The Role of Phytotechnology in the Rehabilitation of the BHPBilliton Cannington Ag-Pb-Zn Mine

Thesis submitted by

Scott Maurice KEELING BSc, Dip Sci, MSc

in March 2005

For the degree of Doctor of Philosophy
in the School of Earth Sciences
James Cook University

STATEMENT OF ACCESS

I, the undersigned, author of this work, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

I do not wish to place any further restriction on access to this work.

_____ _____

Signature Date

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary eduction. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

_____ _____

Signature Date

ELECTRONIC COPY

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library is an accurate copy of the print thesis submitted, within the limits of the technology available.

_____ _____

Signature Date

Preface

Significant contributions from the following persons were crucial to the progress of this study. Mr Peter Whitehead and Dr Paul Nelson provided generous support and advice in matter of earth science. Associate Professor Paul Gadek and Dr Peter Franks provided vital access to plant growth facilities. Mr Gary Warren contributed his detailed botanical knowledge of northern Australia. Dr Michael Liddell provided essential laboratory equipment and personnel, namely the technical services of Mr Robert Ennis-Thomas. Dr Michael Steele provided statistical advice for the project. Mr David Godwin provided expertise in experimental design. In addition to funding the project, the Environment Department of the Cannington mine also provided travel and accommodation to and from its operational centres. The Advanced Analytical Centre (AAC) at James Cook University and the Townsville and Brisbane offices of Australian Laboratory Services Ltd provided analytical services for this research project.

The following publications resulting from this research project are currently in press or have been accepted for publication at this time;

Keeling, S.M., 2001, Heavy metal phytoextraction: Turning waste into ore. International Conference on Green Processing 2002. Conference Proceeding of the Australasian Institute of Mining and Metallurgy, p. 137-146

Keeling, S.M. and Werren, G. Phytoremediation: The uptake of metals and metalloids by Rhodes Grass grown on metal contaminated soil. Remediation Journal. In press

Keeling, S.M. Passive uptake of Ag, As, Cd, Pb and Zn by subtropical Australian pasture plant species: implications for the revegetation of metal contaminated soils at mine sites. The Rangeland Journal. Accepted

Acknowledgements

I thank Professor Tim Bell and Associate Professor Bernd Lottermoser, School of Earth Sciences, James Cook University for the opportunity to undertake a PhD project in biogeochemistry. Many thanks are also due to the staff and students of the Faculty of Science, Engineering and Information Technology at Cairns and Townsville for their support over the past 4 years. I am grateful for your contributions and have enjoyed my time in Far North Queensland.

This project would not have been possible without the financial support and cooperation of the Environment Department, BHPBilliton Cannington Ag-Pb-Zn Mine, in particular Mr Ross Wilson and Steve Malone. Your support has made it possible for me to fulfil a dream and no amount of beer is going to make up for that. I sincerely hope that these findings, in some small way, help you towards solving land rehabilitation problems that may arise at Cannington.

I also humbly acknowledge receipt of the following grants and awards that made it possible to undertake this research project; (a) James Cook University Earth Science Studentship, (b) Australian Postgraduate Association Industry (APAI) Linkage, (c) Australian Research Council research grant (LP0219428), (d) James Cook University Doctoral Merits Research Scheme and (e) Australasian Institute of Mining and Metallurgy Gold 88 Endowment.

Finally, I thank the friends I have made in Cairns for all the riotous fun, insect repellent and barbequed treats, I shall miss you all very much.

Abstract

Phytotechnology utilises the unique biochemical processes of plants to manage and remediate contaminants such as heavy metals, hydrocarbons, radionuclides and pesticides from soil and water. The use of *in situ* biological systems to rehabilitate large volumes of contaminated soil has enormous potential for application around the globe, particularly in the mining and metal production industries. This study investigated the use of two phytotechnologies (pastoral vegetation covers and chemically-assisted phytoextraction) as environmental tools to manage mine tailings and soil contaminated with mine tailings at the BHPBilliton Cannington Ag-Pb-Zn Mine. The study was conducted in accordance with the mine's Environmental Management Overview System (EMOS) and employed the Australian and New Zealand Environment and Conservation Council (ANZECC) Investigation Guidelines for heavy metal and metalloid contamination of industrial and commercial soil.

Selected pasture plant species (*Chloris gayana*, *Crotalaria novae-hollandiae*, *Cymbopogon ambiguus*, *Cymbopogon bombycinus*, *Cyperus victoriensis*, *Gomphrena canescens* and *Triodia molesta*) were cultivated in soil contaminated with mine tailings (60 μg Ag g^{-1}, 2039 μg As g^{-1}, 30 μg Cd g^{-1}, 11950 μg Pb g^{-1} and 4150 μg Zn g^{-1}). The addition of 5 wt% to 35 wt% mine tailings to uncontaminated soil significantly improved the biomass production of *Chloris gayana*. In contrast, the biomass production of the remaining species (all native pasture plants) was significantly reduced on soil contaminated with 5 wt% to 35 wt% mine tailings. The pasture plant species accumulated low concentrations of heavy metals and metalloids from soil contaminated with mine tailings, indicating their suitability for the revegetation of pastoral lands. In addition, limestone amendments to soil contaminated with mine tailings effectively improved the revegetation potential of *Cymbopogon ambiguus*, *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae* on soil contaminated with mine

tailings, in addition to reducing the uptake of heavy metals and metalloids by the plants.

The chemically-assisted phytoremediation of soil contaminated with mine tailings was investigated using *Chloris gayana*, *Crotalaria novae-hollandiae*, *Cymbopogon bombycinus* and *Cyperus victoriensis* and soil amendments of EDTA, DTPA, EDDS, ammonium thiosulphate, ammonium thiocyanate and thiourea. Plant uptake of heavy metals and metalloids resulting from the application of the soil amendments indicated that, based upon published models for the technology, no pasture plant species would be suitable for the chemically-assisted phytoremediation of contaminated soil at the Cannington mine. *Crotalaria novae-hollandiae* and *Cyperus victoriensis*, however, did tolerate the effects of ongoing soil treatments with EDTA and EDDS, while accumulating modest quantities of heavy metals and metalloids, suggesting that vegetation covers with these plants could be used to phytoremediate low levels of soil contamination.

The leaching of Ag, Pb and Zn from mine tailings using weekly amendments of low-ionic-strength solutions of EDTA, ammonium thiosulphate, ammonium thiocyanate, thiourea and sodium cyanate was investigated over a three-month period. EDTA, ammonium thiosulphate and ammonium thiocyanate leached significant quantities of metals from the mine tailings over an approximate eight-week leaching period. EDTA solutions were found to dissolve large quantities of Pb (28.1%) and Zn (12.6%) from the mine tailings. Zinc dissolution was also high using a solution of ammonium thiosulphate (12.1%) and Ag dissolution was only notable using an ammonium thiocyanate solution (83.7%). The data indicate that chemical leaching of the Cannington mine tailings using low-ionic-strength solutions may remove a large proportion of the wastes contained heavy metals thus increasing metal production at the site, in addition to decontaminating a hazardous mine waste material.

This research project concludes that the pasture plant species investigated are highly suited to the revegetation of soil contaminated with mine tailings. In addition, the study concludes that the native pasture plant species that were deemed appropriate for phytotechnology applications at the Cannington mine are not suitable for the chemically-assisted phytoremediation of soil contaminated with mine tailings. The study also concludes that periodic leaching of the mine tailings using chemical reagents employed for phytoextraction applications has the potential to elevate metal production by reprocessing the waste while also reducing its toxicity and environmental risk.

