and chemical properties of background soil, soil-1 and soil-2.

Soil Properties	Background soil	Soil-1	Soil-2
Clay (%)	28	24	35
Sand (%)	62	76	65
Total C (%)	0.17	0.15	0.29
Organic C (%)	0.09	0.10	0.19
Inorganic C (%)	0.05	0.05	0.1
EC (mS/m)	29	32	224
CEC ($cmol_c/kg$)	6.2	7.28	14.55
pH	7.45	7.36	7.65
Density ($g\ cm^{-3}$)	1.30	1.30	1.30

Total trace element concentrations in the different soils were determined with XRF (*cf.* 4.2.5). The concentrations (Table 5.2) in soil-1 that were above the background concentrations were arsenic (As), barium (Ba), copper (Cu), molybdenum (Mo), rubidium (Rb), thorium (Th), uranium (U) and vanadium (V).

However, the U, Mo and As concentrations in soil-1 were also above the normal ranges measured for metals in soils (Bowen, 1979), while U and Mo were above the critical soil concentrations (Kabata-Pendias and Pendias, 1984; Table 5.2).

The total U, Mo and As concentrations increased substantially following the addition of pulverized U ore to soil-1 (soil-2). The increases were 7-, 42- and 5-fold for U, Mo and As, respectively. These concentrations were also above the critical ranges measured for metals in soils (Kataba-Pendias and Pendias, 1984; Table 5.2).

Table 5.2 Trace element concentrations (mg kg^{-1} soil) of the different soils.

Element	Background	Soil-1	Soil-2		Normal range[1]	Critical range[2]
As	2.1	11	56		0.1 - 40	20
Ba	655	687	674		na	na
Cu	28	30	46		30	50
Co	4.0	2.7	8.8		na	na
Cr	33	31	42		34	75
Ga	15	15	17		na	na
Mo	1.5	4.1	173		0.1 - 40	2
Nb	9	9	10		na	na
Ni	17	17	19		2 - 750	100
Pb	16	18	22		42	100
Rb	111	112	127		na	na
Sc	11	8	10		na	na
Sr	126	119	132		na	na
Th	9	9	10		na	na
U	7	30	205		0.7 - 9	1
V	61	63	69		100	50
Y	23	22	23		na	na
Zn	45	44	50		50	70
Zr	274	221	223		na	na

[1] The normal soil concentration is an average derived for world soils. Data from Bowen (1979).
[2] The critical soil total concentration is the values above which toxicity is considered to be possible. Data from Kataba-Pendias and Pendias (1984).

The U, Mo and As levels directly bioavailable for plant uptake (water soluble fraction) were obtained by washing the soils with distilled water and analyzing cleared supernatants for U, Mo and As using ICP-OES (*cf.* 4.2.6). No U, Mo or As could be detected in the background soil above the lowest limit of detection (LLD: Table 5.3). A comparison between soil-1 and soil-2 revealed that the latter contained substantially more U, Mo and As in the water soluble fraction (Table 5.3). The preparation of soil-2, by mixing pulverized-ore with soil-1, resulted in substantial increases in the water-soluble fractions, especially for U (9.4-fold) and Mo (67.5-fold).

Table 5.3 Water-soluble concentrations of U, Mo and As in background-, low-level- (soil-1) and high-level (soil-2) containing soils collected at a depth of 20 cm. (± = standard deviation). n = 3

	Water soluble metal concentration (mg kg^{-1} soil)			**Ratio**
Metal	**Background**	**Soil-1**	**Soil-2**	**Soil-1:Soil-2**
U	<5	0.7 ±0	6.6 ±0.76	1 : 9.4
Mo	<0.4	0.8 ±0.12	54 ±5.0	1 : 67.5
As	<1	0.22 ±0.050	0.49 ±0.071	1 : 2.23

Consequently, the potential bioavailable bound U, Mo and As concentrations in the different soils were determined after acid digestion and analysis by ICP-OES (*cf.* 4.2.7). No U, Mo or As could be detected in the background soil above the lowest limit of detection (LLD: Table 5.4). The acid digested U, Mo and As concentrations in soil-1 were clearly elevated when compared to the background soil. However, in comparison with soil-1, substantial increases of 6-fold for U, 30-fold for Mo and 5.5-fold for As, were measured in soil-2.

Table 5.4 Acid digested concentrations of U, Mo and As in background-, low-level- (soil-1) and high-level (soil-2) containing soils collected at a depth of 20 cm. (± = standard deviation). n = 3.

	Acid digested metal concentration (mg kg^{-1} soil)			Ratio
Metal	**Background**	**Soil-1**	**Soil-2**	**Soil-1:Soil-2**
U	<5	28 ±5	168 ±16	1:6
Mo	<0.4	4.1 ±0.6	125 ±12	1:30
As	<1	8.9 ±1.2	49 ±0.8	1:5.5

5.2 Citric acid desorption of soil metals

Soil-1 and soil-2 were subjected to increasing concentrations of citric acid in solution (0 to 25 mmol kg^{-1} soil) to examine the ability of citric acid to act as a chelating agent on U, Mo and As. The effect was investigated by measuring the changes in the pH and U, Mo and As content in the soil solution (*cf.* 4.2.9).

Both soil-1 and soil-2 are strongly alkaline, but following the addition of 25 mmol citric acid kg^{-1} soil, a decrease of up to 3.5 pH units were observed in soil-1, whilst a decrease of only 1 pH unit was observed in soil-2 (Figure 5.1). This indicated that soil-2 has a greater capacity to resist acidification than soil-1.

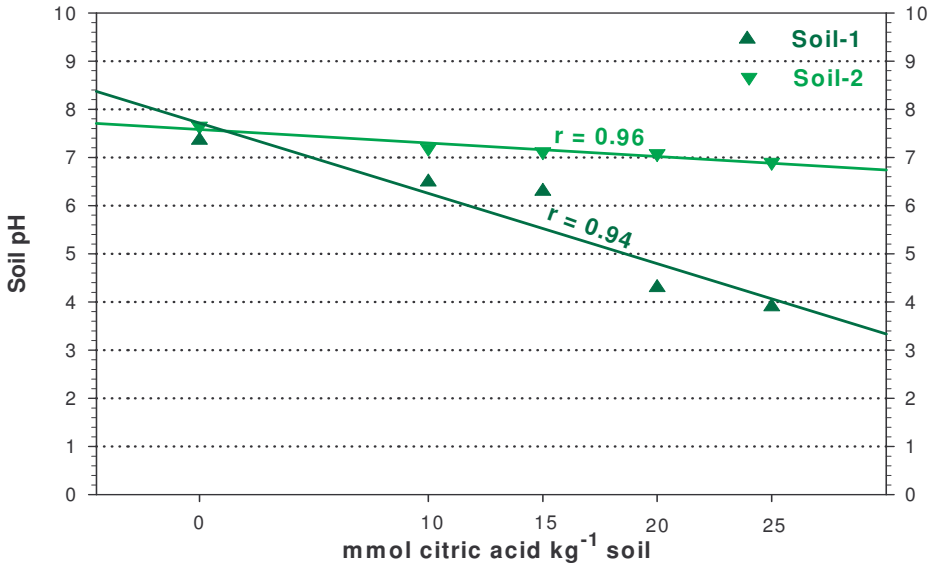

Figure 5.1 Changes in pH values of soil-1 and soil-2 following citric acid additions ranging from 0 to 25 mmol citric acid kg^{-1} soil.

Citric acid addition also proved to be effective in desorbing U, Mo and As from soil-1 and soil-2 (Figure 5.2a to c). Addition of citric acid linearly desorbed U, Mo and As from both soils as the concentration of citric acid increased.

Metal desorption (Figure 5.2a to c) was found to be the greatest at the highest concentration of citric acid used (25 mmol citric acid kg^{-1} soil). Addition of 25 mmol citric acid kg^{-1} soil, increased U solubilization 27-fold, Mo 2.4-fold and As 1.8-fold in soil-1 (Table 5.5). Similarly, the solubilization of U, Mo and As in soil-2 increased 8.3-fold, 1.7-fold and 1.6-fold, respectively. Hence, the degree of metal desorption was greater from soil-1 than soil-2, which can probably be attributed to the greater buffer capacity and total C of soil-2 (Figure 5.1).

Table 5.5 Changes in the metal concentrations (mg kg^{-1} soil) and ratios between soil-1 and soil-2 after desorption with 25 mmol citric acid kg^{-1} soil. (± = standard deviation). n = 3.

	Changes in metal concentrations (mg kg -1) after desorption with 25 mmol citric acid kg^{-1} soil					
	Soil-1			**Soil-2**		
Metal	**0 mmol kg^{-1}**	**25 mmol kg^{-1}**	**Ratio**	**0 mmol kg^{-1}**	**25 mmol kg^{-1}**	**Ratio**
U	0.7 ±0	19 ±2.0	1 : 27	6.6 ±0.76	55 ±6.0	1 : 8.3
Mo	0.8 ±0.12	1.9 ±0.21	1 : 2.4	54 ±5.0	90 ±5.5	1 : 1.7
As	0.22 ±0.050	0.39 ±0.051	1 : 1.8	0.49 ±0.071	0.79 ±0.028	1 : 1.6

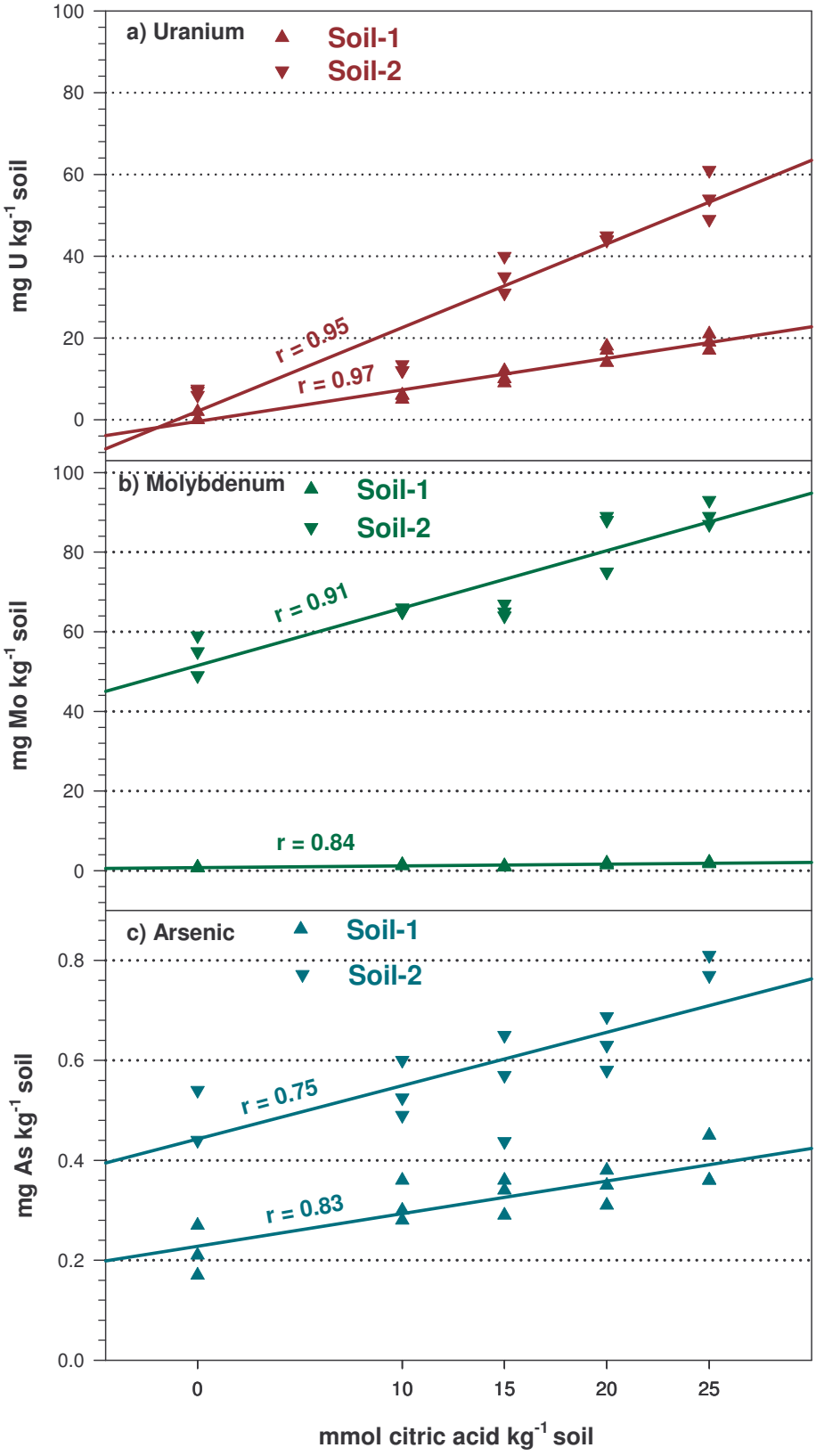

Figure 5.2a to c Efficiency of citric acid additions (0 to 25 mmol citric acid kg^{-1} soil) in enhancing U (a), Mo (b) and As (c) desorption from soil-1 and soil-2. n = 3.

5.3 Citric acid induced metal accumulation in *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus* shoots

5.3.1 Shoot accumulation of heavy metals

The test plants were treated with 25 mmol citric acid kg^{-1} soil in the greenhouse trial since metal desorption was the most pronounced at this concentration (Figure 5.2a to c). Plants from each species grown in soil-1 and soil-2 were randomly selected after 35 days of growth for one of two citric acid treatments. The treatments were: Treatment A (single addition of 25 mmol citric acid kg^{-1} soil) and Treatment B (successive additions of 12.5 mmol citric acid kg^{-1} soil, three days apart). The remaining plants from each species were watered with the same volume of distilled water and regarded as control plants. Each treatment was replicated three times for each species (*cf.* Figure 4.1). Plant shoots were harvested after 7 days following the above treatments. Plant shoots were air dried, acid digested and the shoot metal content analyzed using ICP-OES (*cf.* 4.2.14).

5.3.1.1 Uranium (U) accumulation

Compared to the control plants, both Treatment A (single citric acid addition) and Treatment B (successive citric acid additions) resulted in higher U concentrations in plant shoots grown in both soil-1 and soil-2 (Figure 5.3 A). The concentrations were, however, higher when citric acid was added successively in both soils. Moreover, plants accumulated the highest U concentrations in their shoots when grown in soil-1. The largest U accumulation was observed in *B. juncea* 211000 (1788 mg U kg^{-1} dry biomass), which was 85-times the concentration of the control plants, when citric acid was added successively (Treatment B).

5.3.1.2 Molybdenum (Mo) accumulation

Control plants of *B. juncea* (cultivars: 211000 and 426308) attained higher concentrations of Mo when grown in soil-1 than in soil-2, whilst in contrast, *H. annuus* revealed higher concentrations when grown in soil-2 (Figure 5.3 B).

Treatments A and B resulted in Mo concentrations being greater in all the plant species compared to the control plants. Similar to the U concentrations, Treatment B also resulted in the highest shoot-Mo concentrations for both soils. The largest Mo accumulation was again observed in *B. juncea* 211000 (467 mg Mo kg^{-1} dry biomass) which increased 2.3-fold following the successive addition of citric acid (Treatment B) to soil-1.

5.3.1.3 Arsenic (As) accumulation

No As in plant shoots grown in soil-2 could be detected above the lowest limit of detection (LLD: <1 mg kg^{-1}). Similar to U and Mo accumulation, the As concentrations in plant shoots grown in soil-1 were greater following treatments A and B with the highest concentration measured for Treatment B (Figure 5.3 C). The largest As accumulation was again observed in *B. juncea* 211000 (24 mg kg^{-1}), which increased 11-fold following the successive addition of citric acid (Treatment B).