Glossary of Terms

Chelate: A large molecular weight organic compound, such as EDTA, DTPA and EDDS, having the ability to form soluble complexes with metallic ions (SSSA, 2004).

Chlorinated solvents: Organic solvent containing chlorine atoms, e.g., methylene chloride and 1,1,1-trichloromethane, which are used in aerosol spray containers and in traffic paint (SSSA, 2004).

Ligand: A low molecular weight molecule or ion capable of sharing an electron pair during bonding, such as sulphate (SO_4^{2-}), thiosulphate ($S_2O_3^{2-}$), cyanate (OCN^-) and thiocyanate (SCN^-) (SSSA, 2004).

Metallothioneins (MTs): Low molecular weight proteins and polypeptides involved in the intracellular fixation and regulation of zinc and copper in plants and in neutralising the effects of toxic elements such as cadmium and mercury (SSSA, 2004).

PAHs: Polyaromatic hydrocarbons (SSSA, 2004).

PCB: Polychlorinated biphenyl; a pathogenic and teratogenic industrial compound used as a heat-transfer agent; PCBs may accumulate in human or animal tissue (SSSA, 2004).

Phytochelatin (PCs): Any of a group of plant peptides that bind metals (Cd, Zn, Cu, Pb, Hg) and play important roles in the detoxification of heavy metals (particularly Cd) in plants (BioTech, 2004).

Pyrrolizidine alkaloids: A group of alkaloids characterized by a nitrogen-containing necine, occurring mainly in specimens of the Boraginaceae, Compositeae and Leguminosae plant families (Brown, 2004).

Table of Contents

Section Page

List of Figures

Number Page

List of Tables

Number Page

List of Abbreviations

AAC: Advanced Analytical Centre

AAS: Atomic Adsorption Spectrometry

ALS: Australian Laboratory Services

ANZECC: Australian and New Zealand Environmental Conservation Council

CAP: Chemically-assisted phytoextraction

CDTA: 1,2-Cyclohexanediaminetetraacetic Acid

CEC: Cation exchange capacity

dH_2O: deionised water

DTPA: Diethylenetriaminepentaacetic Acid

DW: Dry weight

E: Dilution factor

EC: Electrical conductivity

EDDS: Ethylenediaminedissuccinatic Acid

EDTA: Ethylenediaminetetraacetic Acid

EGTA: Ethylene Glycol Bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic Acid

EMOS: Environmental Management Overview System

GFAAS: Graphite furnace atomic absorption spectrometry

GXR-3: Geochemical reference material

HEDTA: N-Hydroxyethylenediamine-N,N',N'-tetracetic Acid

HOAc.: Acetic acid

HNO_3: Nitric acid

ICP MS: Inductively coupled plasma mass spectrometry

JCU: James Cook University

LS: Limestone

M: million

NaCN: Sodium cyanide

NH_4HCl: Ammonium hypochlorite

NH_4OAc: Ammonium acetate

NH_4NO_3: Ammonium nitrate

OSM: Osmocote fertiliser

oz: Ounce

P: plant concentration ($\mu g\ g^{-1}$)

PGE: Platinum group element

PRF: Process Residue Facility

SCN: Ammonium thiocyanate

ST: Soil-Tailings mixture

STL: Soil-Tailings-Limestone mixture

TEC: Total element concentration

THIO: Thiourea

TPA: Tonnes per annum

TPP: Triphosphate fertiliser

TSP: Ammonium thiosulphate

USEPA: United States Environmental Protection Agency

XRD: X-ray diffraction

Chapter 1. Study Overview

1.1. Introductory statement

Soil is the biologically active surficial zone of weathering rock and sedimentary parent materials upon which terrestrial plant life exists (Hunt, 1972). Soil formation is a slow geological process and as such, soil is a highly valued natural resource. Soil containing low concentrations of heavy metals is formed from parent materials devoid of metallic substances, while high concentrations of heavy metals in soil may result from metalliferous parent materials or from anthropogenic sources. Consequently, there is considerable variation in the total concentration of heavy metals in soil (Table 1.1).

The presence of abundant heavy metals in soil does not necessarily denote an ecological problem. Contamination is defined as the introduction of micro-organisms, chemicals, toxic substances, wastes or wastewaters into air, water and soil in concentrations that makes the medium unfit for future use (USEPA, 2004). Pollution, on the other hand, is generally the presence of a substance in the environment above a specific concentration that prevents the functioning of natural processes and produces undesirable environmental health effects (USEPA, 2004). Polluted land contains elevated concentrations of a contaminant above a critical soil concentration (Table 1.1), however, the presence of contamination does not necessarily constitute pollution.

Therefore, the terms *polluted* and *contaminated* may be incorrectly applied to soil that contain heavy metal loads. For example, the calamine flora of central Europe inhabit gossanous (Zn, Cd and Pb) soil, as do the 'copper flowers' of the Shaban Copper Arc, Zaire (Brooks *et al.*, 1980; Brooks, 1998).

Table 1.1. The relative abundance ($\mu g\ g^{-1}$) of a selection of trace elements in soil (Alloway, 1995; ANZECC, 1999).

THIS TABLE HAS BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS

Contamination of soil from anthropogenic sources such as mining and smelting of metallic ores cannot be avoided. Human activity has contaminated thousands of soil profiles with heavy metals across the globe. Remedial technologes are actively sought for the effective management and detoxification of soil contaminated with heavy metals. An example of the scale of research and implementation required to deal with this growing environmental challenge can be taken from the United States Environmental Protection Agency which estimated that, as a result of human activities, over US$300 billion is required to remediate 217,000 national sites contaminated with heavy metals (USEPA, 1997). It should be noted that this estimate has been determined using existing remedial technologes (such as excavation and burial) that are often prohibitively expensive to implement.

The remediation of contaminated soil and metalliferous waste materials is an essential component of modern mine site rehabilitation practices. Mine operators are obliged to minimise the environmental impact of their activities and to take measures to protect the environment and future land use capability from the effects of mining. Traditional methods for the rehabilitation of grossly contaminated soil and metalliferous mine wastes may involve any one of a number of expensive engineering solutions. For example, contaminated soil may be excavated and chemically leached to remove the contaminant and then replaced at the site (e.g. Vandevivere *et al.*, 2001a; Kos and Lestan, 2003). Alternatively, contaminated soil and metalliferous mine wastes may be excavated and stored in engineered repositories protected from the environment by multi-layer capping systems (e.g. Bonaparte and Giroud, 1996; Ayres *et al.*, 2003). Electrokinetic extraction is one of the few remedial technologies that actually seeks to deplete ground contamination *in situ* without drastically disturbing the physical and biological character of the host soil (e.g. Gent *et al.*, 2004). This costly technique involves passing a low voltage electrical current through a moist contaminated material over long periods of time and collecting the heavy metal slime deposited upon the cathode. This is a highly specialised rehabilitation technique with a limited number of environmental applications. The question remains; 'how can we remediate large areas of low to moderate levels of soil contamination and not destroy the valuable character of the soil in the process?'

Mining operators in Australia, and globally, are constantly striving to lower the expense of rehabilitating mine tenements to meet defined post-mining land use capabilities. Of equal importance to mine operators is the requirement to promote and maintain local biological diversity at these sites. The act of returning mine tenements to pastoral land uses post-mining must be undertaken with a firm knowledge of the local botanical community available for revegetation. Indeed, the EMOS for the Cannington mine stipulates that 'only native, or tenement hosted, flora are to be used in revegetation programs' (BHPBilliton, 2002). While this philosophy is vital in protecting the environments where mining occurs, it also places significant limitations on what is genuinely achievable from the land rehabilitation process. In many situations where contaminated soil could be effectively rehabilitated, unrealistic environmental objectives may precluded this from happening and the soil will be lost through the use of an engineering solution. For example, the Cannington mine's EMOS indicates that acceptable levels of heavy metals in rehabilitated soil will be similar to those of the soil before mining began. Since the soil contains naturally very low concentrations of heavy metals at the Cannington site, any contamination will be extremely difficult to reduce to these 'pre-mining' levels. Therefore, it is highly likely that current rehabilitation strategies for the Cannington mine site will lead to extensive stripping of contaminated topsoil.

1.2. Phytotechnology

The application of higher plants to perform remedial functions in

contaminated soil and waters has been termed *phytotechnology* (McGrath *et al.*, 1993). Phytotechnology applications range from simply revegetating contaminated soil with plants to employing specialist plants and soil amendments to extract or modify soil toxins for environmental (*phytoremediation*) or economic (*phytomining*) purposes (Cunningham *et al.*, 1995; Keeling, 2000). The development of this field has signalled a major shift in the accepted wisdom behind best practices in the land rehabilitation industry. By placing a high value on the preservation of soil and their structure, phytotechnologies directly address land and water contamination using *in situ* biological systems rather than invasive mechanical or chemical methods.

Considered to be 'soft' or 'low impact' remediation, phytotechnologies are developing to directly focus upon specific economic and environmental challenges for the rehabilitation of contaminated lands (e.g. Brown, 1995; Chaney *et al.*, 1997; USEPA, 2000a; Robinson *et al.*, 2003). These biological rehabilitation strategies (described below) are in many cases barely beyond initial treatability stages for field implementation. However, successful field demonstrations of several of these techniques have indicated that under certain conditions phytotechnologies can be highly successful (e.g. Rock, 1997; Kos and Lestan, 2003). The various biological rehabilitation strategies can be defined as follows,

1. Phytoextraction technologies: based upon plant uptake of contaminants by roots and the translocation of these contaminants to harvestable plant

tissues (e.g. Morrison, 1980; Chaney, 1983). Phytoextraction is most often applied to metal-contaminated soil and may occur via natural plant processes or is facilitated by chemical amendments (e.g. Cunningham *et al.*, 1995; Huang *et al.*, 1997a; Blaylock, 1999).

2. Rhizofiltration: the absorption and translocation of contaminants by plant roots from aqueous media and groundwater. Rhizofiltration first requires the containment of aqueous contamination, in which vegetation is cultivated (e.g. Wolverton, 1975; Outridge and Noller, 1991; Dushenkov *et al.*, 1995). Contaminants are then removed by physically removing the plants.

3. Phytostabilisation: the use of plants and plant roots to prevent contaminant migration via wind and water erosion, leaching, and soil dispersion (e.g. Ma *et al.*, 1993; Cunningham and Berti, 2000).

4. Rhizodegradation: the breakdown of an organic contaminant in soil through microbial activity that is enhanced by the presence of plant roots (e.g. Shimp *et al.*, 1993; Narayanan *et al.*, 1995; Schnoor *et al.*, 1995).

5. Phytodegradation: the breakdown of contaminants taken up by plants through metabolic processes within the plant, or the breakdown of contaminants external to the plant through the effect of phytochemicals (such as enzymes) (e.g. Nellessen and Fletcher, 1993; Schnoor *et al.*, 1995; Cunningham *et al.*, 1997).

6. Phytovolatilisation: the uptake and transpiration of a contaminant by a plant with release of the contaminant or a modified form of the contaminant to the atmosphere via evapotranspiration (e.g. Chaney *et al.*, 1997).

7. Hydraulic control: the use of plants to intercept groundwater flows in order to contain or control the migration of contaminated groundwater plumes (e.g. Sheppard and Evenden, 1985; Gatliff, 1994).

8. Vegetative covers: long-term, self-sustaining plant communities grown in materials that pose an environmental risk. A vegetative cover may reduce that risk to an acceptable level and generally requires minimal maintenance (e.g. Naidu and Harwood, 1997; Mentis, 2001; Bruce *et al.*, 2003).

9. Riparian buffers: installed along streams and riverbanks to control surface runoff into and groundwater contamination of river systems. Riparian barriers are also highly effective for controlling the migration of groundwater plumes (e.g. Licht and Schnoor, 1993).

The phytotechnologies (Table 1.2) most applicable to the mining and metals industries that are under consideration in this study include the use of phytoextraction and vegetative covers. These techniques will be considered in this study because they represent logical extensions of traditional rehabilitation practices. Furthermore, these techniques are essentially outlined in the Cannington mine's EMOS as solutions to land rehabilitation problems

or could be easily integrated into the mine's rehabilitation process.

Table 1.2. A breakdown and description of the techniques collectively termed phytotechnology (USEPA, 2000a).

THIS TABLE HAS BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS

1.2.1. Heavy metal bioavailability in soil

Heavy metals must be soluble in the rhizosphere for effective plant uptake to occur (Raskin and Ensley, 2000). Dissolved heavy metals are absorbed across the root membrane and transported via xylem sap to storage (disposal) sites in biologically inactive plant tissues (Morrison, 1980; Robinson, 1997). The fraction of the total heavy-metal load of a soil that is readily available for

plant absorption is termed *bioavailable* (Thornton, 1999).

The bioavailability of heavy metals can be a natural function of the soil, affected by cover vegetation, or can be artificially promoted using chemical or mineral amendments. The 'toxicity' of a contaminated soil is therefore dependent upon the level of heavy metal bioavailability and is strongly influenced by the soil's physical and chemical characteristics. Physical properties such as water and air availability and soil strength indirectly constrain heavy metal bioavailability by affecting root and plant growth (Robinson, 1997). Chemical properties such as pH, nutrient status, contaminant type and concentration affect metal absorption directly (Ernst, 1996). To further complicate matters, the exudation of phytochemicals by plants into the rhizosphere may promote or inhibit heavy metal bioavailability. For example, many plant species are able to modify rhizospheric pH conditions by as much as two units in materials immediately adjacent to root tissues (Marschner, 1995). In some situations these exudates are very similar in nature to known geochemical ligands such as thiosulphate and thiocyanate (Aplin, 1976; Niedzwiedz-Siegien, 1998).

The only truly quantitative means of determining a plant species response to heavy metal bioavailability in soil is to cultivate the species *in situ*. However, *in situ* investigations are time-consuming, expensive and often impractical due to the infrastructure required to investigate the process. Numerous chemical extractions have been investigated to estimate the bioavailability of heavy

metals in soil, including distilled water (e.g. Singh and Narwal, 1984), 1 M NH_4OAc (e.g. Haq *et al.*, 1980; Ernst, 1996), EDTA and DTPA (e.g. Haq *et al.*, 1980), 0.5 M $CaCl_2$ (e.g. Whitten and Richie, 1991) and 1 M NH_4NO_3 (e.g. McGrath *et al.*, 1997). EDTA and DTPA act as chelating agents in soil, whereas the other reagents model natural plant exudates and natural solvents. Estimating the plant-available concentrations of heavy metals in soil using chemical extractions is an extremely useful and inexpensive means of predicting the potential risk to biological systems posed by a contaminant. If the extractability of heavy metals in the soil is low, then the material would have a low environmental risk and may be considered as simply contaminated. Conversely, if the extractability of a contaminant is high and impacts upon local biological systems then the soil may be considered polluted and remediation must be undertaken.