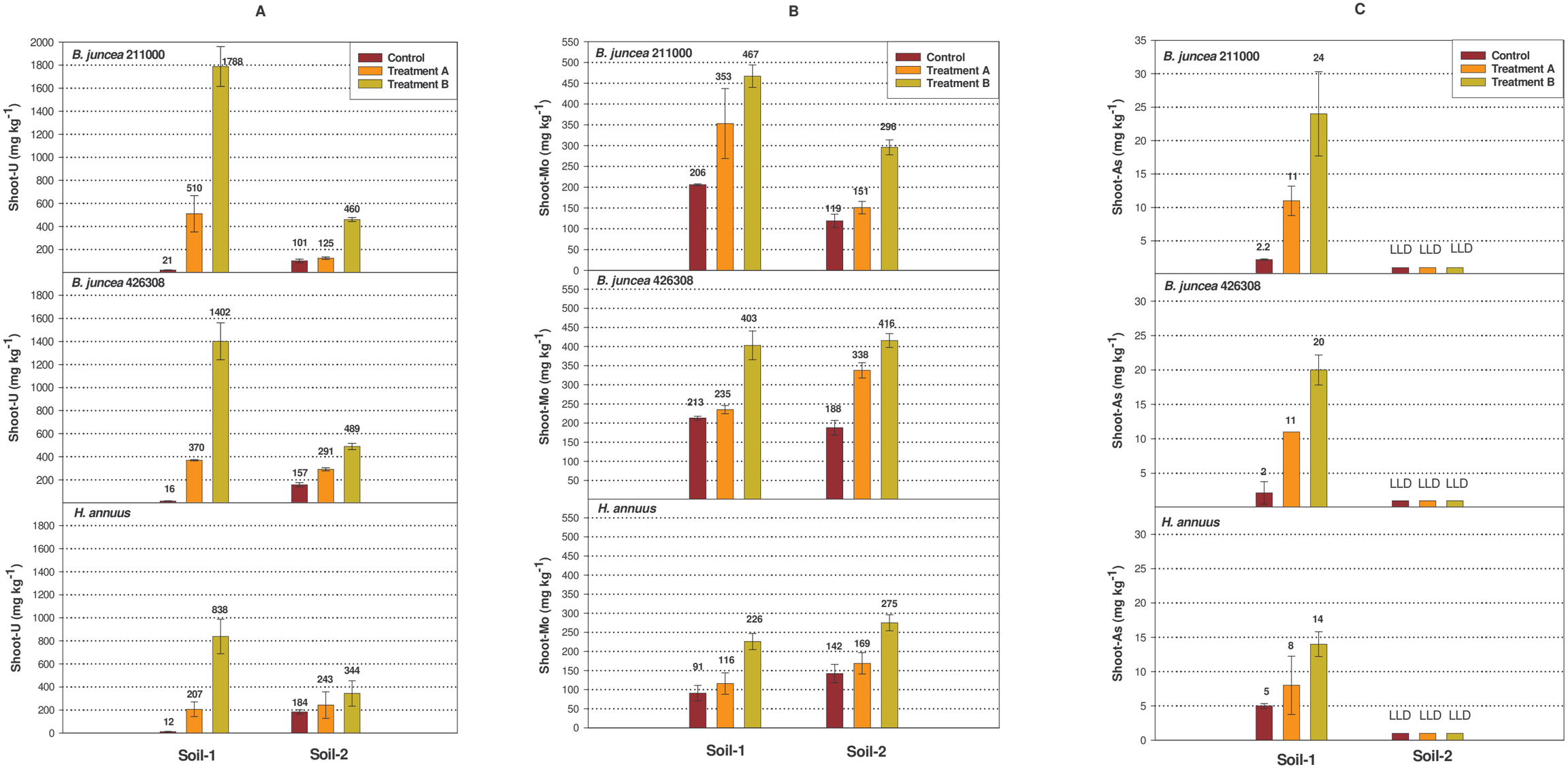

Figure 5.3 A to C Uranium (A), molybdenum (B) and arsenic (C) accumulation in *B. juncea* (cultivars: 211000 and 426308) and *H. annuus* grown in soil-1 and soil-2 amended with 25 mmol- (Treatment A) and 2 x 12.5 mmol citric acid kg^{-1} soil (Treatment B). n = 3

5.4 The effect of citric acid on soil pH during pot trials

On the day of shoot harvests, soil samples were taken and the soil pH measured (*cf.* 4.2.12). Additions of citric acid resulted in pH decreases (Table 5.5) of up to 1.7 units for soil-1 and 1.0 unit for soil-2, when compared to control soils (0 mmol citric acid kg^{-1} soil). The pH of the citric acid soil extracts measured in the desorption studies (25 mmol citric acid kg^{-1} soil) were, however, considerably lower (Figure 5.1). Probably due to the decomposition of citric acid, the pH of soil at the time of plant harvest was higher (less acidic) than the soil pH in the desorption studies (desorption time - 16 hours).

Table 5.6 Changes in soil pH following harvesting of plants grown for 42 days in control and citric acid amended soils.

	Soil pH		
	Control	**Treatment A**	**Treatment B**
Soil-1	7.27	5.53	5.50
Soil-2	7.68	6.68	6.70

5.5 Bioaccumulating potential of *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus*

The soil metal-to-shoot transfer factor, defined as the concentration of a metal in the shoot divided by the concentration of a metal in the soil, is used to evaluate the potential of metal-bioaccumulation in plants (Baker *et al*.,1994; Vandenhove *et al*., 2001) and to calculate the Site Specific Phytoextraction Potential (SSP) of plant species (*cf.* 4.2.15).

The majority of transfer factors (TFs) calculated for the treated plants grown in soil-2 were less compared to the TFs for plants grown in soil-1 (Table 5.7). No As TFs could be obtained for plants grown in soil-2 due to As being undetectable in these plant shoots (Figure 5.3 C). Compared to control plants, the TFs obtained for all plants grown in soil-1 and soil-2 increased following treatments A and B, but substantially more for treatment B in soil-1.

These results (Table 5.7) suggest that the potential of *B. juncea* (211000 and 426308) and *H. annuus* to bioaccumulate U and As without the addition of citric acid is low. These results could indicate strong binding of U and As onto soil particles rendering U and As unavailable for plant uptake under normal soil conditions. When citric acid was added, however, the plants' potential to bioaccumulate U and As increases. The plants, especially *B. juncea* 211000, show good potential for Mo bioaccumulation without the additions of citric acid. These high TFs observed for Mo, compared to the TFs for U and As, in control soils may be due to the greater levels of water-soluble concentrations found for Mo (Table 5.3). Similar to U and As, large increases in Mo TFs were subsequently observed following citric acid additions to soil-1.

Table 5.7 Soil metal-to-shoot transfer factors* for *B. juncea (*cultivars: 211000 and 426308) and *H. annuus* grown in soil-1 and soil-2.

	Metal-to-shoot-transfer factors					
	U		**Mo**		**As**	
	Soil-1	**Soil-2**	**Soil-1**	**Soil-2**	**Soil-1**	**Soil-2**
	Control					
***B. juncea* 211000**	0.75	0.6	50	0.94	0.2	-
***B. juncea* 426308**	0.55	0.93	52	1.5	0.4	-
H. annuus	0.4	1.1	22	1.14	0.5	-
	Treatment A					
***B. juncea* 211000**	18	0.74	86	1.2	1.2	-
***B. juncea* 426308**	13	1.7	57	2.7	1.2	-
H. annuus	7.4	1.4	33	1.4	0.9	-
	Treatment B					
***B. juncea* 211000**	64	2.7	114	2.4	2.9	-
***B. juncea* 426308**	50	2.9	98	3.3	2.2	-
H. annuus	30	2.0	57	2.1	1.5	-

*Concentration of metal in the shoot/concentration of metal in the soil

5.6 Shoot dry weight yields and indices of tolerance for *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus* grown in soil-1 and soil-2

An index of tolerance for each species, calculated as the *biomass of a species grown in the metal contaminated soil* / *the biomass of the plant species grown in the background soil* x 100 (Baker *et al*., 1994), were used to evaluate the phytotoxicity effects on plants grown in soil-1 and soil-2 (*cf.* Figure 5.4a to f and Figure 5.7a to f).

5.6.1 Yield and indices of tolerance for plants grown in soil-1

The dry weight yields for the control plants and plants treated with citric acid were markedly less than their background-grown counterparts (Figure 5.4a to f). Successive additions of citric acid (Treatment B) resulted in even lower shoot dry weights, which were less than 50% of its background grown counterparts. Positive correlations were found between U, Mo and As shoot concentrations and the indices of tolerance of plants (Figure 5.5a to i), suggesting that decreases in shoot dry weights occurred as a result of toxicity due to increased shoot-metal concentrations. See figure 5.6i to iii for images of test plants grown in soil-1.

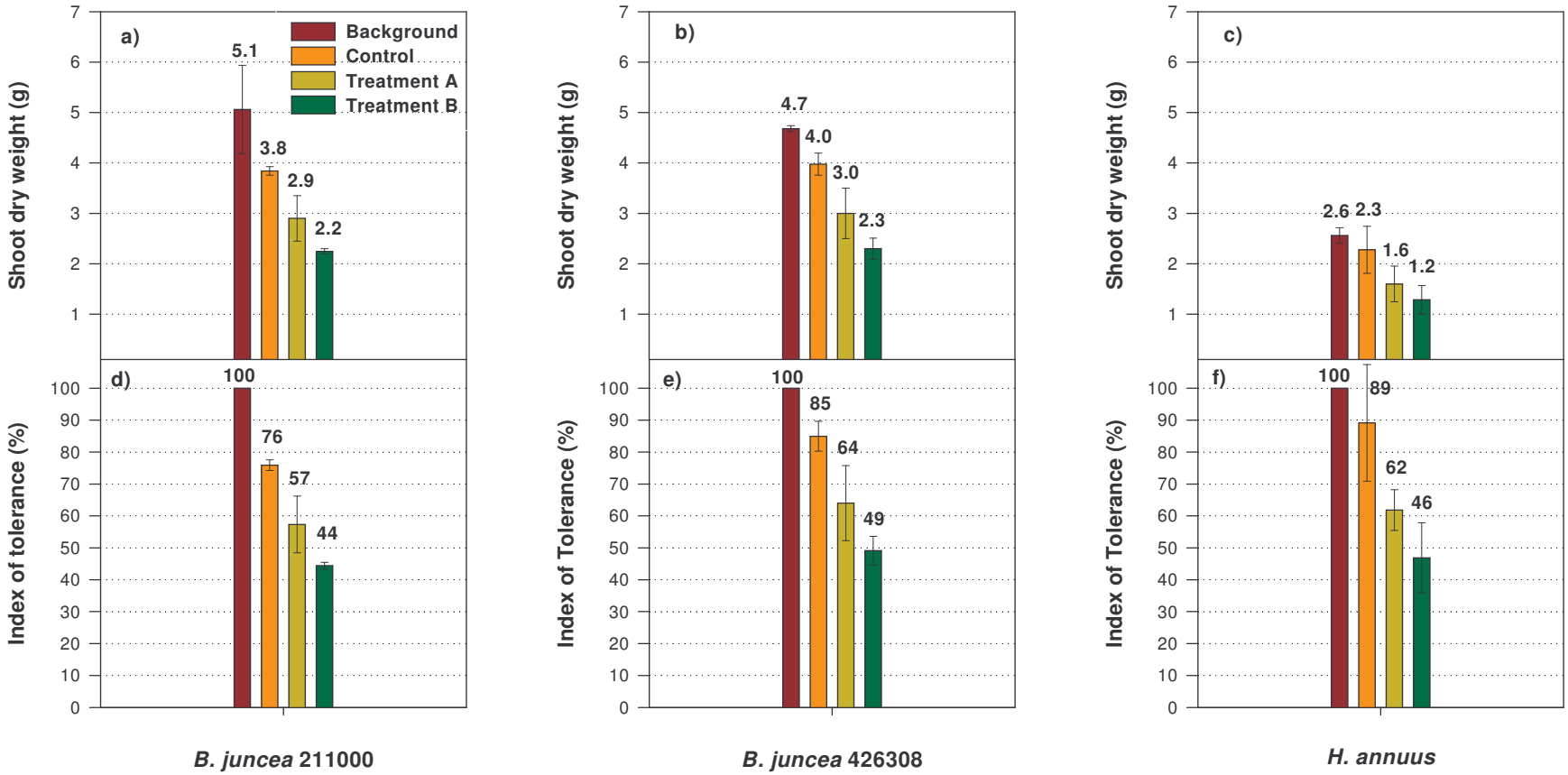

Figure 5.4a to f Shoot dry weights and indices of tolerance for *B. juncea* 211000 (a and d), *B. juncea* 426308 (b and e) and *H. annuus* (c and f) grown in the background and soil-1 with various treatments (Control, 0 mmol citric acid kg^{-1} soil; Treatment A, 25 mmol citric acid kg^{-1}soil; and Treatment B, 2 x 12.5 mmol citric acid kg^{-1} soil, added three days apart). n = 3

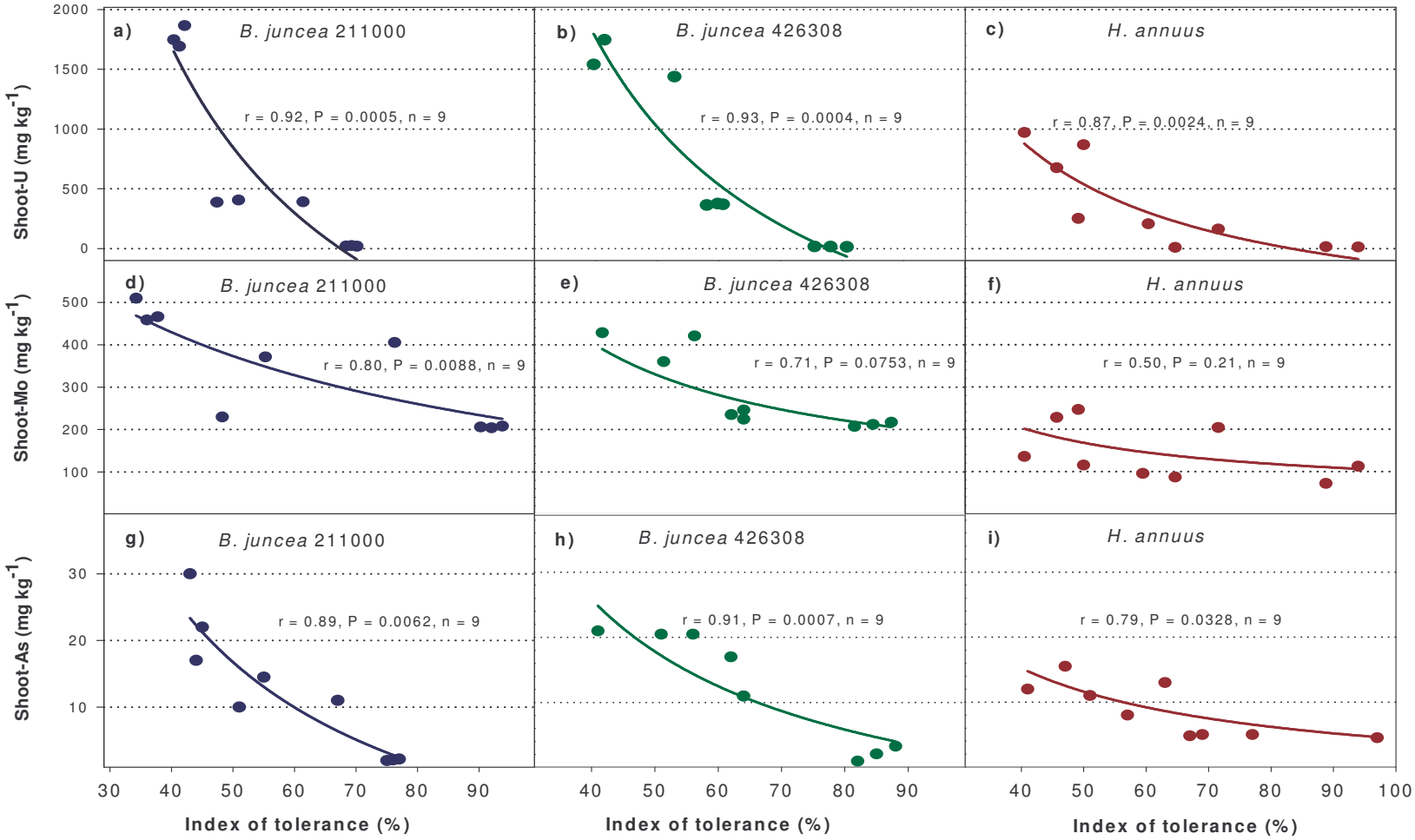

Figure 5.5a to i Relationships between shoot-U (a to c), -Mo (d to f) and -As (g to h) concentrations and the index of tolerance (%) for each species grown in soil-1 (inverse first order polynomial regression).

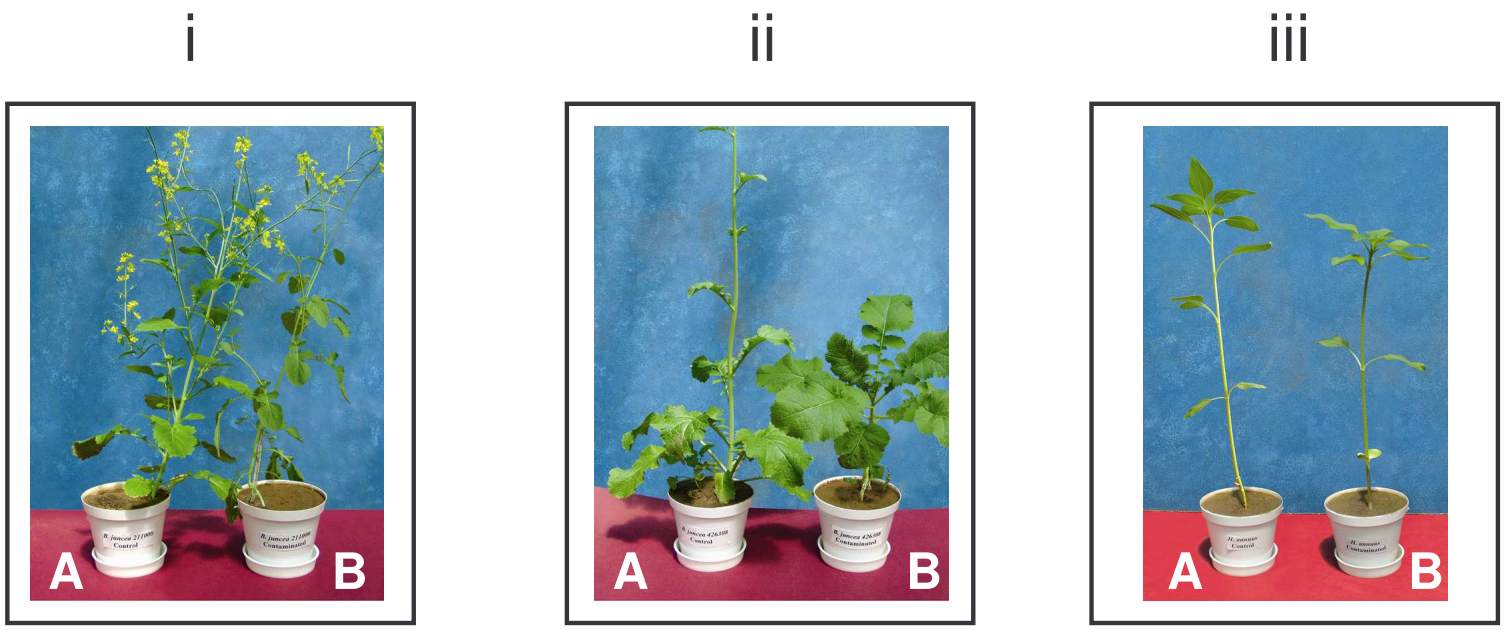

Figure 5.6i to iii *Brassica juncea* 211000 (i), *B. juncea* 426308 (ii) and *H. annuus* (iii) grown in the background soil and soil-1 before citric acid additions, 35 days after transplantation. A = Background, B = Soil-1.