1.2.2. Manipulating heavy metal bioavailability in soil

Soil amendments such as organic matter, phosphate or carbonate rock and red mud can be used to regulate the dissolution of heavy metals in contaminated soil (e.g. Ma *et al.*, 1997; Li *et al.*, 2000; Bernal *et al.*, 2002). The application of alkaline mineral amendments or materials containing organic matter lowers heavy metal bioavailability by promoting the formation of insoluble metal-bearing mineral phases or poorly soluble organometallic complexes (McGrath *et al.*, 1994). These techniques result in no net reduction in the contaminant load of a soil; however, this may be acceptable in order to establish a vegetation cover over a contaminated soil.

The bioavailability of heavy metals in soil may be elevated using a variety of chemical and mineral amendments. The addition of elemental S has been shown to lower soil pH and, in doing so, raises the bioavailability and phytoextraction of heavy metals from soil (Kayser *et al.*, 2000). The excessive use of ammonium-containing fertilisers is also known to lower soil pH via nitrification which may elevate heavy metal bioavailability (Sumner, 2000). However, it is the use of chelating agents, such as EDTA, DTPA and EDDS, which offer the greatest potential for dissolving a range of heavy metals in contaminated soil and for promoting their uptake by plants (e.g. Norvell, 1984; Li and Shuman, 1996; Wu *et al.*, 1999; Kos and Lestan, 2003).

1.3. The phytoextraction of heavy metals by plants

The process of phytoextraction may be thought of as a 'solar-driven biological pump' for removing low to moderate concentrations of contaminations in soil by sequestering them in relatively small volumes of plant material at low cost (Glass, 2000). The prospect of literally 'farming' heavy metals from contaminated soil and sub-economic mineralisations using phytoextraction technologies has generated considerable research activity worldwide (e.g. McGrath, 1998; Blaylock and Huang, 2000; Lasat, 2002). Prior to the discovery that soil amendments such as chelates could promote heavy metal phytoextraction, phytoextraction research was confined to the examination of naturally-occurring heavy-metal accumulating plant species.

Rare examples of extreme natural accumulations of heavy metals in plants (hyperaccumulation) are known (e.g. Brooks and Radford, 1978; Reeves and Brooks, 1983; Morrey *et al.*, 1989; Brooks, 1998) with new examples still being discovered (Ma *et al.*, 2001; Bidwell *et al.*, 2002). However, the global distribution of hyperaccumulators, their growth habits and the types of metals phytoaccumulated by them often exclude many of these plant species from practical application. For example, over 300 species of Ni-hyperaccumulating plants (containing >10000 μg g^{-1} Ni dry wt) have been described of which Australia contains two: *Hybanthus floribundus* (10000 μg g^{-1} Ni, dry wt) and *Stackhousia tryonii* (22000 μg g^{-1} Ni, dry wt) (Severne and Brooks, 1972; Cole, 1973; Batianoff *et al.*, 1990). *Stackhousia tryonii* has only been successfully cultivated from tissue cultures, and successful propagation of *H. floribundas* can only be achieved by first soaking the seed in 'smoke' water (Anderson, 2000; Bhatia *et al.*, 2002). Furthermore, there have been no reported hyperaccumulators of Pb (>10000 μg g^{-1} Pb, dry wt), although several species of the European genus *Thlaspi* have been reported to accumulate large concentrations of Pb, in addition to Cd and Zn (Baker *et al.*, 1994). These species are not currently permitted in Australia because of biosecurity concerns (AQIS, 2005).

Plant species that do not naturally accumulate the high concentrations of heavy metals and metalloids desired for effective phytoextraction can be forced to do so using chemical amendments. A chemical reagent (e.g. EDTA) can be added to soil to dissolve insoluble heavy metals placing them in the

soil solution. Plants may then accumulate the dissolved heavy metals from interstitial pore fluids in soil by evapotranspirative water consumption (Nicholas and Egan, 1975). Alternatively, the newly formed metallic complex, or the chemical reagent itself, may degrade the root membrane resulting in metals flooding into plant roots (Wu *et al.*, 1999). Chemically-assisted phytoextraction may be considered a passive uptake process whereby phytochelatins and metallothioneins are artificially introduced into the rhizosphere (Anderson, 2000).

1.3.1. Plant uptake response to heavy metals in soil

The biological effect of heavy metals contained in contaminated soil may be explained by one of three classical plant response curves (Figure 1.1). The *hyperaccumulator* response indicates physiologically active metal absorption; the *indicator* response indicates the ability to regulate metal absorption; whereas the *excluder* response indicates physiologically controlled resistance to metal absorption at low concentrations followed by no control over metal uptake at higher concentration (Baker, 1981).

Figure 1-1. Classical plant response to increasing heavy metal concentrations in soil (Baker, 1981).

The exclusion of heavy metals from uptake is preferable for vegetative covers where pastoral land use capabilities are required from the rehabilitation process, since the biomass represents a primary component in the food chain. Alternatively, for phytoextractive purposes, the heavy metal concentrations in plants should reflect concentrations observed in hyperaccumulating species. However, as mentioned previously hyperaccumulation is rare and metal specific, so in many situations the use of chemical amendments must be employed to force plants that normally exclude or indicate heavy metal concentrations in soil to respond with accumulator behaviour.

1.4. Phytoextraction: phytoremediation and phytomining

Heavy metal bioavailability and resultant phytoaccumulation govern the efficiency of a phytoextraction operation. Since the mass of accumulated

metals in plant tissues is directly proportional to plant weight (Brooks, 1998), the use of high biomass producing species is critical (Cooper *et al.*, 1999). Ideally, one would seek to use a plant that displayed a high biomass production potential and the 'hyperaccumulator' response, should such a species be available.

Heavy metal hyperaccumulating plants have been investigated as tools for the phytoremediation of a range of Cd, Pb and Zn contaminated soils (Chaney, 1983; Baker and Brooks, 1989; Salt and Kramer, 2000). By cultivating crops of hyperaccumulating plant species on contaminated soil and harvesting the metal-rich biomass, numerous authors indicated significant potential cost reductions compared to conventional land rehabilitation practices (e.g. McGrath *et al.*, 1993; Brown *et al.*, 1994; Huang and Cunningham, 1996).

The cost of remediating Pb-contaminated soil was estimated to be approximately US$280,000 ha^{-1} for phytoremediation compared to approximately US$2.5 million ha^{-1} for traditional rehabilitation technologies such as excavation or burial (Cunningham and Berti, 2000). By comparison, the installation of vegetative covers for phytostabilisation and a pastoral land use in the Australian coal industry has been estimated to cost approximately AU$25,000 ha^{-1} (Grigg *et al.*, 2000).

The concept of 'phytomining' is the recovery of valuable metals from metalliferous soil and soil-like materials and has been investigated at

greenhouse and plot trial scale (e.g. Kelly and Guerin, 1994; Brooks *et al.*, 1998; Nicks and Chambers, 1998; Leblanc *et al.*, 1999). This biotechnology could potentially recover metals from sub-economic mineralisations and metalliferous waste materials. Once captured in harvestable plant tissues, the metals would be recovered for sale via thermal reduction and smelting (Nicks and Chambers, 1995). Alternatively, should valuable organometallic phytochemicals be isolated in the wet biomass, these compounds could be selectively extracted and sold. This premise would also apply to metal-bearing plant materials derived from phytoremediation.

The production of plant biomass from a phytoextraction program represents a carbon sequestration technology. Carbon sequestration may provide additional incentives and benefits to the rehabilitation process by assisting with reducing net carbon emissions at a particular site, or within the greater corporate structure.