5.6.2 Yield and indices of tolerance for plants grown in soil-2

Compared to the background grown plants, substantial decreases in the dry weights of all plant shoots were observed (Figure 5.7a to f). These yield decreases were more severe than for plants grown in soil-1 (compare Figure 5.4a to f and Figure 5.7a to f). Positive correlations were also found to exist between shoot indices of tolerance and shoot-U, -Mo and -As concentrations (Figure 5.8a to f), but was of less significance than the relationships for soil-1 (compare Figure 5.5a to i and Figure 5.7a to f). See figure 5.9i to iii for images of test plants grown in soil-2.

5.7 Potential annual yield of *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus*

Based on the plant shoot dry weights (DW) of plants grown in the background soil (Figures 5.4), potential yields (kg ha^{-1} year) on Rietkuil 307 can be predicted (Table 5.8). Yield predictions are based on three sequential harvests of 8 weeks apart with a plant density of 36 plants m^{-2} (20 cm apart).

It is predicted that both cultivars of *B. juncea* will yield approximately 5 t ha^{-1}, whilst *H. annuus* will only produce half of that. These predicted dry weights are required to calculate the Site-Specific Phytoextraction Potential (SSP) for each species.

Table 5.8 Predicted annual dry weight yields (t ha^{-1}) of *B. juncea* (cultivars: 211000 and 426308) and *H. annuus* grown on Rietkuil 307.

Species	**Dry weight yield (t ha^{-1})**
Brassica juncea 211000	5.51
Brassica juncea 426308	5.08
Helianthus annuus	2.81

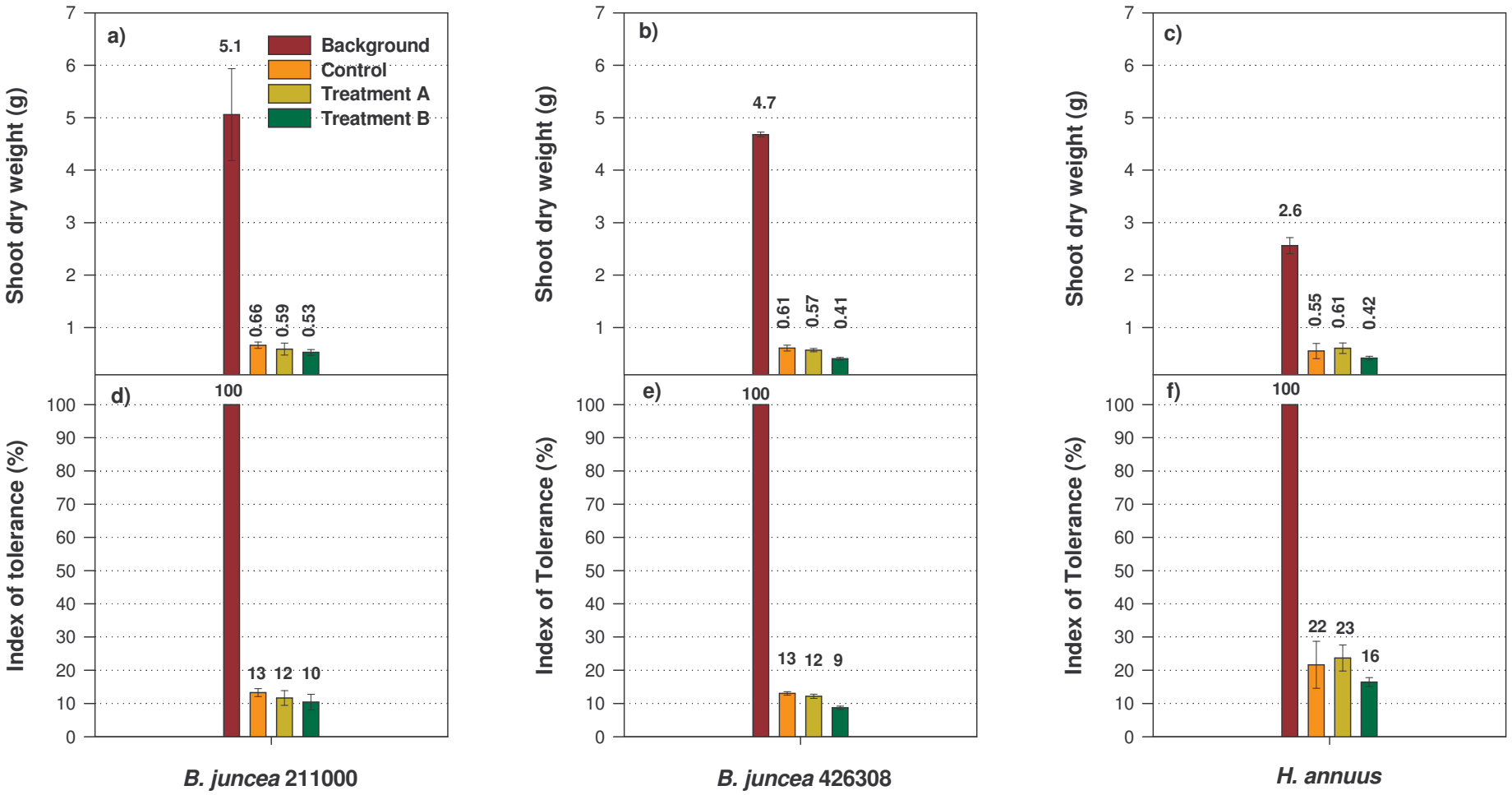

Figure 5.7a to f Shoot dry weights and indices of tolerance for *B. juncea* 211000 (a and d), *B. juncea* 426308 (b and e) and *H. annuus* (c and f) grown in the background and soil-2 with various added treatments (Control, 0 mmol citric acid kg^{-1} soil; Treatment A, 25 mmol citric acid kg^{-1}soil; and Treatment B, 2 x 12.5 mmol citric acid kg^{-1} soil, added three days apart). n = 3

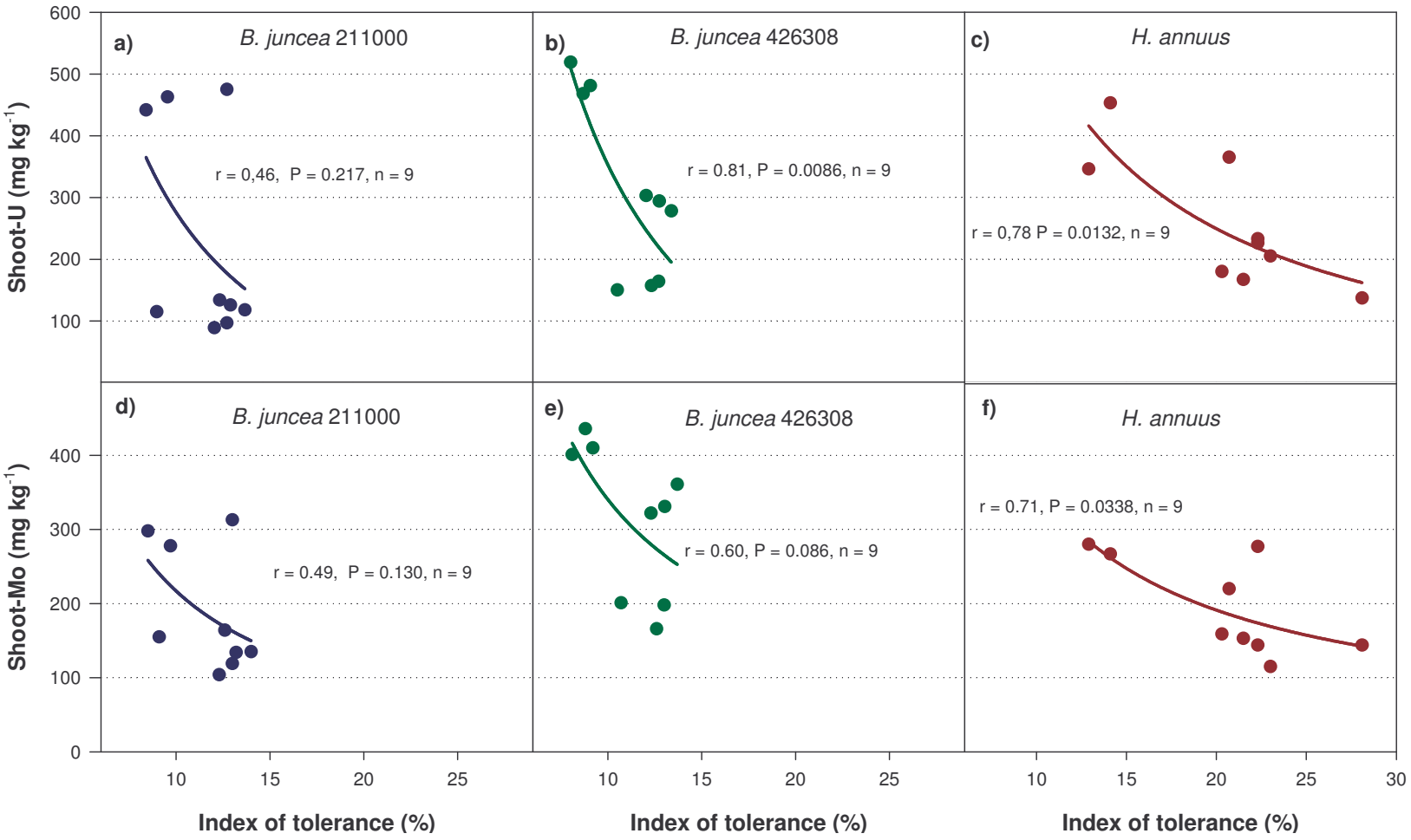

Figure 5.8a to f Relationships between shoot-U (a to c) and -Mo (d to f) concentrations and the index of tolerance (%) for each plant species grown in soil-2 (inverse first order polynomial regression).

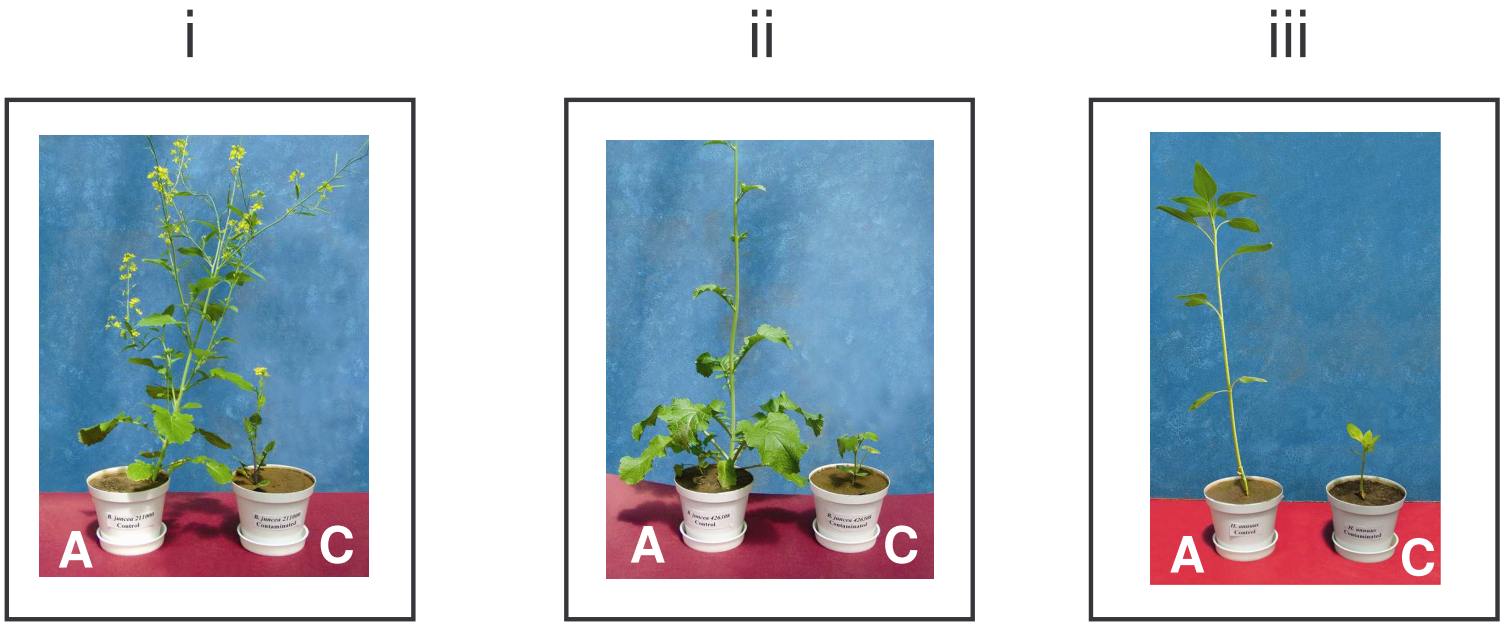

Figure 5.9i to iii *Brassica juncea* 211000 (i), *B. juncea* 426308 (ii) and *H. annuus* (iii) grown in the background soil and soil-2 before citric acid additions, 35 days after transplantation. A = Background, C = Soil-2.

5.8 The Site-Specific Phytoextraction Potential of *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus* from low- and high-level metal contaminated soils

The Site-Specific Phytoextraction Potential (SSP) is an index of a plant's metal removal potential from one hectare of soil after one year (Hinton, 2005). The SSP is calculated using the metal to shoot transfer factor, the concentration of the metal in the soil, the predicted shoot dry weight yield (kg ha^{-1} $year^{-1}$) and their respective indices of tolerance.

For example, the SSP of *B. juncea* 211000 for U grown in soil-1, with Treatment B, was calculated using equation 1 (*cf.* 4.2.15):

$$\mathbf{SPP} = \frac{\mathbf{[5510\ kg\ ha^{-1}\ x\ 44\%]\ x\ 64\ x\ 28\ (mg\ U\ kg^{-1}soil)}}{\mathbf{10^6}}$$

$$\mathbf{= 4.34\ kg\ U\ ha^{-1}}$$

where **5510 kg ha^{-1}** represents the potential shoot dry weight (DW) yield of *B. juncea* 211000 grown in background soil with three sequential harvests (Table 5.8); **44%** is the index of tolerance obtained by *B. juncea* 211000 grown in soil-1 with Treatment B (Figure 5.4d); **64** is the shoot-U transfer factor achieved by *B. juncea* 211000 in soil-1 with Treatment B (Table 5.7); **28 mg U kg^{-1}** represents the acid digested U concentration in soil-1 (Table 5.4); and **10^6** represents a conversion factor for converting mg to kg. The SSP answer indicates that *B. juncea* 211000 will be able to remove 4.34 kg U ha^{-1} $year^{-1}$ under given conditions.

The SSP of *B. juncea* (cultivars: 21100 and 426308) and *H. annuus* for U, Mo and As removal from soil-1 and soil-2 (Table 5.9) illustrate that large amounts of U and As can be removed following citric acid additions (treatments A and B), especially following the successive additions of citric acid (Treatment B). Because of the metal toxicities experienced by plants grown in soil-2 (Figure 5.9i to iii), considerably greater amounts of U were removed from soil-1 than

soil-2. No value could be obtained for As removal in soil-2 since no shoot-As concentrations could be detected for plants grown in this soil. Because of the decreased dry weight production of *B. juncea* 426308 and *H. annuus* grown in soil-1 (Figure 5.4b and c), the increased Mo accumulation by shoots following citric acid additions (Figure 5.3 B) did not directly translate into equivalent increases in metal removal. Similar to U removal, considerably less Mo can be removed from soil-2 than soil-1, probably because of plant-metal toxicity.

Table 5.9 Site-Specific Phytoextraction Potentials* (SSP) of *B. juncea* (cultivar: 211000 and 426308) and *H. annuus* for U, Mo and As removal (kg ha^{1}) from soil-1 and soil-2.

	Site-Specific Phytoextraction Potential (SSP) for:					
	***B. juncea* 211000**		***B. juncea* 426308**		***H. annuus***	
	Soil-1	**Soil-2**	**Soil-1**	**Soil-2**	**Soil-1**	**Soil-2**
Treatments	U removal (kg ha^{-1})					
Control	0.088	0.010	0.066	0.019	0.028	0.014
Treatment A[1]	1.58	0.014	1.18	0.031	0.36	0.026
Treatment B[2]	4.34	0.042	3.48	0.037	1.09	0.035
	Mo removal (kg ha^{-1})					
Control	0.858	0.071	0.94	0.133	0.225	0.064
Treatment A[1]	1.11	0.10	0.760	0.223	0.426	0.096
Treatment B[2]	1.13	0.165	1.0	0.189	0.302	0.162
	As removal (kg ha^{-1})					
Control	0.0074	-	0.015	-	0.011	-
Treatment A[1]	0.033	-	0.035	-	0.014	-
Treatment B[2]	0.063	-	0.049	-	0.017	-

* SSP (kg ha^{-1}) = [DW yield (kg ha^{-1}) x index of tolerance (%)] x TF x Csoil (mg U kg^{-1}) / CF
[1] 25 mmol citric acid kg^{-1} soil
[2] 2 x 12.5 mmol citric acid kg^{-1} soil

5.9 Years required to phytoextract U, Mo and As from soil-1 and soil-2

The number of years required to phytoextract metals (U, Mo and As) from soil-1 and soil-2 to the background levels can be predicted based on the data from the total metal concentrations per hectare (*cf.* Appendix A3) and the SSP.

For example, the number of years required for *B. juncea* 211000, with three sequential harvests per year, to decrease the U levels in soil-1 using successive applications of citric acid (treatment B), can be calculated using equation 2 (*cf.* 4.2.15):

$$\textbf{Predicted years} = \frac{\textbf{72.8 kg U ha}^{-1} - \textbf{13 kg U ha}^{-1}}{\textbf{4.34}}$$

$$= \textbf{13.8 years}$$

where **72.8 kg U ha^{-1}** represents the predicted total concentration of U per hectare in soil-1 (*cf.* Appendix A3) based on the acid digested metal concentration measured in the soil (mg U kg^{-1} soil; table 5.4), at a soil depth of 20 cm and a soil density of 1.30 kg dm^{-3} (Table 5.1); **13 kg U ha^{-1}** is the background soil metal concentrations per hectare calculated similar to the above (*cf.* Appendix A3). It should be noted that none of the background levels were above the lowest limit of detection as measured by the ICP-OES and for this reason, the lowest limits of detection (LLD) were regarded as the background levels (U = 13-, Mo = 1.04-; As = 2.6 kg^{-1} ha^{-1} , *cf.* Appendix A3). The value of **4.34** represents the SSP for *B. juncea* 211000. The answer predicts that it will take *B. juncea* 211000, based on three sequential harvests per year, 13.8 years to decrease U from soil-1 to background levels.

The number of years required to decrease U, Mo and As concentrations in soil-1 and soil-2 to background levels are presented in Table 5.9.

The years required to decontaminate soil-1 to background levels are substantially less than would be required for metal decontamination from the higher contaminated soil (soil-2). This can be explained by the considerably low dry matter production of the plants grown in soil-2 (Figure 5.7a to f) and subsequent low metal accumulation because of metal toxicity. Both cultivars of *B. juncea* (211000 and 426308) appeared to be good candidates, in conjunction with successive additions of citric acid, to remove U and Mo from soil-1 collected from Rietkuil 307. In addition, both cultivars also showed that they are more efficient in their metal removal potential than *H. annuus*. However, *B. juncea* 211000 illustrated the best potential to decontaminate U and Mo levels in the soil from Rietkuil 307. The predictions made suggest that it will take *B. juncea* 211000, in conjunction with successive additions of citric acid (Treatment B), approximately 14 and 9 years to decontaminate U and Mo from soil-1 (at a depth of 20 cm), respectively. Due to the low accumulation of As in plants and long remediation times, phytoextraction would not be practical for the removal of As from soil-1.

Table 5.10 Years required to phytoextract U, Mo and As from soil-1 and soil-2 to background metal levels (soil-depth 20 cm; soil density 1.3 kg dm^{-3}).

	Years predicted to remediate soil-1 and soil-2 using:					
	***B. juncea* 211000**		***B. juncea* 426308**		***H. annuus***	
	Soil-1	**Soil-2**	**Soil-1**	**Soil-2**	**Soil-1**	**Soil-2**
Treatments	Years required[1] for U phytoextraction to background concentrations[2]					
Control	670	42 380	906	22 305	2 136	30 286
Treatment A[3]	38	30 214	50	13 671	166	16 308
Treatment B[4]	14	10 090	17	11 459	55	12 114
	Years required[1] for Mo phytoextraction to background concentrations[2]					
Control	11	4 563	10	2 436	43	5 062
Treatment A[3]	9	3 240	13	1 453	23	2 745
Treatment B[4]	9	1 963	10	1 714	32	2 000
	Years required[1] for As phytoextraction to background concentrations[2]					
Control	2 770	-	1 367	-	1 864	-
Treatment A[3]	621	-	586	-	1 464	-
Treatment B[4]	325	-	418	-	1 206	-

[1] Metal concentration in contaminated soil (kg ha^{-1}) – metal concentration in background soil (kg ha^{-1}) / SPP
[2] U = 5 mg kg^{-1}; Mo = 0.4 mg kg^{-1}; As = 1 mg kg^{-1}
[3] 25 mmol citric acid kg^{-1} soil
[4] 2 x 12.5 mmol acid kg^{-1} soil

CHAPTER 6

DISCUSSION

6.1 Heavy metal contamination in Rietkuil 307 topsoil

The uranium (U) ore stockpiles on Rietkuil 307 in the Beaufort West district of the Karoo Uranium Province is a major source of heavy metal contamination in the topsoil. The U, molybdenum (Mo) and arsenic (As) concentrations in the soil, sampled in the vicinity of the ore stockpiles (soil-1) are higher than the background soil concentrations for the area (*cf.* Table 5.2). The concentrations of U, Mo and As were also above the normal ranges for metals in soils (Bowen, 1979) while U and Mo exceed critical toxicity levels (Kataba-Pendias and Pendias, 1984; *cf.* Table 5.2)

Scholtz (2003) reported that heavy metal contamination on Rietkuil 307 is suppressed by the lack of run-off due to the dry climate. Rahn *et al*. (1996) performed a study on environmental impacts resulting from U mining in the Black Hills, United States of America. He stated that the release of contaminants from U ore stockpiles is limited due to the dry climate of the region which receives an annual precipitation of 400 to 600 mm. Harries and Ritchie (1983) studied the pollution levels in the runoff from U mine tailings in northern Australia and proved that the amount of leachate released is dependant on the rainfall (1500 mm annually). Beaufort West receives an annual rainfall of 236 mm (South African Weather Service, 1990; *cf.* Appendix A2). An increase in precipitation and subsequent run-off from the ore stockpiles could result in greater metal leachate into the topsoil and groundwater. This supplied the rationale for mixing pulverized U ore with soil-1 (soil-2). Soil-2 thus represents a highly contaminated soil, which may occur in this area due to long term failure to remove or rehabilitate the U ore stockpiles (*cf.* 4.2.3).

Through the process of biomagnification, heavy metal contamination in topsoil poses potential harmful effects to organisms occupying positions in higher trophic levels. Biomagnification occurs when a non-degradable metal becomes concentrated in the tissues of organisms at higher trophic levels of a food chain. Diamond *et al*. (1989) reported that U compounds can cause cellular injury and tubular necrosis in a variety of mammalian organs. Similarly, high Mo

concentrations in ruminant tissue can cause a fatal Cu deficiency known as molybdenosis (Albasel and Pratt, 1989; Mullen *et al*., 2005). Mullen *et al*. (2005) reported that a concentration of >10 mg Mo kg^{-1} forage can cause molybdenosis. Arsenic has been reported as carcinogenic and especially toxic to the skin, lung and bladder in mammalian tissue (Sheppard *et al*., 1992; Ng *et al*., 2003; ATSDR, 2005; U.S. Department of Health, 2005).

The water soluble U, Mo and As concentrations measured for the low-level (soil-1) and high-level (soil-2) contaminated soils showed that substantially higher concentrations of U, Mo and As were extracted from soil-2 than from soil-1 (Table 5.3). This is clearly indicative of the potential threat regarding toxicity levels and possible biomagnification in different organisms of the region.

Given the environmental concentrations of especially U, Mo and As, and its bioavailability and threat to the environment (*cf*. Appendix A1), the rehabilitation of the trial mining site on Rietkuil 307 should be viewed as a priority.

6.2 Phytoextraction as a soil remediation technology

Phytoextraction is a soil remediation technology that makes use of plant species to extract metals from contaminated soils. When using non-hyperaccumulators as phytoextractors, one of the greatest factors limiting the success of this technology is the solubility of metals in the soil solution. Since plants can only accumulate metals in the labile fraction of the soil, the success of phytoextraction would be restricted by the unavailability of soil metals.

It has been reported that under normal soil conditions, the mobility of U is relatively low and non-hyperaccumulating plant species are not capable of accumulating the required rate of U accumulation for phytoextraction (Huang *et al*., 1998; Vandenhove *et al*., 2001; Shahandeh and Hossner, 2002a and b). Therefore, the artificial solubilization of heavy metals in soil by adding chelates is proposed to enhance the efficiency of metal phytoextraction through the formation of bioavailable metal-chelate complexes or through the dissolution of

soil minerals by acidification (Chen *et al*., 1995; Huang *et al*., 1998; Ebbs *et al*., 1998; Shahandeh and Hossner, 2002a; Vandenhove *et al*., 2001), which increases metal bioavailability and the translocation of metal-chelate-complexes from root to shoot (Blaylock *et al*., 1997). One such chelating agent which has proven to be successful and environmentally friendly is citric acid. Huang *et al*. (1998) reported a 1000-fold increase in the U concentration in *Brassica juncea* shoots following the addition of citric acid to the soil medium. Several researchers (Huang *et al*., 1998; Qualls and Haines, 1992; Jones *et al*., 1996; Jones and Darrah, 1994) reported that citric acid is more environmentally friendly than other chelating agents, due to its rapid biodegradability.

The specific objective of this study was, therefore, to determine whether citric acid induced phytoextraction would be a viable soil remediation technology to decrease U, Mo and As levels in contaminated soil (soil-1) on Rietkui 307 in the Karoo Uranium Province, South Africa. A further objective was to determine how effective phytoextraction would be if the metal concentrations in the soil were to increase (soil-2) as a result of long term failure to remove the U ore stockpiles.

Plant-metal accumulation and responses as a result thereof, are important criteria to use in phytoextraction since it provide some estimation of the amount of metals that can be removed per year (Site-Specific Phytoextraction Potential) as well as the time needed for remediation.

An ideal plant for phytoextraction should have high biomass production combined with a superior capacity to tolerate, accumulate and translocate metals and have the ability to cope and survive in the climate where it is to be grown (*cf.* Figure 1.5). *Brassica juncea* cultivars were selected because of its ability to extract large amounts of U from soil and the subsequent translocation of U to shoots with citric acid addition (Huang *et al.,* 1998; Shahandeh and Hossner, 2002a; Schmidt, 2003; Vandenhove *et al*., 2001; Salt *et al*., 1995a), its ability to adapt to extreme weather conditions (Turner *et al*., 2001; Gunasekera, 2003; Kimber and McGregor, 1995; Oram and Kirk, 1992), extensive and deep

rooting system, vigorous seedling growth and ground covering ability (Gunasekera, 2003). *Helianthus annuus* was chosen for its high U accumulation in roots (Shahandeh and Hossner, 2002b; Dushenkov, 1997) and has been described as metal- and drought tolerant with high yield potential (Schmidt, 2003; Johnston, 2002).

6.3 Citric acid desorption of soil metals

To determine the efficiency of citric acid to desorb soil-bound U, Mo and As, both soil-1 and soil-2 were subjected to increasing concentrations of citric acid solutions, which ranged from 0 to 25 mmol citric acid kg^{-1} soil. It was demonstrated (*cf.* Figure 5.2a to c) that citric acid was efficient in enhancing U, Mo and As solubilization in soils, which were the most pronounced at the highest concentration of citric acid used. Compared to the control, U desorption increased 27- and 8.3-fold for soil-1 and soil-2, respectively (*cf.* Table 5.5). For all the metals tested, the degree of U desorption was found to be the highest. This supported previous findings that citric acid has a higher chelating capacity for U compared to any other metal (Huang *et al*., 1998; Shahandeh and Hossner, 2002a, b; Blaylock *et al.*, 1997).

In addition, a greater degree of U, Mo and As concentrations were desorbed from soil-1 than soil-2 with the addition of 25 mmol citric acid kg^{-1} soil (*cf.* Table 5.5). This could be attributed to the lower buffer capacity of soil-1 (*cf.* Figure 5.1), which probably resulted in a lesser degree of metal adsorption onto soil particles, compared to soil-2. The buffer capacity of soils determines the ability of soils to resist acidification. Barrow (1986) stated that at a high soil pH, cations may precipitate out of solution onto soil solids, making it unavailable for plant uptake. Soil-2 clearly had a greater capacity to resist acidification than soil-1 (*cf.* Figure 5.1). This could be attributed to the increase in clay-, organic- and inorganic carbon content, as well as the subsequent increase in cation exchange capacity (CEC) (*cf.* Table 5.1). With the lower resistance to acidification observed in soil-1, H^+ concentrations can increase rapidly and cations must compete with the extra H^+ for positions on CEC sites. This will

result in an increase in cation desorption and solubility in the soil solution (Vandenhove *et al*., 2001; Shahandeh and Hossner, 2002b; Pilon-Smits, 2005).

It was demonstrated that the U, Mo and As concentrations in the soil solution increased linearly upon the addition of increasing concentrations of citric acid (*cf.* Figure 5.2a to c). This suggests that when the soil pH is lowered by the addition of citric acid (*cf.* Figure 5.1), even metals such as the predominantly anion species, Mo (Reisenauer *et al*., 1962; Williams and Thornton, 1973) and As (Sadiq *et al*., 1983; Jones *et al*., 1997; Darland and Inskeep, 1997;), are also released into the soil solution. Therefore, it appeared that citric acid not only formed soluble chelate complexes with U, but apparently to some extent with Mo and As as well.

The citric acid desorptions of U, Mo and As were successful in this study and the greatest rates were achieved with the highest concentration of 25 mmol citric acid kg^{-1} soil added. Consequently, this concentration was chosen to be used in the pot trials.

6.4 Citric acid induced metal accumulation in *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus* shoots

6.4.1 Shoot accumulation of heavy metals

Citric acid-induced metal accumulation in *Brassica juncea* (cultivars 211000 and 426308) and *Helianthus annuus* shoots were evaluated in a greenhouse trial to determine the Site Specific Phytoextraction Potential (SSP) from low-level (soil-1) and high level (soil-2) U, Mo and As contaminated soils. After 35 days of growth in the metal contaminated soils, three subsets of each species grown in soil-1 and soil-2 received one of three treatments (*cf*. Figure 4.1). These treatments consisted of a single addition of 25 mmol citric acid kg^{-1} soil (Treatment A); a successive addition of 12.5 mmol citric acid kg^{-1} soil, applied three days apart (Treatment B); and distilled water which were regarded as control plants. All plants were harvested 7 days after these treatments (42 days

after transplantation). Several researchers have reported that metal accumulation is rapid following citric acid treatment and that maximum concentrations in plant tissues are reached in approximately three days (Huang *et al*., 1998; Vandenhove *et al*., 2001). Furthermore, Vandenhove *et al*. (2001) reported that plants died soon after citric acid additions and should be harvested within 7 days of citric acid addition.

The results obtained in this study (*cf.* Figure 5.3 A to C) confirm the findings of various reports which suggested that due to the formation of bioavailable U-citrate complexes, metal translocation to plants shoots is enhanced (Huang *et al*., 1998; Ebbs *et al*., 1998; Vandenhove *et al*., 2001; Shahandeh and Hossner, 2002b). It was demonstrated that the shoot-U, -Mo and -As concentrations in all the plants evaluated increased with successive additions of citric acid (Treatment B). High increases were especially observed for the plants grown in soil-1. The largest increase in shoot-U, -Mo and -As concentrations were observed in *B. juncea* 211000 grown in soil-1, which increased 85-, 2.3- and 11-fold, respectively after successive additions of citric acid.

6.4.1.1 Uranium (U) accumulation

In control soils, with no citric acid additions, plants were not capable of accumulating U, but following citric acid additions, marked increases were observed (*cf*. Figure 5.3 A). Soil amendments are needed to enhance the mobility of U in soils and several researchers have used citric acid to increase U bioavailability due to its high binding capacity for U (Huang *et al*., 1998; Shahandeh and Hossner, 2002a).

Huang *et al*. (1998) reported an increase in the shoot-U concentration in *B. juncea* 426308 of more than a 1000-fold, from 5 mg kg^{-1} DW to more than 5000 mg kg^{-1} DW, following a single addition of 20 mmol citric acid kg^{-1} soil containing 750 mg U kg^{-1} soil. They attributed this enhanced U accumulation to the chelation of U by citric acid, forming soluble U-citrate complexes. In another study, Shahandeh and Hossner (2002a) demonstrated that a single addition of

20 mmol citric acid kg^{-1} soil (600 mg U kg^{-1} soil) increased U in *B. juncea* 426308 shoots 140-fold, from 10 mg U kg^{-1} DW to more than 1400 mg U kg^{-1} DW.

Considerably lower shoot-U concentrations were, however, obtained in the present study (*cf.* Figure 5.3 A). With a single addition of 25 mmol citric acid kg^{-1} soil (Treatment A), shoot-U concentrations in *B. juncea* 426308, *B. juncea* 211000 and *H. annuus* increased only 23-, 24- and 17-fold, respectively (*cf.* Figure 5.3 A). This lower accumulation of U, when compared to the studies of Huang *et al*. (1998) and Shahandeh and Hossner (2002a), may be attributed to the greater acid digested U concentrations in the soils of their respective studies. The acid digestible U concentration of soil-1 was only 28 mg U kg^{-1} soil compared to 750 mg U kg^{-1} soil (Huang *et al*., 1998) and 600 mg U kg^{-1} soil (Shahandeh and Hossner, 2002a) used by the authors.