1.5. Chemically-assisted phytoextraction

The dissolution of heavy metals in soil using chelates varies for different soil conditions. For example, different mineral forms of a contaminant, such as phosphate, carbonate and sulphide compounds can result in significantly different uptake responses by plants (Anderson, 2000). In addition, the presence of other labile elements can interfere with the performance of chemical amendments used to dissolve heavy metals in soil and also the

uptake response observed in the plants (Keeling, 2000).

EDTA is a common agricultural chemical used since the 1960s as a soil additive to improve micronutrient bioavailability (Kabata-Pendias and Pendias, 1984). Overuse of EDTA in agriculture and its long biological half-life in soil has led to dissolved contaminants leaching into groundwater (Means *et al.*, 1980; Kinnersley, 1993). Conversely, the persistence of EDTA in soil-like materials has been utilised to leach heavy metals from contaminated soil using successive treatment of the reagent (Tejowulun and Hendershot, 1998). In addition to EDTA, a number of other heavy metal chelating agents have been investigated for phytoextractive purposes: CDTA, DTPA, EGTA, HEDTA, citric acid, and malic acid (USEPA, 2000b). Aside from the environmental concerns, EDTA has, until recently, proved to be the most effective means of promoting plant uptake of Pb, and other base metals and metalloids (e.g. As, Cd, Co, Cu, Se and Zn) (Blaylock, 2000; Chaney *et al.*, 2000). Chelate concentrations reported thus far have indicated that at least 0.001 M EDTA per kg of soil is required before the concentration of Pb in plant tissues is elevated to a viable phytoremediation level (>10000 $\mu g\ g^{-1}$ Pb, dry biomass) (Raskin and Ensley, 2000).

The paucity of hyperaccumulation in the botanical record has resulted in the development of CAP as the means to remediating heavy metal contaminants from soil. The uptake of heavy metals by non-accumulating plant species is facilitated by applying chemical reagents to the revegetated soil. Chelates

(SSSA, 2004) have been demonstrated to increase the bioavailability of Pb in soil 100-fold (Raskin and Ensley, 2000). *Zea mays* (maize) was reported to accumulate >10000 μg Pb g^{-1} (DW) from a contaminated soil amended with the chelate EDTA (Huang and Cunningham, 1996). This prompted numerous authors to demonstrate that applications of chelating agents could promote heavy metal phytoextraction by a range of well-known agricultural plant species (e.g. Blaylock *et al.*, 1997; Chaney *et al.*, 1997; Huang *et al.*, 1997a; Ebbs and Kochian, 1998; Deram *et al.*, 2000; Sarret *et al.*, 2001). Rufus Chaney determined that one chemically-assisted phytoextraction treatment to a soil depth of 0.25 m (approximately 6250 tonnes) with 0.01 M EDTA per kg soil (3.52 g kg^{-1}, 7 tonnes of reagent per hectare) would cost approximately US$30,300 ha^{-1} (US$5 per tonne) (Chaney, 2000). Blaylock and Huang (2000) confirmed that 0.01 M EDTA kg^{-1} is crucial to promote viable Pb concentrations (>10000 μg Pb g^{-1}) in plants.

Those species which accumulated >10000 μg Pb g^{-1} in harvestable plant tissues and produced >20 tonnes of biomass per hectare per year (equivalent to 200 kg Pb per hectare per year) were deemed suitable for phytoremediation projects (Raskin and Ensley, 2000). Lead uptake of 10000 μg g^{-1} (DW) has not been detected to occur in any natural heavy-metal-accumulating plant species, and biomass production of 20 t ha^{-1} has only been inferred for one hyperaccumulator (*Berkheya coddii*), and only then under careful management (Robinson *et al.*, 1997). Even if two crops could be cultivated on a contaminated soil each year, the required biomass production

would be 10 t ha^{-1} $crop^{-1}$ (containing >10000 μg Pb g^{-1}). This level of biomass production is virtually unseen among pasture plant species. Therefore, even though these performance indicators for Pb phytoremediation have been achieved, and are repeatable, they only serve to demonstrate that the technology can succeed using well-understood and carefully bred crop species.

Virtually all the plant species investigated for phytoextractive applications have been common agricultural crop species (e.g., Oat - *Avena sativa*, Barley - *Hordeum vulgare* and Indian Mustard - *Brassica juncea*) or known heavy-metal-hyperaccumulating plant species (Huang and Cunningham, 1996; Ebbs and Kochian, 1998; Raven *et al.*, 1998). These species have been bred to exhibit specific traits for agricultural purposes resulting in reduced genetic diversity. Therefore, crops of these species require a high degree of management compared to native pastures (e.g. Kochian, 2000; Lasat, 2002). In contrast, the evolution of highly successful Australian pasture plants has occurred in an environment where increased adaptability (genetic diversity) has often been a competitive advantage (James, 1981). This suggests that considerable variation in metal tolerance and the uptake of heavy metals can be expected in Australian pasture plant species when grown on contaminated soil. Furthermore, genetic variation between different geographic populations of these plant species implies that their growth response to heavy metal contamination in soil may be dramatically different. Scant information is available regarding heavy metal tolerance and the CAP potential of Australian

pasture plant species. A study of the vegetation colonising the Mary Kathleen uranium mine in Queensland indicated that the base-metal and radionuclide concentrations of many resident pasture plant species were elevated compared to background values (Costelloe, 2003). It is, therefore, appropriate that research of some of these native pasture genera should be conducted in Australia, given the oft-lamented dictum of 'ecological sustainability' in land rehabilitation.

Finally, the economics of phytoremediation must be considered given industry's prime motivation of cost reduction through innovation. Standard cost information is not readily available for phytoremediation because the field is an emerging technology. As a result, the ability to develop cost comparisons and to estimate project feasibilities is strongly controlled by site specific influences, such as a remote location. A thorough assessment of the technologies applicability and an accurate cost comparison to conventional treatment technologies are critical in determining the economic potential of phytoremediation. Care must be taken when comparing the process costs of phytoremediation with those of traditional treatment technologies because financial assessments may include a variety of unique system costs such as :

1. Project Design
    - Development funding
    - Site characterization
    - Treatability and pilot testing
2. Installation costs
    - Site preparation
    - Soil preparation

- Infrastructure
- Cultivation
- Maintenance

3. Operating costs
    - Personnel
    - Crop management
    - Biomass disposal
    - Monitoring
4. Site decommissioning

A review of the economics of phytoremediation reported by Ensley (2000) estimated that the conventional excavation-landfill approach to soil remediation commonly used in the United States would cost between US$150 to US$350 per tonne (equivalent to approximately US$1.1M to US$2.5M per hectare). By comparison, CAP of Pb contaminated soil was estimated to cost between US$20-US$80 per tonne (equivalent to approximately US$150,000 to US$580,000 per hectare). The latter estimate also included the cost of off-site disposal of biomass as a hazardous waste. Cunningham and Ow (1996) estimated that the cost of excavation and disposal of Pb contaminated soil from a 4.9 hectare site was US$12 million. By comparison, decontamination of the site by soil washing was estimated to cost US$6.3 million and the cost of phytoremediating the site was estimated to be US$200,000. Therefore, the rehabilitation of soil contaminated with heavy metals using phytotechnology indicates that significant cost reductions are available compared to contemporary remediation technologies.

1.6. Research goal

The goal of this study was to evaluate recent developments in phytotechnology and hydrometallurgy as tools to manage contaminated soil and mine tailings at the BHPBilliton Cannington Ag-Pb-Zn Mine. The study was essentially a 'green fields' investigation of the potential of both native and exotic pasture plant species (Table 1.3) to revegetate and phytoremediate undiluted mine tailings and soil contaminated with mine tailings. In addition, the geochemical nature of the mine tailings produced at Cannington supported an evaluation of the chemical regents currently employed in phytotechnology as alternatives to contemporary heap leaching reagents.