Huang *et al*. (1998) also reported that U concentrations in the shoots of *B. juncea* 426308 increased with greater soil-U concentrations. They reported that shoots of *B. juncea* 426308 accumulated greater levels of U when grown in soil with a concentration of 750 mg U kg^{-1} soil than in a soil of 280 mg U kg^{-1} soil. This would suggest greater U concentrations in plant shoots grown in soil-2 than when grown in soil-1 in the present study. The contrary was, however, observed (Figure 5.3 A). The reason for this is probably the toxicity experienced by the plants as a result of the multiple metal contaminants in soil-2 (U, Mo and As), which were not present in the soils used by Huang *et al*. (1998).

Moreover, Huang *et al*. (1998) evaluated both cultivars of *B. juncea* (cultivars 211000 and 426308) for its U accumulating potential and found that *B. juncea* 426308 accumulated the most U in its shoots. In contrast, however, it was found in the present study that *B. juncea* 211000 accumulated the most U in its shoots (*cf*. Figure 5.3 A).

6.4.1.2 Molybdenum (Mo) accumulation

Citric acid additions also enhanced Mo desorption from soils (*cf*. Table 5.2b), and subsequent accumulation thereof in plant shoots (*cf.* Figure 5.3 B), but were less pronounced than for U accumulation (*cf.* Figure 5.3 A). Of all the plants evaluated, *B. juncea* 211000, as with U, showed the greatest capability to accumulate Mo in its shoots.

No information regarding the formation of soluble Mo-citrate complexes is available in the literature and the mechanism for the increased Mo accumulation in plant shoots remains unclear. Williams and Thornton (1973) did, however, report that EDTA forms soluble chelate complexes with loosely bound Mo-organic complexes. The high Mo concentrations in the control plant shoots (no citric acid addition) grown in soil-1 and soil-2 (*cf.* Figure 5.3 B) at pH 7.36 and 7.65, respectively, suggest that Mo is very soluble at an alkaline soil pH. This is consistent with the observations of Barshad (1951) who reported that hydroxyl ions (OH^-) may compete with Mo ions for positions on anion exchange sites at a soil pH above 7, thus increasing its bioavailability (Williams and Thornton, 1973).

Moreover, the higher Mo concentrations measured in plant shoots following citric acid additions and the subsequent decrease in soil pH, could have caused the dissolution of Mo minerals, resulting in the release of bound or adsorbed Mo into the soil solution. Molybdenum apparently associates readily with organic matter, and with Ca-, Al-, Fe- and Mn-oxides (Reisenhauer *et al*., 1962; Karimian and Cox, 1978). Gahoonia *et al*. (1992) found that a low soil pH increased the concentrations of Mo in the soil solution, as was also found in the present study. The acidity caused by citric acid in solution results from the three carboxyl groups, each of which can lose a proton to form the citrate anion (Johnson *et al*., 1996). These anions apparently mobilize substantial amounts of Mo (MoO_4^{2-}) from Ca-, Al-, Fe- and Mn-oxides through mechanisms of anion exchange (Geelhoed *et al*., 1999).

6.4.1.3 Arsenic (As) accumulation

Arsenic accumulation in plant shoots was relatively low compared to U and Mo accumulation following citric acid additions (*cf.* Figure 5.3 C). This can be attributed to the low desorption of As into the soil solution (Smith *et al*., 1998; Jain *et al*., 1999). Arsenic mobility in soils is low due to the strong adsorption onto soil particles (Smith *et al*., 1998; Jain *et al*., 1999) and the level of accumulation by terrestrial plants is subsequently low, even when grown on soil containing high concentrations of As (Pitten *et al*., 1999).

The pattern for As accumulation in the shoots of all plants (*cf.* Figure 5.3 C) was similar to that obtained for U and Mo (*cf.* Figure 5.3 A and B). It was again *B. juncea* 211000 grown in soil-1, which showed the best potential to accumulate As when citric acid was added successively (*cf.* Figure 5.3 C).

Similar to the shoot-Mo accumulation and desorption in the present study, the dissolution of As minerals at low soil pH could have caused increases in the bioavailability of As or could have occurred as a result of anion exchange with citrate. Li *et al*. (1997) documented that P, an As analogue (*cf.* 3.3.2), was mobilized from P sources such as Ca-, Al-, and Fe-phosphates with the addition of citric acid to the soil. They attributed this to mechanisms of anion exchange.

6.4.2 Single citric acid application vs. successive additions

Several reports exist (Huang *et al*., 1998; Qualls and Haines, 1992; Jones *et al*., 1996; Jones and Darrah, 1994) stating that citric acid is an environmentally friendly chelate to use for phytoextraction due to its rapid biodegradation rate. Huang *et al*. (1998) reported that U accumulation in *B. juncea* reached the highest levels after three days of citric acid addition, whereafter concentrations remained unchanged until harvesting on day seven. Jones and Darrah (1994) estimated that the half-life of citric acid is 12 hours.

Results presented in this study (*cf.* Figure 5.3 A to C) indicate that successive applications of citric acid, three days apart (Treatment B), enhanced shoot concentrations of U, Mo and As in all the plants more than a single application of citric acid (Treatment A). The lower shoot concentrations obtained with Treatment A can possibly be attributed to rapid biodegradation rates of citric acid, which subsequently reduced the availability of soluble metal-chelate complexes in the soil solution (Huang *et al.*, 1998; Jones and Darrah, 1994). Thus, it appeared that less bioavailable metal-citrate complexes were available for plant uptake in soils that received Treatment A resulting in lower concentrations of the metals in plant shoots (*cf.* Figure 5.3 A to C). These results further suggest that the enhanced shoot-U, -Mo and -As concentrations occurred as a result of soluble metal-citrate complexes formed increasing its bioavailability.

Furthermore, the pH of the control soil, soil-1 and soil-2 was measured on the day of plant harvest. Compared to control treatments (0 mmol citric acid kg^{-1} soil) additions of citric acid resulted in pH decreases of approximately 1.7 units for soil-1 and 1.0 unit for soil-2 (*cf.* Table 5.6). The pH of the soil extracts measured in the desorption studies (25 mmol citric acid kg^{-1} soil) were, however, considerably lower for soil-1 (*cf.* Figure 5.1). Thus, the soil pH at the time of plant harvest (*cf.* Table 5.6) were higher (less acidic) than the soil pH in the desorption studies (desorption time - 16 hours), further suggesting that citric acid is rapidly biodegradable in soils.

Consecutive citric acid additions at lower concentrations can therefore also alleviate the potential risks of heavy metal migration to the groundwater by reducing the amount of chelate and soluble metals in solution at any one time. In this approach, chelate applications could be optimized to meet plant water requirements and subsequent metal uptake in solution.

6.5 Bioaccumulating potential of *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus*

Metal-to-shoot transfer factors (TFs) are used to determine whether a plant has the ability to bioaccumulate heavy metals (pollutants) against a concentration gradient from contaminated soils. Baker (1981) proposed that a transfer factor less than or equal to 1 is not an indication of bioaccumulation potential. None of the plants grown in control soils show any potential for U and As bioaccumulation (*cf.* Table 5.7). This may be due to the unavailability of these metals in soil-1 and soil-2, probably as a result of adsorption onto soil particles.

However, when citric acid was added to the soils the potential of all the plants to bioaccumulate U and As increased (*cf.* Table 5.7). This can be explained by the enhanced bioavailability of U and As following desorption by citric acid (*cf.* Figure 5.2a to c). This resulted in increased shoot metal concentrations for all the plants, as the metals became more available in the soil solution through the chelating action of citric acid. This phenomenon was the most pronounced for U bioaccumulation; especially for *B. juncea* 211000 grown in soil-1 with successive additions of citric acid (*cf.* Table 5.7).

The Mo TFs obtained for the plants grown in the control soil-1 was, however, an indication of bioaccumulation (*cf.* Table 5.7). Accumulation was strongly against the concentration gradient and this might even be an indication of Mo hyperaccumulation (*cf.* Table 5.7). This may be attributed to the level of bioavailable Mo in the water soluble fraction of soil-1 (*cf.* Table 5.3), which lies within the acceptable ranges for soils (Bowen, 1979). Addition of citric acid, either as a single dose (Treatment A) or successive doses (Treatment B), enhanced this phenomenon.

However, the water soluble concentration of Mo in soil-2 is considerably higher, approximately 68-fold, than in soil-1 (*cf.* Table 5.3), which exceeds the critical range for Mo (Bowen, 1979). The shoot-metal concentration of Mo measured is consistently lower in plants grown in soil-2. This also resulted in TFs for Mo of

approximately 1.0 indicating that all the plants loses the ability to bioaccumulate Mo against a concentration gradient in this highly contaminated soil (soil-2). This could be attributed to multiple toxic effects of different metals contributing to the lower yields for plants grown in soil-2 (*cf.* Figure 5.7a to f).

6.6 Dry weight yields and indices of tolerance for *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus* grown in soil-1 and soil-2

Plant stress as a result of metal toxicity is a crucial parameter to consider when evaluating phytoextraction for metal removal. Since the potential of phytoextraction depends on accumulation of metals in shoots and on shoot biomass production, the final rate limiting step to consider is metal toxicity towards plant growth.

Vandenhove *et al.* (2001) and Rodríguez *et al*. (2006) found linear relationships between the concentration of U in the shoots of *B. juncea and H. annuus* and the U in the soil solution. Thus, together with the greater desorption levels of U, Mo and As from soil-2 into the soil solution, and the greater acid digested metal concentrations, compared to soil-1, it was expected that plants grown in soil-2 should have accumulated greater concentrations of metals than plants grown in soil-1. However, the majority of plants accumulated more metals when grown in soil-1 compared to plants grown in soil-2 (*cf.* Figure 5.3 A to C).

A possible reason for this lesser accumulation of metals in plants grown in soil-2 could be the result of toxicity effects of multiple metal contaminants on plant metabolism and function. The biomass of plants grown in soil-2 (*cf.* Figure 5.9i to iii) was suppressed and dry weight yields were considerably less than the background and soil-1 grown plants (*cf.* Figure 5.7a to f). The plants were also stunted, dwarfed and showed various other toxic symptoms (*cf.* Figure 5.9i to iii). According to Baker (1981) and Duquène *et al*. (2005), exclusion or restricted transport of elements in plants may occur in response to high concentrations of

heavy metals in soil. Furthermore, high soil-metal concentrations reduce photosynthesis and alter enzyme and protein production which ultimately affect nutrient transport (Assche and Clijsters, 1990; Reichman, 2002).

High soil metal concentrations may, therefore, suppress root growth and development. A greater development of plant roots and subsequent greater root surface areas of plants grown in soil-1, could have resulted in roots "exploring" the potted soil more intensely and allowed for greater accumulation and translocation of soluble metal-chelates. Sheppard *et al*. (2005) reported a predicted-no-effect-concentration (PNEC) value of less than 100 mg U kg^{-1} for microorganisms in soil contaminated with U. Benson *et al*. (1981), Ying *et al*. (2004) and Al Agely *et al*. (2005) reported that mycorrhizal symbiosis may be involved in As and P plant uptake. Therefore, a reason for As being undetectable in shoots grown in soil-2 (*cf.* Figure 5.3 C) could be related to the high U concentration of 168 mg kg^{-1} in soil-2 which may have reduced mycorrhizal fungi growth and subsequent As uptake.

Moreover, addition of citric acid to plants grown in soil-1 resulted in marked shoot biomass decreases (*cf.* Figure 5.4a to f). The decrease in soil pH (*cf.* Figure 5.1) may partially account for this decrease in shoot-dry weight production. Vandenhove *et al*. (2001) documented that the addition of 25 mmol citric acid kg^{-1} soil resulted in yield decreases in *B. juncea* and suggested that this were caused by the decrease in soil pH. However, when an equivalent amount of citric acid was added at pH 7 (adjusted with KOH) plant growth was also inhibited implying that the decreased dry weight production was not primarily due to a low pH but rather to an increase in metal uptake (Vandenhove *et al*., 2001).

Positive relationships were obtained between the shoot-U, -Mo and -As concentrations and the indices of tolerance (*cf.* Figure 5.5a to i) for all the plants grown in soil-1 in the present study. These results suggest that the major cause for the dry weight decreases was due to the increase in U, Mo and As to toxic concentrations in shoots. Similar trends were reported by Vandenhove *et al*.

(2001), Shahandeh and Hossner (2002b) and Rodríguez *et al*. (2006) for *B. juncea* and *H. annuus*.

In addition, the shoot dry weights of all the plants grown in soil-2 (*cf.* Figure 5.7a to f) were substantially lower compared to the plants grown in the background soil and soil-1. Positive relationships between shoot-U, -Mo and -As concentrations and the indices of tolerance were also obtained for these plants (*cf.* Figure 5.8i to iii) which, further, suggest that plant toxicity was experienced. These correlations were, however, less significant when compared to plants grown in soil-1 (compare Figure 5.5i to iii and 5.8i to iii). All the plants grown in soil-2 exhibited severe toxic symptoms including stunted and dwarfed growth, violet colouring of stems, leaf curling and necrosis of leaves (*cf*. Figure 5.9i to iii). Similar toxic symptoms were reported by Deuel and Swoboda (1972). They reported a 55% decrease in *Glycine max* (soybean) shoot biomass when grown in 12.5 mg As kg^{-1} soil. Leaf wilting, violet coloration (increased anthocyanin), root discoloration and cell plasmolysis were the main As toxicity symptoms (Deuel and Swoboda, 1979).

Broad ranges of U and As concentrations in soils that cause toxic effects to plants have been reported. These variations can be attributed to differences in soil characteristics which would determine the bioavailability of these metals in soils. It is, therefore, not enough to know the contamination level through total metal content in soil since high concentrations of these metals in soils would not automatically imply their release and assimilation by plants. Rodríguez *et al*. (2006) suggested that it may be more suitable to consider the concentration of metals in the soil solution rather than the total or acid digested metal concentrations in soil, as a parameter of toxicity.

Marked differences in the water soluble metal concentrations between soil-1 and soil-2 were measured in the present study (*cf.* Table 5.3). The largest difference was measured for Mo which was 67-fold higher in the soil solution of soil-2 than in soil-1 (*cf.* Table 5.3). A considerable variation also existed in the acid digested Mo concentration between soil-1 and soil-2 (1:30 ratio; *cf.* Table

5.4). Thus, the Mo leachate from the ore stockpiles on Rietkuil 307 is very mobile and as a result does not readily accumulate in the topsoil. For this reason Mo is a potential source of groundwater contamination. Therefore, the removal of the U ore stockpiles on Rietkuil 307 should be the primary objective regarding rehabilitation.

6.7 The Site-Specific Phytoextraction Potential of *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus* from low- and high-level metal contaminated soils

The Site Specific Phytoextraction Potentials (SSP) of *Brassica juncea* (cultivars: 211000 and 426308) and *Helianthus annuus* were determined by considering several factors (*cf.* 4.2.13) which included:

- the acid digested metal concentrations in the soil;
- the metal accumulation in the plant shoots, before and after citric acid additions;
- the soil metal-to-shoot transfer factors;
- the indices of tolerance for the plant species;
- the potential biomass yield of the plant species per annum.

The Site-Specific Phytoextraction Potential (an index of a plant's metal removal potential from a hectare of soil after one year) and the number of years required to phytoextract metals to background concentrations can be calculated (*cf.* 4.2.15). The results obtained (*cf.* Table 5.9 and Table 5.10) were based on the metal accumulation by young plants with maximum growth potential. Marschner (1995) reported a decline in mineral nutrient content and dry matter as plants aged and it is believed that plants will extract more metals in a growing season following consecutive sowing and harvests of immature plants compared to a single harvest of fully mature plants. It should, however, be noted that the initial shoot yields in the greenhouse study were obtained following irrigation and soil fertilization. To achieve similar growth potentials of plants in the field, it is

suggested that soil be fertilized and that the plants are irrigated to field capacity when necessary from the uncontaminated (Scholtz, 2003) irrigation dam on Rietkuil 307 (*cf.* Figures 1.3 and 1.4).

Beaufort West in the Karoo Uranium Province, South Africa lies in a summer rainfall area, characterized by hot summers and cold winters and receives a low annual rainfall of 236 mm and is regarded as semi-desert (South African Weather Service, 1990; *cf.* Appendix A2). Based on available data and a field situation with a 6 month growing season for crops (three crops at 9 week intervals) in the Beaufort West region, dry weight yields of 5.51, 5.08 and 2.81 tonnes per annum were predicted for *B. juncea* 211000, *B. juncea* 426308 and *H. annuus*, respectively (*cf.* Table 5.8).

According to the Site-Specific Phytoextraction Potential (SSP) it would be impractical to use phytoextraction for the remediation of U and As from the low-level metal contaminated soil (soil-1) on Rietkuil 307 without the additions of citric acid (*cf.* Table 5.10). In the case of *B. juncea* 211000, for example, based on the amount of U and As phytoextracted annually (*cf.* Table 5.9), it will take 670 years and 2770 years, respectively for U and As to be extracted to background levels in soil-1 without the addition of citric acid.

Although treatment A may have resulted in negative effects on the biomass production of plants grown in soil-1 (*cf.* Figure 5.4a to f), the number of years required to remove the U and As from soil-1 decreased substantially with this treatment (*cf.* Table 5.10). However, even with the highest U and As removal rates achieved by *B. juncea* 211000, with treatment A, the number of years required is still too long to make phytoextraction a practical remediation strategy on Rietkuil 307 (*cf.* Table 5.10).

The successive additions of citric acid to soil-1 (treatment B) decreased shoot biomass even further (*cf.* Figure 5.4a to f) but the high increase in metal concentrations (*cf.* Figure 5.3 A and C) resulted in a drastic decrease in the number of years predicted to phytoextract U and As from soil-1 (*cf.* Table 5.10).

Both the *B. juncea* cultivars (211000 and 426308) could be used for the phytoextraction of U from soil-1. It was predicted that it would take 14 years and 17 years respectively, to decontaminate the U levels in soil-1 to background concentrations using *B. juncea* 211000 and *B. juncea* 426308, in conjunction with successive citric acid treatments (Treatment B; *cf*. Table 5.10).

As a result of the apparent hyperaccumulation of Mo in the control soil-1, without the addition of citric acid, it is also feasible to phytoextract Mo using *B. juncea* 211000 (10 years) and *B. juncea* 426308 (11 years) from soil-1 (*cf.* Table 5.10). Furthermore, the successive addition of citric acid (Treatment B) not only enhanced Mo concentrations in the shoots of *B. juncea* 211000 and *B. juncea* 426308 but also reduced the amount of years needed to decontaminate the Mo in soil-1 to background concentrations (*cf*. Table 5.10).

Due to the low bioavailability of As and its subsequent low accumulation in plants, phytoextraction would not be a practical technology for the removal of As from soil-1. These results confirm the conclusions made by Sadiq (1986) and Sheppard (1992) when they reported that As has very low solubility in soils. The results obtained in this study confirm the conclusions made by Sheppard (1992) whom recommended that the remediation of As contaminated soils would probably have to involve soil excavation.

Although citric acid (treatment A and B), resulted in increased metal accumulation in plant shoots grown in soil-2 compared to the control (*cf.* Figure 5.3 A to C), the accumulation and dry weight production (*cf.* Figure 5.7a to f) were, however, insufficient to suggest that U, Mo and As phytoextraction by B. *juncea* (cultivars: 211000 and 426308) and *H. annuus* would be a practical decontamination alternative to current remediation technologies. At the highest extraction rate, more than 10 000 years will be required to decrease U levels in soil-2 to background levels and more than 1 400 years to decontaminate Mo to background levels (*cf*. Table 5.10).

The results obtained in this study show that both *B. juncea* cultivars (211000 and 426308) are superior in their U, Mo and As accumulating potential compared to *H. annuus*. Furthermore, the addition of successive citric acid treatments is required to attain sufficient extraction levels by the *B. juncea* cultivars (211000 and 426308), which could make the phytoextraction of U as remediation option on Rietkuil 307 soil feasible. In so doing, the low-level Mo contamination in Rietkuil 307 soil will simultaneously be reduced to background concentrations.

CHAPTER 7

CONCLUSIONS

7.1 Practical issues regarding the implementation of induced phytoextraction

Induced phytoextraction is a potential remediation strategy that can be used to decontaminate soils contaminated with inorganic pollutants. It is a low cost, *in situ*, solar driven technology that makes use of vascular plants and solubilizing agents to accumulate and translocate metals from roots to shoots. Harvesting the plant shoots can permanently remove these contaminants from the soil. Before this strategy is employed successfully, some practical issues require evaluation including:

1. Removal of the contamination source;
2. Soil evaluation and amendment screening for enhanced metal solubilization;
3. Estimating the rate of contaminant accumulation in plant shoots and the time required for remediation;
4. The level of contamination;
5. Field studies to determine the rate of removal in the field opposed to greenhouse trials;
6. Potential toxicity towards grazing animals;
7. Risk assessments of solubilized metals leaching into soil profile and into groundwater;
8. Disposal of plant biomass.

i) Removal of the contamination source

The removal of the U ore stockpiles, present as a result of inadequate rehabilitation following U trial mining on Rietkuil 307, should be a primary objective with regard to soil remediation. Scholtz (2003) did an assessment of the potential toxic influences of uranium trial mining on Rietkuil 307 and suggested that a long term remediation goal should include the removal of the ore stockpiles. He suggested that the stockpiles should be sold and recycled, buried or replaced by refilling the open mine pit which is the original source of

the ore. These actions will essentially remove the source of contamination, but since metals cannot be broken down, the elevated metal concentrations in topsoil would still present potential hazards for animals and humans.

ii) Soil evaluation and amendment screening for enhanced metal solubilization

An evaluation of Rietkuil 307 soil revealed that U, Mo and As concentrations were above the normal and critical metal levels published for soils (Bowen, 1979). Plants accumulate metals from the labile fraction of the soil and if phytoextraction is considered as an alternative soil remediation strategy, metal bioavailability would need to be manipulated. Citric acid was used as a soil amendment in the present study and the results proved it effective in chelating U, Mo and As from the soil solid phase into the soil solution. The studies also revealed that citric acid has a high chelating capacity for U.

iii) Estimating the rate of contaminant accumulation and time required for remediation

Of the plant species tested, *Brassica juncea* 211000 proved to be the most effective in accumulating U, Mo and As from Rietkuil 307 soil. It was estimated that *B. juncea* 211000 will be able to remove U and Mo in the contaminated soil on Rietkuil 307, to background soil concentrations within 14 and 9 years, respectively. However, due to the low solubility of As, phytoextraction will not be an effective remediation technology for As contamination on Rietkuil 307 soil.

iv) The level of contamination

This study revealed that citric acid induced phytoextraction of U and Mo is effective on low-level contaminated soil (soil-1). However, phytoextraction may not be applied to soils with high U, Mo and As concentrations due to the low biomass production and low metal accumulation in plant shoots grown in the high-level metal contaminated soil (soil-2). Thus, phytoextraction would not be

a feasible approach on high-level metal concentrated soils due to the long remediation times and toxicity to plants.

v) Field studies to determine the rate of removal in the field opposed to greenhouse trials

Before phytoextraction is implemented, however, it is suggested that field trials follow greenhouse studies to evaluate whether similar metal accumulation and growth will be achieved. Most studies performed on induced phytoextraction involved greenhouse pot experiments with soil, either collected from contaminated sites (Huang *et al*., 1998; Cooper *et al*., 1999), or artificially contaminated with the desired metal (Ebbs *et al*., 1998; Shahandeh and Hossner 2002a and b). However, uptake of heavy metals by plants under controlled greenhouse conditions could differ substantially from plant uptake in the field. For example, Kayser *et al*. (2000) showed that three times less Cd was found in tobacco (*Nicotiana tabacum*) and sunflower (*Helianthus annuus*) and seven times less Cd in Indian mustard (*Brassica juncea*) when these species were grown in the field compared to pot experiments. On average, Cd removal from soils in the field was only 20% compared to the pot experiments. Several explanations for this lesser removal were presented: (i) in most pot experiments the soil was artificially enriched with soluble heavy metal salts, increasing the variability of results and the probability of achieving extraordinary high heavy metal-plant concentrations; (ii) the soluble heavy metal concentrations of the soils were lower in the field versus pot experiments; (iii) the efficiency of soil amendments is higher in pot experiments because plant roots explore potted soil more intensely and are always in contact with the soil amendments.

vi) Potential toxicity towards grazing animals

Because phytoextraction involves toxic pollutants to accumulate in above ground plant tissues, these plant materials may pose health hazards to wildlife. Pilon-Smits (2005) suggested that the potential toxicity of plant tissues can be

tested in a laboratory digestibility study using model organisms or *in vitro* simulations of animal digestion systems. The author, furthermore, suggested that exposure of wildlife to contaminated areas may be minimized by fencing, netting, noise and/or scarecrows.

vii) Risk assessments of solubilized metals leaching into soil profile and into groundwater

The use of soil amendments to increase metal bioavailability and subsequent plant uptake may also increase metal leaching into the groundwater following a rainfall event. It is recommended that rapid biodegradable amendments such as organic agents be used. When the results of this study are compared to the citric acid degradation patterns reported by Huang *et al*. (1998), Qualls and Haines (1992), Jones *et al*. (1996) and Jones and Darrah (1994), it suggest that citric acid rapidly biodegraded following additions to soil-1 and soil-2. Thereby, soluble metal-chelate complexes will be reduced, minimizing the risk of groundwater contamination. Furthermore, the addition of smaller dosages of citric acid more often may decrease the amount of metal leachate. Kayser *et al*. (2000) also suggested that by placing amendments directly into the rooting zone, instead of mixing into the entire field, will lower the amount of amendment required to enhance metal accumulation. Similarly, a dense root mat will increase accumulation of solubilized metal accumulation which would consequently decrease metal leaching.

The analysis of heavy metal concentrations in leachate produced by excess water supplied in phytoextraction pot experiments may be an easy way to quantify metal leaching from the soil columns. The frequency and amount of these induced leaching events can be adapted to the number of heavy rainfall events of an area (Schmidt, 2003). Bischoff *et al*. (1999), furthermore, suggested that monitoring boxes which are filled with ion exchange resins and placed under the rooting zone of plants can be used to quantify metal leachate in the field.

viii) Disposal of harvested plant biomass

The incineration of plant tissue can further concentrate the metals which reduce the amount of biomass for disposal. Chaney *et al*. (2000) proposed that the value of the reclaimed metal may provide an additional incentive for phytoextraction. They showed that the metals in the biomass could be recovered (phytomining) and was shown to balance the cost of phytoextraction.

7.2 Concluding remarks

The results from this study suggest that citric acid induced phytoextraction using the two cultivars of *B. juncea* (211000 and 426308) could be a feasible remediation technology to decontaminate the low-level U and Mo contaminated soil (soil-1) on Rietkuil 307. However, due to the low accumulation of As in plant-shoots, phytoextraction would not be practical for the removal of As from this soil. Furthermore, phytoextraction from high-level U, Mo and As contaminated soils (soil-2) would not be a practical technology due to the low biomass yield produced by the plant shoots as a result of toxicity. Long term failure to remove the U ore stockpiles could result in more elevated metal concentrations in the topsoil which would require other means of remediation than phytoextraction.

7.3 Further studies

The success of phytoextraction as an alternative decontamination technology is limited by four factors: the total amount of potentially bioavailable metals in soil; the rate of metal transfer from soil solution to plant roots; the rate of metal translocation from roots to shoots; the ability of plants to tolerate large concentrations of metals in soil or plant tissue; and the ability of plants to cope with climatic extremes. These factors generally influence the amount of time needed to phytoextract metals to background or acceptable soil levels.

With genetic engineering, plants can be manipulated to accumulate, translocate and tolerate heavy metals, thus creating the ideal transgenic plant for environmental cleanup in the shortest possible time (Pilon-Smits, 2005; Bennett, 2003; Persans *et al*., 2001). For instance, genes can be isolated from metal hyperaccumulators and inserted into fast growing high biomass plant species (Persans *et al*. 2001). It has been suggested that phytoextraction would become commercially available if metal removal and -tolerance properties of hyperaccumulator plants, such as *Thlapsi caerulescens* (Brown *et al*., 1995; Bennett, 2003) or *Pteris vittata* (Ma *et al*., 2001), could be transferred into fast growing, high biomass producing crop species.

Furthermore, a system which incorporates crop rotation could be implemented on soils with multiple metal contaminations which includes As. For instance, the As hyperaccumulator, *P. vittata* can be used in conjunction with *B. juncea* to phytoextract U and As from contaminated soils.

The amount of years to decontaminate the metal contaminated soils was predicted based upon the metal concentrations of young plants with maximum growth potential. Before the implementation of phytoextraction, however, it should be investigated whether similar metal concentrations would be achieved if citric acid is applied to fully matured plants. In addition, given the toxic effect of citric acid and metal accumulations on young plants' biomass production, it should be investigated whether similar toxic effects would be observed in fully mature plants following citric acid additions.

Moreover, continuous citric acid additions and harvesting of plant shoots on soils could have a negative effect on seed germination in the long term and the effects of continuous citric acid applications to soils require further investigation.

CHAPTER 8

REFERENCES

Abedin, M.J., Feldmann, J. and Meharg, A.A. 2002. Uptake kinetics of arsenic species in rice plants. *Plant Physiology* 128: 1120-28.

Agency for Toxic Substances and Disease Registry (ATSDR). 2005. Toxicological Profile for Arsenic (Draft for Public Comment). Atlanta, GA: U.S. Department of Public Health and Human Services, Public Health Service.

Ahumuda, I., Mendoza, J., Navarrete, E. and Ascar, L. 1999. Sequential extraction of heavy metals in soils irrigated with wastewater. *Communications in Soil Science and Plant Analysis* 30: 1507-1519.

Al Agely, A. Sylvia, D.M. and Ma, L.Q. 2005. Mycorrhizae increase arsenic uptake by the hyperaccumulator chinese brake fern (*Pteris vittata* L.). *Journal of Environmental Quality* 34: 2181-2186.

Albasel, N. and Pratt, P.F. 1989. Guidelines for molybdenum in irrigation waters. *Journal of Environmental Quality* 18: 259-264.

Allen, E.B. 1988. The reconstruction of disturbed arid lands: An ecological approach. *In*: Glenn, E.P., Waugh, W.J., Moore, D., Mckeon C. and Nelson, S.G. 2001. Revegetation of an abandoned uranium millsite on the Colorado Plateau, Arizona. *Journal of Environmental Quality* 30: 1154-1162.

Ammerman, C.B., Fontenot, J.P., Spivey-Fox, M., Hutcinson, H.D., Lepore, P., Stowe, H.D., Thompson, J.D. and Ullrey, D.E. 1980. Mineral tolerance of domestic animals. National Academy of Sciences, Washington D.C., pp. 578.

Anderson, A.J. 1956. Molybdenum as fertilizer. *Advances in Agronomy* 8: 163-202.

Anderson, C.W.N., Brooks, R.R., Stewart, R.B. and Simcock, R. 1998. Harvesting a crop of gold in plants. *Nature* 395: 353-554.

Anderson, C.W.N., Brooks, R.R., Chiarucci, A., Lacoste, C.J., Leblanc, M., Robinson, B.H., Simcock, R. and Stewart, R.B. 1999. Phytomining for nickel, thallium and gold. *Journal of Geochemical Exploration* 67: 407-415.

Assche, F. and Clijsters, H. 1990. Effects of metals on enzyme activity in plants. *Plant and Cell Environment* 24: 1-15.

Athar, M. and Vohora, S.B. 1995. Heavy metals and environment. New Age International Publishers Limited, New Delhi, pp. 216.

Axelsen, K.B. and Palmgren, M.G. 2001. Inventory of the superfamily of P-type ion pumps in *Arabidopsis*. *Plant Physiology* 126: 696-706.

Baker, A.J.M. 1981. Accumulators and excluders – strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* 3: 643-654.

Baker, A.J.M. and Brooks, R.R. 1989. Terrestrial higher plants which hyperaccumulate chemical elements – a review of their distribution, ecology and phytochemistry. *Biorecovery* 1: 81-126.

Baker, A.J.M., McGrath, S.P., Sidoli, C.M.D. and Reeves, R.D. 1994. The possibility of in situ metal decontamination of polluted soils using crops of metal-accumulating plants – a feasibility study. *Resources Conservation and Recycling* 11: 41-49.

Baker, A.J.M., Mcgrath, S.P., Reeves, R.D. and Smith, J.A.C. 2000. Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. *In*: *Phytoremediation of Contaminated Soil and Water*. Terry, N. and Bañuelos, G. (eds.). Lewis, Boca Raton, Florida, pp. 85-108.

Barrow, N.J. 1974. Effect of previous additions of phosphate on phosphate adsorption by soils. *Soil Science* 118: 82-89.

Barrow, N.J. 1986. Testing a mechanistic model. IV. Describing the effects of pH on zinc retention by soils. *Journal of Soil Science* 37: 295-303.

Barshad, I. 1951. Factors affecting the molybdenum content of pasture plants. *Soil Science* 71: 297-313.

Bennett, L.E., Burkhead, J.L., Hale, K.L., Terry, N., Pilon, M. and Pilon-Smits, E.A.H. 2003. Analysis of transgenic Indian mustard plants for phytoremediation of metal contaminated mine tailings. *Journal of Environmental Qua*lity 32: 432-440.

Benson, A.A., Cooney, R.V. and Herrera-Lasso, J.M. 1981. Arsenic metabolism in algae and higher plants. *Journal of Plant Nutrition* 3: 285-292.

Blaylock, M. J., Salt, D.E., Dushenkov, S., Zakaharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D. and Raskin, I. 1997. Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents. *Environmental Science and Technology* 31: 860-865.

Bliss, L.T. and Meyerhof, D.P. 1987. Uranium concentrations in air near a Canadian uranium refinery. *Atmospheric Environment* 21: 165-172.

Bischoff, W.A., Siemens, J. and Kaupenjohann, M. 1999. Solutes transport into groundwater – A survey on field methods under consideration of preferential flow. (In German with English abstract). *Wasser Boden* 51: 37-42.

Boulding, J.R. 1994. Description and sampling of contaminated soils. 2nd Edition, Lewis Publishers.

Bowen, H.J.M. 1979. Environmental chemistry of the elements. *In*: *Heavy metals in soils*. Alloway, B.J., (ed.). Blackie. Glasgow and London, pp. 339.

Brooks, R.R. 1972. Geobotany and biogeochemistry in mineral exploration. Harper and Row publishers, pp. 290.

Brooks, R.R., Lee, J., Reeves, R.D. and Jaffré, T. 1977. Detection of nickleliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration* 7: 49-57.

Brown, S.L., Chaney, R.L., Angle, J.S. and Baker, A.M. 1995. Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. *Soil Science Society of America Journal* 59: 125-133.

Brune, A., Urbach, W. and Dietz, K-J. 1994. Compartementation and transport of zinc in barley primary leaves as basic mechanisms involved in zinc tolerance. *Plant, Cell and Environment* 17: 153-162.

Cannon, H.L. 1971. The use of plant indicators in ground water surveys, geologic mapping, and mineral prospecting. *Taxon* 20: 227-256.