In a broad context, the global distribution of some of the plant species under investigation (Section 2.7 to 2.10) makes the study relevant to the remediation of metalliferous soils in tropical to temperate climate zones across North and South America, Southern and Central Africa, Asia and Indonesia (Watson and Dallwitz, 1992). Furthermore, BHPBilliton (the collaborative partner) believed that the emergent science of phytotechnology might produce significant positive outcomes for financially sustainable environmental management practices at the Cannington mine, and for other operations worldwide. Finally, BHPBilliton sought to identify technologies capable of reducing the environmental risks associated with the storage of highly metalliferous mine tailings.

Table 1.3. The taxonomy of plant species selected for this study (ANBG, 2004)

THIS TABLE HAS BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS

1.6.1. Specific aims

The specific aims of this study were designed to meet the research goal and are presented below in form of research questions:

1. Is it possible to effectively revegetate soil contaminated with mine tailings and undiluted mine tailings at the BHPBilliton Cannington Ag-Pb-Zn Mine with local pasture plant species?

2. How effective are known heavy metal chelating agents and thio-compounds at dissolving metals and metalloids contained in undiluted mine tailings and soil contaminated with mine tailings at the BHPBilliton Cannington Ag-Pb-Zn Mine?

3. Is it possible to use pasture plant species and the principles of chemically-assisted phytoextraction to phytoremediate soil

contaminated with mine tailings or to phytomine undiluted mine tailings at the BHPBilliton Cannington Ag-Pb-Zn Mine?

4. What are the limitations to effective application of phytotechnology at the BHPBilliton Cannington Ag-Pb-Zn Mine?

In order to answer these specific research questions, six phytotechnology experiments and one hydrometallurgical experiment were conducted, based upon currently published methodologies (cf. Blaylock *et al.*, 1997; Huang *et al.*, 1997a; Wu *et al.*, 1999; Feng and Van Deventer, 2002; Greet and Smart, 2002; Greman *et al.*, 2003). In addition, numerous complementary solvent extractions and sequential extractions were conducted in support of the above experiments. A synthesis of the results of these seven experiments was used to answer the specific research questions and form the basis of the thesis outlined below.

1.7. Thesis outline

The study was divided into a number of discrete investigations to determine specific environmental outcomes for the Environmental Department of the Cannington mine. Each of the investigations, set out in this thesis, constitutes a publication already in submission or in preparation for submission to international or Australian scientific journals. Furthermore, the progression of the chapters serves to demonstrate a methodology suitable for application

to any metalliferous mining operation wishing to undertake revegetation and soil remediation projects with native pasture plant species.

Chapter 3 - Phytoextraction of As, Ag, Cd, Pb, and Zn by Subtropical Australian Pasture Plant Species Grown on Soil Contaminated with Mine Tailings

The aim of this study was to determine the revegetation potential of a selection of native Mitchell Grassland plant species grown on a range of soil contaminated with mine tailings. The plant species investigated (*Triodia molesta* - Spinifex, *Gomphrena canescens* - Bachelor Button, *Cyperus victoriensis* - Channel Nutgrass, *Crotalaria novae-hollandiae* - New Holland Rattlepod, *Cymbopogon ambiguus* - Scent Grass, and *Cymbopogon bombycinus* - Lemon Scented Grass) are endemic to the Cannington mine tenement and thus satisfied the mine's EMOS for land rehabilitation. In addition, the plants' natural abilities to accumulate heavy metals and metalloids were determined to estimate the potential fodder toxicity risks associated with the desired future land use capability. Amendments of limestone were also investigated as a means of reducing both the level of tailings-induced substrate toxicity and the levels of heavy metals and metalloids accumulated by the plants.

Chapter 4 - The Uptake of Metals and Metalloids by Rhodes Grass (*Chloris gayana* Kunth cv. 'Pioneer') Grown on Mine Tailings and Soil Contaminated with Mine Tailings

Due to the limited success of revegetating grossly contaminated soil with native pasture plants (Chapter 3) and after consultation with the Environment Department at the Cannington mine, a revegetation study was undertaken using the introduced pasture species Rhodes Grass (*Chloris gayana* Kunth cv. 'Pioneer'). Experimentation with this species, although contradicting the mine's current environmental regulations, was undertaken for two reasons. Firstly, the species is a commonly introduced pasture plant in the Australian landscape and many other countries worldwide (Watson and Dallwitz, 1992) indicating that land management practices for the species were mature in a number of countries where BHP Billiton currently operate. Secondly, the species displays a remarkable ability to tolerate extremely high concentrations of heavy metals in soil (Harwood *et al.*, 1999) suggesting that controlled use of the species may result in short-term environmental gains for the Cannington mine, and other sites operated by BHP Billiton. Therefore, with an objective of revegetating undiluted mine tailings, it was deemed appropriate for the investigation.

Chapter 5 - Chemically Assisted Phytoremediation of Soil Contaminated with Mine Tailings using Australian Subtropical Pasture Plant Species

Having determined the revegetation potentials of a selection of native pasture plant species (Chapter 3), the next step was to evaluate their potentials to phytoextract heavy metals and metalloids from soil contaminated with mine tailings using a range of chemical amendments. Since the metal concentration

in plant tissues is proportional to plant weight, the three highest biomass producing species (*Cyperus victoriensis*, *Crotalaria novae-hollandiae*, and *Cymbopogon bombycinus*) were investigated. This study complied with the biological and environmental frameworks contained in the Cannington mine's EMOS and thus represents an accurate assessment of the application of both regulatory systems to the rehabilitation of contaminated soil at the mine site.

Chapter 6 - Chemically Assisted Phytoextraction of Metals and Metalloids from Mine Tailings and Soil Contaminated with Mine Tailings using Rhodes grass (*Chloris gayana Kunth* cv. Pioneer)

This study was conducted subsequent to determining the revegetation potential of this species indicated in Chapter 4. Firstly, the effectiveness of using *Chloris gayana* to phytoremediate heavy metals and metalloids from a soil contaminated with 12.5 wt% mine tailings was examined. Secondly, the use of fertiliser amendments was investigated to directly revegetate undiluted mine tailings. Chemical reagents were then employed to promote heavy metal phytoextraction from the revegetated tailings. It is hoped that this investigation might provide the Environment Department at the Cannington mine with a means of directly revegetating 125 hectares of exposed tailings dam surfaces designated for construction at the mine. This would significantly reduce the environmental risk posed by the tailings to the surrounding landscape prior to installation of the proposed store and release cover system. In addition, it was hoped that the CAP of commodity metals

contained in the mine waste, and their subsequent decontamination, might provide the Cannington mine with alternative strategies for managing and rehabilitating this mine waste.

Chapter 7 – The Leaching of Ag, Pb and Zn from Cannington Mine Tailings using Novel Chemical Reagents

This study aimed to determine the long-term heavy metal leaching potential of low ionic strength solutions of chelates and thio-compounds to dissolve commodity metals (Ag, Pb and Zn) from Cannington mine tailings. It is estimated that the Process Residue Facilities tailings dam complex currently contains approximately 8 M oz Ag, 44,000 tonnes Pb, and 12,700 tonnes Zn with a combined value of US$113 million[*]. This investigation was designed to determine a means of accessing the residual concentrations of commodity metals contained in the mine tailings thus elevating the mine's waste to resource status. Tailings dam design and its de-watering infrastructure indicates that metal recovery could be based upon conventional heap leaching technologies of 'ore' that has already been mined, crushed and deposited in a leach tank. It is hoped that this study might provide the Environment Department at the Cannington mine with a means of reducing tailings toxicity *in situ* and potentially generating a revenue stream from the extraction of valuable metals from the mine's waste.