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewer, E.P., Angle, J.S. and Baker, A.J.M. 1997. Phytoremediation of soil metals. *Current Opinion in Biotechnology* 8: 279-284.

Chaney, R.L., Li, Y.M., Brown, S.L., Homer, F.A., Malik, M., Angle, J.S., Baker, A.J.M., Reeves, R.D. and Chin, M. 2000. Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress. *In*: *Phytoremediation of Contaminated Soil and Water*. Terry, N. and Bañuelos, G., (eds.). Lewis, Boca Raton, Florida, pp. 129-158.

Chen, T., Macauley, E. and Hong, A. 1995. Selection and test of effective chelators for removal of heavy metals from contaminated soils. *Canadian Journal of Civil Engineering* 22: 1185-1197.

Chen, M. and Ma, L.Q. 1998. Comparison of four USEPA digestion methods for trace metal analysis using certified and Florida soils. *Journal of Environmental Quality* 27:1294-1300.

Chen, H.M., Zeng, C.R., Tu, C. and Shen, Z.G. 2000. Chemical methods and phytoremediation of soil contaminated with heavy metals. *Chemosphere* 41: 229-234.

Chen, T.B., Wei, C.Y., Huang, Z.C., Huang, Q.F. and Lu, Q.G. 2002. Arsenic hyperaccumulator *Pteris vittata* L. and its arsenic accumulation. *Chinese Science Bulletin* 47: 902–905.

Chen, B.D., Zhu, Y-G. and Smith, F.A. 2006. Effects of arbuscular mycorrhizal inoculation on uranium and arsenic accumulation by Chinese brake fern (*Pteris vittata* L.) from uranium mining-impacted soil. *Chemosphere* 62: 1464-1473.

Clarkson, D.T. and Luttge, U. 1989. Mineral nutrition: divalent cations, transport and compartmentation. *Progress in Botany* 51: 93-112.

Cobbett, C.S. and Goldsbrough, P.B. 2000. Mechanisms of metal resistance: phytochelatins and metallothioneins. In: *Phytoremediation of Toxic Metals – Using Plants to clean up the Environment*. Raskin, I. and Ensley, B.D., (eds.). Wiley, New York, pp. 247-271.

Cole, D.I., Labuschagne, L.S. and Söhnge, A.P.G. 1991. Aeroradiometric survey for uranium and ground water follow up in the main Karoo Basin. Memoir 76 of the Geological survey. Department of mineral and energy affairs, pp. 145.

Cooper, E.M., Sims, J.T., Cunningham, S.D., Huang, J.W. and Berti, W.R. 1999. Chelate-Assisted Phytoextraction of Lead from Contaminated Soils. *Journal of Environmental Quality* 28: 1709-1719.

Cunningham, S.D., Berti, W.R. and Huang, J.W. 1995. Phytoremediation of contaminated soils. *Trends in Biotechnology* 13: 398-403.

Darland, J.E. and Inskeep W.P. 1997. Effects of pH and phosphate competition on the transport of arsenate. *Journal of Environmental Quality* 26:1133-1139.

Deuel, L.E. and Swoboda, A.R. 1972. Arsenic toxicity to cotton and soybeans. *Journal of Environmental Quality* 1:317-320.

Diamond, G.L., Morrow, P.E., Panner, B.J., Gelein, R.M. and Baggs, R.B. 1989. Reversible uranyl fluoride nephrotoxicity in the Long Evans rat. *Fundamental and Applied Toxicology* 13: 65-78.

Djingova, R. and Kuleff, I. 2000. Instrumental techniques for trace analysis. *In*: *Trace elements: Their distribution and effects in the environment*. Vernet, J.P. (ed.). Elsevier Science Ltd., United Kingdom, pp. 146.

Dodge, C.J. and Francis, A.K. 1994. Photodegradation of uranium-citrate complex with uranium recovery. *Environmental Science and Technology* 28: 1300-1306.

Doucleff, M. and Terry, N. 2002. Pumping out the arsenic. *Nature Biotechnology* 20:1094-1095.

Duffey, R.B. 2005. Sustainable futures using nuclear energy. *Progress in Nuclear Energy* 47: 535-543.

Dushenkov, S., Kapulnik, Y., Blaylock, M., Sorochiskey, B., Rakin, I. and Ensley, B. 1997. Phytoremediation: a novel approach to an old problem. *In*: *Global Environmental Biotechnology*. Wise, D.L., (ed.). Elsevier Science Ltd. The Netherlands, pp. 563-572.

Duquène, L., Vandenhove, H., Tack, F., van der Avoort, E., Wannijn, J. and van Hees, M. 2005. Phytoavailability of uranium: influence of plant species and soil characteristics. *In*: *Uranium in the Environment*. Merkel, B.J. and Hasche-Berger, A. (eds.). Freiberg, Germany, pp. 476-469.

Department of Water Affairs and Forestry (DWAF). 1996. *South African Water Quality Guidelines (*2nd ed*)*. Volume 1: Domestic Use pp.197.

Department of Water Affairs and Forestry (DWAF). 1996. *South African Water Quality Guidelines (*2nd ed*)*. Volume 4: Agricultural Water Use: Irrigation pp. 199.

Department of Water Affairs and Forestry (DWAF). 1996. *South African Water Quality Guidelines (*2nd ed*)*. Volume 5: Livestock Watering pp. 163.

Department of Water Affairs and Forestry (DWAF). 1996. *South African Water Quality Guidelines (*2nd ed*)*. Volume 6: Agricultural Water Use: Aquaculture pp. 163.

Department of Water Affairs and Forestry (DWAF). 1996. *South African Water Quality Guidelines (*2nd ed*)*. Volume 7: Aquatic Ecosystems pp. 159.

Ebbs, S.D., Brady, D.J. and Kochian, L.V. 1998. Role of uranium speciation in the uptake and translocation by plants. *Journal of Experimental Botany* 49: 1183-1190.

Elliot, H.A. and Shields, G.A. 1988. Comparative evaluation of residual and total metal analyses in polluted soils. *Communications in Soil Science and Plant Analyses* 19: 1907-1915.

Ernst, W.H.O., Verkleij, J.A.C. and Schat, H. 1992. Metal tolerance in plants. *Acta Botanica Neederlandica* 41: 229-248.

Fargasova, A. 1994. Effect of Pb, Cd, Hg, As and Cr on germination and root growth of *Sinapis alba* seeds. *Bulletin of Environmental Contamination and Toxicology* 52: 452-456.

Foth, H.D. 1990. *Fundamentals of soil science.* 8th *edition.* John Wiley & Sons, New York, pp. 360.

Francis, C.W., Timpson, M.E. and Wilson, J.H. 1999. Bench- and pilot-scale studies relating to the removal of uranium from uranium-contaminated soils using carbonate and citrate lixiviants. *Journal of Hazardous Materials* 66: 67-87.

Gahoonia, T.S., Claassen, N. and Jungk, A. 1992. Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. *Plant and Soil* 140: 241-248.

Gee, G.W. and Bauder, J.W. 1986. Particle-size Analysis. Methods of soil analysis. Part 1. Physical and mineralogical methods. *In*: *Agronomy*. Klute, A., (ed.). Madison, WI, pp. 383-411.

Geelhoed, J.S., van Riemsdijk, W.H. and Findenegg, G.R. 1999. Simulation of the effect of citrate exudation from roots on the plant availability of phosphate adsorbed on goethite. *European Journal of Soil Science* 50: 379-390.

Goldsbrough, P.B. 2000. Metal tolerance in plants: the role of phytochelatins and metallothioneins. In: *Phytoremediation of Contaminated Soil and Water*. Terry, N. and Bañuelos, G., (eds.). Lewis, Boca Raton, Florida, pp. 221-234.

Gulati, K.L., Oswal, M.C. and Nagpaul, K.K. 1980. Assimilation of uranium by wheat and tomato plants. *Plant and Soil* 55: 55-59.

Gunasekera, C.P. 2003. Adaptation of Indian mustard (*Brassica juncea* L.) to short season dryland Mediterranean-type of environments. Ph.D thesis, Curtin University of Technology, Sri Lanka, pp. 168.

Hamon, R.E., Lorenz, S.E., Holm, P.E., Christensen, T.H. and McGrath, S.P. 1995. Changes in trace metal species and other components of the rhizosphere during growth of radish. *Plant*, *Cell and Environment* 18: 749-756.

Harper, M. and Haswell, S.J. 1988. A comparison of copper, lead and arsenic extraction from polluted and unpolluted soils. *In*: *Heavy metals release in soils*. Selim, H.M. and Sparks, D.L., (eds.). Lewis, Boca Raton, Florida, pp. 207-235.

Harries, J. and Ritchie, A.I.M. 1983. Runoff fraction and pollution levels in runoff from a waste rock dump undergoing pyretic oxidation. *Water, Air and Soil Pollution* 19: 155-170.

Hawkesford, M.J. 2003. Transporter gene families in plants: the sulfate transporter gene family – redundancy or specialization? *Physiologia Plantarum* 117: 155-63.

Hertsgaard, M. 2004. Three Mile Island. *Nation* 278: 7-8.

Hinton, T.G., Knox, A.S., Kaplan, D.I. and Sharitz, R. 2005. Phytoextraction of uranium and thorium by native trees in a contaminated wetland. *Journal of Radioanalytical and Nuclear Chemistry* 264: 417-422.

Hirose, K. and Sugimura, Y. 1981. Concentration of uranium and the activity ratio of $^{234}U/^{238}U$ in surface air-effect of atmospheric burn-up of cosmos-954. *Papers in Meteorology and Geophysics* 32: 317-322.

Hirose, K. and Sugimura, Y. 2003. Chemical speciation of particulate ^{238}U, $^{239,240}Pu$ and Th isotopes in seawater. *Science of the Total Environment* 131: 517-524.

Hossner, L.R., Loepert, R.H., Newton, R.J. and Szaniszlo, P.J. 1998. Literature review: Phytoaccumulation of chromium, uranium and plutonium in plant systems. Amarillo National Resource Centre for Plutonium, Springfield, VA, pp.51.

Howard, B.J., Beresford, N.A. and Hove, K. 1991. Transfer of radiocaesium to ruminants in natural and semi-natural ecosystems and appropriate countermeasures. *Health Physics* 61: 715 -725.

Huang, J.W., Blaylock, M.J. and Kapulnik, Y. 1998. Phytoremediation of uranium-contaminated soils: Role of organic acids in triggering uranium hyperaccumulation by plants. *Environmental Science and Technology* 32: 2004-2008.

ISO 11 265. 1994. Soil quality – Determination of specific electrical conductivity. Geneva, Switzerland.

Jaffré, T., Brooks, R.R., Lee, J. and Reeves, R.D. 1976. *Sebertia acuminate*: a nickel-accumulating plant from New Caledonia. *Science* 193: 579-580.

Jain, A., Raven, K.P. and Loeppert, R.H. 1999. Arsenite and arsenate adsorption on serrihydrite: surface charge reduction and net OH-release stoichiometry. *Environmental Science and Technology* 33: 1179-1184.

Jang, M., Hwang, J.S., Choi, S. and Park, J.K. 2005. Remediation of arsenic-contaminated soils and washing effluents. *Chemosphere* 60: 344-354.

Jarrell, W.M., Page, A.L. and Elseewi, A.A. 1980. Molybdenum in the environment. *In*: *Toxicity summary for molybdenum and molybdenum compounds*. Opresko, D.M. Health and Safety Research Division, Oak Ridge, Tennessee, pp.18.

Johnson, C.M., Stout, P.R., Broyer, T.C. and Carlton, A.B. 1957. Comparative chlorine requirements of different plant species. *Plant and Soil* 8: 337-353.

Johnson, J.F., Allan, D.L., Vance, C.P. and Weiblen, G. 1996. Root carbon dioxide fixation by phosphorus defcient *Lupinus albus*. Contribution to organic acid exudation by proteoid roots. *Plant Physiology* 112: 19-30.

Johnston, A.M., Tanaka, D.L., Miller, P.R., Brandt, S.A., Nielsen, D.C., Lafond, G.P. and Riveland, N.R. 2002. Oilseed crops for semiarid cropping systems in the northern Great Plains. *Agronomy Journal* 94: 231-240.

Jones, D.L. and Darrah, P.R. 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant and Soil* 166: 247-257.

Jones, D.L., Prabowo, A.M. and Kochian, L.V. 1996. Kinetics of malate transport and decomposition in acid soils and isolated bacterial populations: the effect of microorganisms on root exudation of malate under Al stress. *Plant and Soil* 182: 239-247.

Jones, C.A, Inskeep, W.P. and Neuman, D.R. 1997. Arsenic transport in contaminated mine tailings following liming. *Journal of Environmental Quality* 26: 433-439.

Jones, T.L. and Serne, R.J. 1995. Contaminant release from solidified radioactive wastes buried in unsaturated sediments: lysimeter study. *Journal of Environmental Quality* 24: 1063-1073.

Kari, F.G. and Giger, W. 1996. Speciation and fate of ethylendiamintetraacetate (EDTA) in municipal wastewater treatment. *Water Research* 30: 122-134.

Karimian, N. and Cox, F.R. 1978. Adsorption and extractability of molybdenum in relation to some chemical properties of soil. *Soil Science Society of American Journal* 42: 757-761.

Kataba-Pendias, A. and Pendias, H. 1984. Trace elements in soils and plants. *In*: *Heavy metals in soils*. Alloway, B.J., (ed.). Blackie, Glasgow and London, pp. 339.

Kayser, A., Wenger, K., Keller, A., Attinger, W., Felix, H., Gupta, S.K. and Schulin, R. 2000. Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: The use of NTA and sulfur amendments. *Environmental Science and Technology* 34: 1778-1783.

Kimber, D.S. and McGregor, D.I. 1995. The species and their origin, cultivation and world production. *In*: *Brassica oilseeds, production and utilization*. Kimber, D.S., McGregor, D.I. (eds.). CAB Int, Wallingford, pp 1-7.

Kitagishi, K. and Yamane, I. 1981. Heavy Metal Pollution in Soils of Japan. Japan Scientific Society Press, Tokyo, pp. 21-67.

Krebs, R., Gupta, S.K., Furrer, G. and Schulin, R. 1999. Gravel sludge as an immobilizing agent in soils contaminated by heavy metals - a field study. *Water, Air and Soil Pollution* 115: 465-479.

Kumar, N.P.B.A., Dushenkov, V., Motto, H. and Raskin, I. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental Science and Technology* 29: 1232-1238.

Lamas, M., Fleckenstein, J., Schroeter, S., Sparovek, R.M. and Schnug, E. 2002. Determination of uranium by means of ICP-QMS. *Communications in Soil Science and Plant Analysis* 33: 3469-3479.

Laroche, L., Henner, P., Camilleri, V., Morello, M. and Garnier-Laplace, J. 2005. Root uptake of uranium by a higher plant model (*Vaseolus vulgaris*) – bioavailability from soil solution. *Radioprotection* 40: 33-39.

Le Roux, J.P. 1994. Author's reply to discussion — genesis of stratiform U-Mo deposits in the Karoo Basin of South Africa. *Ore Geology Reviews* 9:248-252.

Le Roux, J.P. and Brynard H.J. 1994. A strategy for uranium exploration in the Permo-Triassic Beaufort Group of the main Karoo Basin, South Africa. *Journal of African Earth Science* 18: 245-253.

Li, M., Shinano, T. and Tadano, T. 1997. Disitribution of exudates of lupin roots in the rhizosphere under phosphorus deficient conditions. *Soil Science and Plant Nutrition* 43: 237-245.

Li, Y-M., Chaney, R., Brewer, E., Roseberg, R., Angle, S.J., Baker, A., Reeves, R. and Nelkin, J. 2003. Development of a technology for commercial phytoextraction of nickel: economic and technical considerations. *Plant and Soil* 249: 107-115.

Liator, M. 1995. Uranium isotopes distribution in soils at the Rocky Flats Plant, Colorado. *Journal of Environmental Quality* 24: 314-323.

Liu, D.H., Jiang, W.S., Wang, W., Zhao, F.M. and Liu, C. 1994. Effects of lead on root growth, cell division, and nucleolus of *Allium cepa*. *Environmental Pollution* 86: 1-4.

Lothenbach, B., Furrer, G. and Schulin, R. 1997. Immobilizaion of heavy metals by polynuclear aluminium and montmoillonite compounds. *Environmental Science and Technology* 31: 1452-1462.

Lozano, J.C., Vera Tome, F., Gomez, Escobar, V. and Blanco Rodríguez, P. 2000. Radiological characterization of a uranium mine with no mining activity. *Applied Radiation and Isotopes* 53: 337-343.

Ma, J.F. and Nomoto, K. 1996. Effective regulation of iron acquisition in graminaceous plants. The role of mugineic acids as phytosiderophores. *Physiologia Plantarum* 97: 609-617.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y. and Kennelley, E.D. 2001. A fern that hyperaccumulates arsenic. *Nature* 409: 579-579.

Macnair, M.R., Tilstone, G.H. and Smith, S.E. 2000. The genetics of metal tolerance and accumulation in higher plants. *In*: *Phytoremediation of Contaminated Soil and Water*. Terry, N. and Bañuelos, G., (eds.). Lewis, Boca Raton, Florida, pp. 235-250.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London, pp. 889.

Mascher, R., Lippmann, B., Holzinger, S. and Bergmann, H. 2002. Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. *Plant Science* 163: 961-969.

Mason, B.J. 1992. Preparation of soil sampling protocols: sampling techniques and strategies. Environmental Research Centre, University of Nevada-Las Vegas, USA, pp. 14.

Mason, C.F.V., Turney, W.R.J.R., Thompson, B.M., Lu, N., Longmire, P.A. and Chisholm-Brause, C.I. 1997. Carbonate leaching of uranium from contaminated soils. *Environmental Science and Technology* 31: 2707-2711.

Meharg, A.A. and Hartley-Whitaker, J. 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytologist* 154: 29-43.
Meharg, A.A. and Macnair, M.R. 1990. An altered phosphate uptake system in arsenate-tolerant *Holcus lanatus* L. *New Phytotologist* 116: 29-35.

Meyer, M.C., Paschke, M.W., McLendon, T. and Price, D. 1998. Decreases in soil microbial function and functional diversity in response to depleted uranium. *Journal of Environmental Quality* 27: 1306-1311.

Minerals Act no. 50 of the Republic of South Africa. 1991.

Mullen, R.W., Raun, W.R., Basta, N.T., Schroder, J.L. and Freeman K.W. 2005. Effect of Long-Term Application of Biosolids on Molybdenum Content and Quality of Winter Wheat Forage. *Journal of Plant Nutrition* 28: 405-420.

Mylona, P.V., Polidoros, A.N. and Scandalios, J.G. 1998. Modulation of antioxidant responses by arsenic in maize. *Free Radical Biology and Medicine* 25: 576-585.

Neunhäuserer, C., Berreck, M. and Insam, H. 2001. Remediation of soils contaminated with molybdenum using soil amendments and phytoremediation. *Water, Air and Soil Pollution* 128: 85-96.

Ng, J.C., Wang, J. and Shraim, A. 2003. A global health problem caused by arsenic from natural sources. *Chemosphere* 52:1353-1359.

Norberg, A.B. and Molin, N. 1983. Toxicity of cadmium, cobalt, uranium and zinc to *Zoogloea ramigera*. *Water Research* 17: 1333-1336.

Oram, R.N. and Kirk, J.T.O. 1992. Breeding Indian mustard varieties for Australian conditions. *In*: Proceedings of the 6th Australian Agronomy Conference. Hutchinson, K.J. and Vickery, P.J. (eds.). pp 467-470.

Page, A.L., Miller, R.S.H. and Keeney, D.R. 1982. Methods of soil analysis, Part 2. Chemical and microbiological properties, 2nd edition, American Society of Agronomy, Inc. Madison, WI.

Persans, M.W., Nieman, K. and Salt, D.E. 2001. Functional activity and role of cation-efflux family members in Ni hyperaccumulation in *Thlapsi goesingense*. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 9995-10000.

Percebois, J. 2003. The peaceful uses of nuclear energy: technologies of the front and back-ends of the fuel cycle. *Energy Policy* 31: 101-108.

Pfeifer, H-R., Derron, M-H., Rey, D., Sclegel, C., Atteia, O., Dalla Piazza, R., Dubois, J-P. and Mandia, Y. 2000. *In*: *Trace elements: their distribution and effects in the environment.* Markert, B. and Friese, K. (eds.). Elsevier Science Ltd, Oxford, United Kingdom, pp. 35.

Pickering, I.J., Prince, R.C., George, M.J., Smith, R.D., George, G.N. and Salt D.E. 2000. Reduction and coordination of arsenic in Indian mustard. *Plant Physiology* 122: 1171-1177.

Pierce, M.L. and Moore, C.B. 1982. Adsorption of arsenite and arsenate on amorphous iron hydroxide. *In*: *Heavy metals release in soils*. Selim, H.M. and Sparks, D.L., (eds.). Lewis, Boca Raton, Florida, pp. 207-235.

Pilon-Smits, E.A.H. 2005. Phytoremediation. *Annual Review of Plant Biology* 56: 15-39.

Pitten, F-A., Müller, G., and König, P. 1999. Risk assessment of former military base contaminated with organoarsenic-based warfare agents: Uptake of arsenic by terrestrial plants. *Science of the Total Environment* 226:237-245.

Qualls, R.G. and Haines, B.L. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution and stream water. *Soil Science Society of America Journal* 56: 578-586.

Ragnarsdottir, K.V. and Charlett, L. 2000. Uranium behaviour in natural environments. *In*: *Environmental mineralogy: microbial interactions, anthropogenic influences, contaminated land and waste management.* Cotter Howells, J.D., Campbell, L.S., Valsami Jones, E. and Batchelder, M. (eds.). Mineralogical Society Series 9, Mineralogical Society, London, pp. 245-289.

Rahn, P.H., Davis, A.D., Webb, C.J. and Nichols, A.D. 1996. Water quality impacts from mining in the Black Hills, South Dakota, USA. *Environmental Geology* 27: 38-53.

Ramos, L., Hernandez, L.M. and Gonzales, M.J. 1994. Sequential fractionation of copper, lead, cadmium and zinc in soils from near Dońana National Park. *Journal of Environmental Quality* 23: 50-57.

Reichman, S.M. 2002. The responses of plants to metal toxicity: a review focusing on copper, manganese and zinc. *Australian Minerals & Energy Environment Foundation*, pp. 54.

Reisenauer, H.M., Tabikh, A.A. and Stout, P.R. 1962. Molybdenum reactions with soils and the hydrous oxides of iron, aluminum, and titanium. *Soil Science Society of America Proceedings* 26: 23-27.

Reuveny, Z. 1977. Derepression of ATP sulfurylase by the sulfate analogs molybdate and selenate in cultured tobacco cells. *Proceedings of the National Academy of Science* 74: 619-22.

Rodríguez, P.B., Tomé, F.V., Fernández, M.P. and Lozano, J.C. 2006. Linearity assumption in soil-to-plant transfer factors of natural uranium and radium in *Helianthus annuus* L. *Science of the Total Environment* 361: 1-7.

Sadiq, M. 1986. Solubility relationships of arsenic in calcareous soils and its uptake by corn. *Plant and Soil* 91: 241-247.

Sadiq, M., Zaidi, T.H. and Mian, A.A. 1983. Environmental behavior of arsenic in soils: theoretical. *Water, Air, and Soil Pollution* 20: 369-377.

Salido, A.L., Hasty, K.L., Lim, J.M. and Butcher, D.J. 2003. Phytoremediation of arsenic and lead in contaminated soil using Chinese brake ferns (*Pteris vittata*) and Indian mustard (*Brassica juncea*). *International Journal of Phytoremediation* 5: 89-103.

Salt, D.E., Blaylock, M., Kumar, N.P.B.A., Dushenkov, V., Ensley, B.D., Chet, I. and Raskin, I. 1995a. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology* 13: 468-475.

Salt, D.E., Prince, R.C., Pickering, I.J. and Raskin, I. 1995b. Mechanisms of cadmium mobility and accumulation in Indian Mustard. *Plant Physiology* 109: 1427-1433.

Schmidt, U. 2003. Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation and leaching of heavy metals. *Journal of Environmental Quality* 32: 1939-1954.

Schneider, P., Voerkelius, S., Nindel, M., Forster, M. and Schreyer, J. 2001. Release of contaminants from uranium mine waste – laboratory and field experiments. *Mine Water and the Environment* 20: 30–38.

Scholtz, N. 2003. Assessment of potential toxic influence of uranium trial mining in the Karoo uranium province. M.Sc thesis, University of the Free State, South Africa, pp. 22-55.

Scholtz, N., Scholtz, O.F. and Potgieter, G.P. 2005. Potential environmental impact resulting from inadequate remediation of uranium mining in the Karoo Uranium Province, South Africa. *In*: *Uranium in the Environment*. Merkel, B.J. and Hasche- Berger, A. (eds.). Springer, Berlin, p. 897.

Schrattenholzer, L. 2004. A roadmap to a sustainable global energy system, *Proceedings of the International Energy Workshop*, Paris.

Shahandeh, H. and Hossner, L.R. 2002a. Enhancement of uranium phytoaccumulation from contaminated soils. *Soil Science* 167: 269-280.

Shahandeh, H. and Hossner, L.R. 2002b. Role of soil properties in phytoaccumulation of uranium. *Water, Air and Soil Pollution* 141: 165-180.

Sheppard, S.C. 1992. Summary of phytotoxic levels of soil arsenic. *Water, Air and Soil Pollution* 64: 539-550.

Sheppard, S.C., Evenden, W.G. and Pollock, R.J. 1989. Uptake of natural radionuclides by field and garden crops. *Canadian Journal of Soil Science* 69: 751-767.

Sheppard, S.C., Evenden, W.G. and Anderson, A.J., 1992. Multiple assays of uranium toxicity in soil. *Environmental Toxicology and Water Quality: An International Journal* 7: 275-294.

Sheppard, S.C., Sheppard., M.I., Gallerand., M.-O. and Sanipelli, B. 2005. Derivation of ecotoxicity thresholds for uranium. *Journal of Environmental Radioactivity* 79: 55-83.

Sheppard, M.I., Thibault, D.H. and Sheppard, S.C. 1985. Concentrations and concentration ratios of U, As and Co in Scots Pine grown in a waste-site soil and an experimentally contaminated soil. *Water, Air and Soil Pollution* 26: 85-94.

Shimwell, D.W. and Laurie, A.E. 1972. Lead and zinc contamination of vegetation in the southern Pennines. *Environmental Pollution* 3: 291-301.

Sims, R.E.H., Rogner, H.H. and Gregory, K. 2003. Carbon emission and mitigation cost comparisons between fossil fuel, nuclear and renewable energy resources for electricity generation. *Energy Policy* 31: 1315-1326.

Skwarzec, B., Boryło, A. and Strumińska, D.I. 2004. Activity disequilibrium between ^{234}U and ^{238}U isotopes in Southern Baltic. *Water Air and Soil Pollution* 159: 165-179.

Smith, E., Naidu, R. and Alston, A.M. 1998. Arsenic in the soil environment: a review. *Advanced Agronomy* 64: 149-195.

South African Weather Service. 1990. Climate statistics for Beaufort West, station nr. 009 21716 for period 1961 to 1990.

Spalding, R.F. and Druliner, A.D. 1981. Groundwater uranium concentration – how high is high? *Studies in Environmental Science* 17: 581-587.

Speer, H.L. 1973. The effect of arsenate and other inhibitors on early events during the germination of lettuce seeds (*Lactuca sativa* L.). *Plant Physiology* 52: 129-133.

Stephen, U.W., Schmidke, I., Stephan, V.W. and Scholtz, G. 1996. The nicotianamine molecule is made-to-measure for complexation of metal micronutrients in plants. *Biometals* 9: 84-90.

Taiz, L. and Zeiger, E. 2002. *Plant Physiology*. Sinauer Associates, (eds.). Sunderland, U.S.A., pp. 690.

Terwelle, H.F. and Slater, E.C. 1967. Uncoupling of respiratory-chain phosphorylation by arsenate. *Biochimica Et Biophysica Acta* 143: 1-7.

Tessier, A., Campbell, P.G.C. and Bisson, M. 1979. Sequential Extraction Procedure for the Speciation of Particulate Trace Metals. *Analytical Chemistry* 51: 844-850.

Turner, B.R. 1979. Excursion guidebook to Geocongress 79, Geology of the uraniferous Beaufort Group near Beaufort West. *Geological Society of South Africa*, pp. 72.

Turner, N.C., Wright, G.C. and Siddique, K.H.M. 2001. Adaption of grain legumes (pulses) to water limited environments. *Advances in Agronomy* 71: 193-231.

U.S. Department of Agriculture (USDA). 2004. Soil survey laboratory methods manual. Version No. 4.0. Soil Survey Investigations Report No. 42. (http://soils.usda.gov/technical/lmm/)

U.S. Department of Health. 1999. Toxicological profile for uranium. Public Health Service Agency for toxic Substances and Disease registry, pp. 462.

U.S. Department of Health. 2005. Toxicological profile for arsenic. Public Health Service Agency for toxic Substances and Disease registry, pp. 481.

USEPA. 1996. Acid digestion of sediments, sludges, and soils. SW-846 Method 3050B. USEPA, Washington, DC.

Vandenhove, H., van Hees, M. and van Winkel, S. 2001. Feasibility of phytoextraction to clean up low-level uranium-contaminated soil. *International Journal of Phytoremediation* 3: 301-320.

Van der Merwe, P.J. 1990. Uranium prospecting in the Main Karoo Basin in retrospect. Vol. 1. Atomic Energy Corporation of South Africa, Reference 147.

Vassil, A.D., Kapulnik, Y., Raskin, I. and Salt, D.E. 1998. The role of EDTA in lead transport and accumulation in Indian Mustard. *Plant Physiology* 117: 447-453.

Volkering, F., Breure, A.M. and Rulkens, W.H. 1998. Microbiological aspects of surfactant use for biological soil remediation. *Biodegradation* 8: 401-417.

Von Wiren, N., Klair, S., Bansal, S., Briat, J.F., Khodr, H., Shiori, T., Leigh, R.A. and Hider, R.C. 1999. Nicotianamine chelates both Fe(III) and Fe(II). Implications for metal transport in plants. *Plant Physiology* 119: 1107-1114.

Waggitt, P.W. 2004. Uranium mine rehabilitation: the story of the South Alligator Valley intervention. *Journal of Environmental Radioactivity* 76: 51-66.

Wang, J.R., Zhao, F.J., Meharg, A.A., Raab, A., Feldmann, J. and Mcgrath, S.P. 2002. Mechanisms of arsenic hyperaccumulation in *Pteris vittata*. Uptake kinetics, interactions with phosphate, and arsenic speciation. *Plant Physiology* 130: 1552-1561.

Wennig, R. and Kirsch, N. 1988. Molybdenum. *In*: *Toxicity summary for molybdenum and molybdenum compounds*. Opresko, D.M. pp.18.

Wenger, K. 2000. Ligand-assisted phytoextraction of heavy metals from contaminated soils using common crop plants. PhD thesis, Swiss Federal Institute of Technology, Zurich, pp. 130.

Williams, C. and Thornton, I. 1973. The use of soil extractants to estimate plant-available molybdenum and selenium in potentially toxic soils. *Plant and Soil* 39: 149-159.

Wu, L., Li, H., Luo, Y.M. and Christie, P. 2004. Nutrients can enhance phytoremediation of copper polluted soil by Indian mustard. *Environmental Geochemistry and Health* 26:331-335.

Ying, Z., Guo, L.D. and Liu, R.J. 2004. Arbuscular mycorrhizal fungi associated with common pteridophytes in Dujiangyan, southwest China. *Mycorrhiza* 14: 25-30.

Zenk, M.H. 1996. Heavy metal detoxification in higher plants – a review. *Gene* 179: 21-30.

Zhu, Y.G., Shaw, G. 2000. Soil contamination with radionuclides and potential remediation. *Chemosphere* 41: 121-128.

APPENDIX A

A1. South African Water Quality Guidelines

Table 1 Target Water Quality Ranges for South Africa (DWAF, 1996).

	Uranium (mg/ℓ)	**Molybdenum (mg/ℓ)**	**Arsenic (mg/ℓ)**
Domestic use	0 - 0.070	n.a.	0 - 0.01
Agricultural water use (irrigation)	≤0.01	≤0.01	≤0.1
Livestock watering	n.a	0 - 0.01	0 – 1.0
Agricultural use (aquaculture)	n.a.	n.a.	0 - 0.05
Aquatic ecosystems	≤0.01	n.a.	n.a.

n.a. = not available

A2. Beaufort West climatological information

Table 2 Climatological information for Beaufort West based on monthly averages for the 30-year period 1961 to 1990 (South African Weather Service, 1990).

Month	Temperature (° C)				Precipitation		
	Highest Recorded	Average Daily Maximum	Average Daily Minimum	Lowest Recorded	Average Monthly (mm)	Average Number of Days with >1mm	Highest 24 Hour Rainfall (mm)
January	41	32	16	8	35	4	50
February	41	31	15	5	30	4	67
March	39	29	14	4	30	5	83
April	36	24	10	0	20	5	30
May	32	21	8	-3	11	3	70
June	29	19	5	-5	8	3	18
July	29	18	4	-6	9	2	34
August	34	20	5	-5	14	3	55
September	36	23	7	-4	12	3	41
October	39	26	10	-1	21	4	48
November	41	28	12	3	27	5	47
December	40	31	14	4	19	3	38
Year	41	25	10	-6	236	43	83

A3. Calculation of soil weight and total metals

For a soil depth of 20 cm and a soil density of 1.3 g cm^{-3}, soil weight ha^{-1} will therefore be:

$$\frac{1.3\ g\ cm^{-3}\ x\ 20\ \ cm\ (100\ cm\ \ m^{-1})^2\ x\ 10000\ m^2\ ha^{-1}}{1000\ \ g\ \ kg^{-1}} = 2.6\ x\ 10^6\ kg\ \ \ ha^{-1}$$

Based thereupon, the concentrations of U, Mo and As per hectare of soil (depth of 20 cm) can be predicted (Table 5.8). For example, the U concentration in soil-1 ha^{-1} can be calculated as follows:

$$\frac{2.6\ x\ 10^6\ kg\ \ soil\ \ ha^{-1}\ x\ 28\ mg\ \ U\ \ kg^{-1}}{10^6} = 72.8\ \ kg\ \ U\ \ ha^{-1}$$

Table 3 Predicted total concentrations of U, Mo and As (kg ha^{-1}) in soil-1, soil-2 and background soil based on initial soil-metal concentrations (mg kg^{-1}), a soil depth of 20 cm and soil densities of 1.3 g cm^{-3}.

Metal	Soil-1	Soil-2	Background
U	72.8	437	<13.0
Mo	10.7	325	<1.04
As	23.1	127	<2.6