[*] Assuming: US$7.20 oz^{-1} Ag, US$980 t^{-1} Pb, US$1020 t^{-1} Zn, 7 years of 2 M TPA production to realise 0.5 M tonnes of concentrates, 65% of tailings solids to backfill, 35% of tailings solids to the repository

Chapter 2. Materials and Methods

2.1. Site description

BHPBilliton owns and operates the Cannington Ag-Pb-Zn mine in central Queensland, Australia (21º 52' 09" S, 140º 55' 10" E). The Cannington mine is located on the eastern flood plain of the Hamilton River, 800 km west-southwest of Townsville, Queensland, Australia (Figure 2.1). Cannington is positioned in a semi-arid climatic zone (250-550 mm annual rainfall) that extends from central Queensland, across the Northern Territory, to the Pilbara region of Western Australia. The mine receives seasonally extreme rainfall events and is prone to surface flooding and sheet wash, which at times has halted the movement of personnel and product to and from the operation.

2.2. Deposit geology and geochemistry

The Cannington deposit is located in the south-east corner of the Proterozoic Mount Isa Block, within the metamorphics of the Lower Middle Proterozoic Eastern Succession (Bodon, 1998). Considered to be a Broken Hill-type deposit, the Cannington orebody is characterised by extreme enrichment of Ag, high levels of Sb, Cd, As, Cu and F and the general absence of pyrite (Walters, 1998). There is no oxidation profile preserved at the basement sub-crop nor any form of chemical or physical surface expression of the deposit

(Walters, 1998).

Figure 2-1. The location of the BHPBilliton Cannington Ag-Pb-Zn Mine, NW Queensland, Australia (Wilson, 2002).

The deposit is divided by faulting into a shallow low-grade Northern Zone and a deeper high-grade and more extensive Southern Zone (Bodon, 1998). The major structural feature of the Southern Zone is an isochnal fold (which dips to the east and plunges to the south) containing a broadly zoned and

faulted sequence of Ag-Pb-Zn, Ag-Pb, and Zn lodes. The orebody is subdivided into five main economic zones for mining (Table 2.1) that contain 10 mineralization types according to the zonation of Pb and Zn and the assemblages of various siliceous and mafic gangue minerals. The major economic sulphides associated with the Cannington deposit are galena and sphalerite (Walters, 1998). The Ag content is principally in the form of freibergite but is also present in a solid solution within galena and native Ag. Metal production at Cannington scheduled to proceed for a further 22 years, accounts for approximately 6% of global Ag output and 7% of global Pb output today. It is therefore testament to the environmental commitment of BHPBilliton that this study has been undertaken at this time.

Table 2.1. Subdivision of the Cannington orebody into economic zones based primarily upon the abundance of sulphide minerals (Walters, 1998).

2.2.1. Mining and production

Cannington is an underground mine accessed via a decline ramp and a shaft for ore recovery (Figure 2.2). The decline ramp functions as an access point for mobile equipment and personnel, as well as one of the main draw points for fresh air to ventilate the underground workings. Mining is conducted from multiple headings simultaneously and from a variety of ore types. The stoping method used for the extraction of the main, thicker hangingwall ore bodies of the deposit is transverse, long hole open stoping. Broken ore from the stopes is loaded from draw points below each stope and either hauled to the surface by truck via the decline ramp or transferred to the hoisting shaft. Tight control of the milling operations 'head grade' requires the stockpiling and blending of various ore types prior to flotation separation. This indicates that the gangue mineral assemblage of the mine tailings, and its geochemistry, is likely to vary over the life of the mine as various ore bodies are brought into production while others are depleted.

Ore processing at Cannington involves several stages designed to separate and then concentrate the valuable minerals contained in the ore. A primary comminution circuit of crushers, grinders and cyclone separators divides the ore into valuable mineral concentrates and gangue to produce Pb-rich and Zn-rich flotation feed stocks. These flotation feed stocks are then pumped into tanks and treated with a variety of reagents to render the valuable minerals hydrophobic and the gangue minerals hydrophilic. Fine air bubbles are introduced into the base of the tanks whereupon hydrophobic particles

attach themselves to the bubbles and float at the top of the tanks as froth.

Figure 2-2. Aerial photograph of the BHPBilliton Cannington Ag-Pb-Zn Mine (2001) indicating the position of mining Infrastructure and the Process Residue Facilities tailing dam complex (Wilson, 2002).

Mineral-rich froth is collected and leached with acid to remove fluorine, a major contaminant of the ore body, from the concentrates. The mineral

concentrates are then sent to horizontal pressure filters for dewatering and a final acid wash to remove any remaining traces of fluorine. The Pb and Zn concentrates are then transported by road and rail to the Townsville Port Facility where they are shipped to smelters in Australia and overseas.

2.3. Regional and local pedology

The Cannington deposit is overlain by approximately 10 to 60 m of recent and Cretaceous sediments (emplaced by the Hamilton River) derived from topographic highs to the west and northwest of the Cannington mine. These sediments have weathered to produce a vertosol soil containing large quantities of expanding clay minerals (≥30 wt%) such as smectite (Isbell, 1996; Sumner, 2000). Vertosol soil generally develops in recent (Quaternary) sediments containing high proportions of calcareous materials (Sumner, 2000). The expanding nature of these clay soils has led to vertosol soil being described as 'self-mulching'; where the shrink-swell nature of clays produces surface cracks and may cause diapirs to form in the soil profile. Vertosol soil occurs in over 100 countries including Australia where it is distributed across approximately eighty million hectares (Figure 2.3). The more youthful soil contained within the Vertosol soil order, occurring on the Cannington mine tenement, is located in close proximity to the Hamilton River.

Figure 2-3. The distribution of soil orders in Australia (Isbell, 1996; ANRA, 2004). The position of the Cannington mine (X) is indicated.

2.4. Vegetation

Vertosol soil supports distinctive grassland plant communities across central and northern Australia (Figure 2.4). The vegetation supported by Vertosol soil is characterised as Mallee Woodlands, Acacia Open Woodlands, Hummock Grasslands and Other (misc.) Grasslands (ANRA, 2004). The Cannington mine tenement is situated in the latter two vegetation groups which contain the Mitchell Grasslands plant association composed of predominantly *Astrebla* and *Themeda* species (Orr, 1975). These vegetation groups host an array of existing and historical base metal mining operations (Figure 2.5) as well as an assortment of precious metal and radionuclide mining operations. It is highly plausible that this study may have a direct influence on ecologically sustainable revegetation and rehabilitation practices within a variety of vegetation and climate zones across Australia including the Mitchell Grasslands.

Figure 2-4. The distribution of major vegetation groups within Australia (ANRA, 2004). The position of the Cannington mine (X) is indicated.

Figure 2-5. The distribution of operating (red) and historical (green) Pb and Zn mines in Australia (GA, 2004). The position of the Cannington mine (✕) is indicated.

2.5. Mine waste management

The Cannington mine produces approximately 1.5 million tonnes of mine tailings annually, of which approximately one third is deposited in the mine's Process Residue Facility (PRF). The final mine tailings drawn from the flotation tanks are thickened and a portion is mixed with cement and used as paste fill in underground voids. The remainder is pumped to the PRF containing a multi-cell tailings dam complex. Effluent from the leach circuit is also pumped to the cells after the pH is adjusted to neutral with the addition of lime. The PRF has a designed surface expression (footprint) of

approximately 125 ha containing three tailings dam cells and one acid effluent pond (BHPBilliton, 2002). At present, the design height of the tailings dam cells is 15 m (Cell One) and 12 m (Cell Two and Three). However, as new ore bodies are brought into production at the mine the eventual height of all the tailings dam cells is likely to be 15 m.

2.5.1. Rehabilitation practices and strategies

The rehabilitation strategy for the Cannington mine site is designed to return all lands disturbed by the operation, except the PRF, to a pastoral land use capability at or shortly after mine closure (BHPBilliton, 2002). This will involve the decommissioning and removal of all mine infrastructure and closure of the mine's decline entrance (portal). Highly contaminated soil, and other hazardous materials, generated at the operation are to be excavated and disposed of in the tailings dam. The land surface will then be revegetated with a collection of native pasture plant species. At present, the Environment Department of the Cannington mine intends to fence off and cap the PRF with a multi-layer store-and-release cover system, which will also support a vegetation cover of native pasture plant species.

2.6. Plant species selection and description

The selection of plant species used in this investigation was based upon a number of criteria.

1. The Cannington mine's EMOS currently indicates that plant species indigenous to the Cannington Mine tenement or at the very least, endemic to the Mitchell Grasslands plant species should be investigated were to be indigenous (BHPBilliton, 2002).

2. The presence of known or inferred heavy-metal-accumulating plant species on the Cannington mine tenement or the greater Mitchell Grasslands region should be identified and included in the study (e.g. Antonovics *et al.*, 1971; Farago *et al.*, 1977).

3. Since metal accumulation in plants is proportional to plant weight (Brooks, 1998), the project required the selection of high-biomass-producing species to maximise the potential recovery of metals and metalloids (Raskin *et al.*, 1994).

4. The plant species selected should represent a diverse collection of species inhabiting a range of positions in the landscape. This was important because of the variety of metalliferous materials (e.g. waste rock stockpiles, tailings dams, contaminated pasture soils) that would require rehabilitation at the mine.

5. Finally, the commercial availability of bulk quantities of viable seed stocks was an important consideration since the eventual aim of the study was to establish a means of remediating or revegetating large surface areas with native plants.

Plant selection progressed by evaluating the species listed in the Cannington mine's vegetation survey with available literature on these species. Once a list of high biomass producing species and any reported heavy metal accumulating species had been compiled, reliable suppliers of seed stocks were sourced or, in two cases, seed was sourced directly from the mine tenement immediately after the 2001-2002 wet season. The following plant species were thus selected for investigation: (a) *Triodia molesta* 'Spinifex', (b) *Cyperus victoriensis* 'Channel Nutgrass', (c) *Cymbopogon ambiguus* 'Scent Grass', (d) *Cymbopogon bombycinus* 'Lemon Scented Grass', (e) *Crotalaria novae-hollandiae* 'New Holland Rattlepod', (f) *Gomphrena canescens* 'Bachelor Button' and (g) *Chloris gayana* Kunth cv pioneer 'Rhodes grass' (Table 1.3). Seed stocks for these species were supplied by Kimseed Ltd, Perth, WA (e) or Southedge Seed Ltd, Mareeba, QLD (c, d, e, f and g). The exceptions were seed of *Gomphrena canescens* and *Cyperus victoriensis*, which were collected from the Cannington mine site. A description of each plant species used in the study and their global and national distributions is briefly outlined below.

2.7. Poaceae

Poaceae contains the monocotyledonous grass species (*Astrebla squarrasa*, *Chloris gayana*, *Cymbopogon ambiguus*, *Cymbopogon bombycinus*, *Themeda triandra* and *Triodia molesta*) collectively referred to as the Gramineae. Approximately 12,000 species of grasses are distributed throughout the world across frigid, temperate, subtropical and tropical climate zones (FloraBase, 2004). The gramineae are herbaceous perennial plants composed of parallel veined leaves extending from terete (circular in cross-section) hollow culms (stalks) (ANBG, 2004). Reproduction is via bisexual flowers and fruit are circular in cross section with the pericarp and testa fused.

2.7.1. Astrebla squarrasa (Bull Mitchell)

Four recorded species of *Astrebla* grasses (*A. lappacea* - curly mitchell, *A. elymoides* - hoop mitchell, *A. pectinata* - barley mitchell and *A squarrasa* - bull mitchell) characterise a Mitchell grassland plant association in Australia (Figure 2.6) (Hall, 1982; Watson and Dallwitz, 1992). The Mitchell grasslands encompass approximately 300,000 km^2 of south-eastern Queensland, the northern and central Northern Territory, the East Kimberley region of Western Australia and northern regions of South Australia.

Astrebla species are characteristically found on grey cracking clay soils within precipitation range across mainland Australia of between 250 mm and 550

mm annually (Waters, 2004). Waters (2004) also suggests that because of sensitivities to overgrazing, the Mitchell grasslands will require conservation planning and action throughout their geographic range in Australia. *Astrebla lappacea* and *A. elymoides* are generally regarded as having better livestock feed values, whereas *A. pectinata* and *A. squarrasa* (Figure 2.7) are regarded as having the least nutrition value (Phelps, 2004). *Astrebla squarrasa* is a coarse, tussocky grass growing to 1.5 m in height and has been recorded in arid and semi-arid regions of Queensland, the Northern Territory and Western Australia (Figure 2.8). This drought-hardy species has been used to revegetate dredged vertosol soil (Chan *et al.*, 1997) and clay capped pyritic mine wastes and recovers well from over grazing by virtue of its exceptional root system (Menzies and Mulligan, 2000; Waters, 2004) .

Figure 2-6. The distribution of the Mitchell grasslands plant association (green) on mainland Australia, generally found across an annual rainfall band of 250 mm to 550 mm. (Orr, 1975). The position of the Cannington mine (✕) is indicated.

Figure 2-7. *Astrebla squarrasa*. Photo D.J. Edinger (ANBG, 2004).

Figure 2-8. The distribution of *Astrebla squarrasa* in Australia (ANBG, 2004). The position of the Cannington mine (✕) is indicated.

2.7.2. *Chloris gayana* (Rhodes grass)

Native to South and East Africa, in areas from 660 to over 2160 m altitudes, *Chloris gayana* (Figure 2.9) has been introduced into India, North America, Australia (especially in Queensland), South America (Brazil, Argentina, and Uruguay), North Africa and the Philippine Islands (Duke, 1983). Rhodes grass is a hardy perennial pasture species introduced into tropical and subtropical environments in Australia (Figure 2.10) and elsewhere, to improve pasture quality in marginally productive regions ('t Mannetje and Kersten, 1992). The species grows to 1.6 m in height and displays common spreading by rooting of rhizomatous stolons (Duke, 1983).

Rhodes grass exhibits a high tolerance to soils of poor nutrient status, high salinity and low pH and is used extensively to revegetate and phytostabilise coal mine wastes and overburden materials in Australia and Africa (Carroll *et al.*, 2000; Grigg *et al.*, 2000; Rethman, 2000). Revegetation of clay-capped pyritic waste at the Rum Jungle U-Cu mine in the Northern Territory using *Chloris gayana* met with limited success (Menzies and Mulligan, 2000). Initially, *Chloris gayana* was the dominant pasture species on the revegetated site, however, an increase in soil permeability caused acidification (pH 4.6) and enrichment of Cu (1600 $\mu g\ g^{-1}$) from the underlaying mine waste resulting in broad-scale dieback (Menzies and Mulligan, 2000; Taylor *et al.*, 2003). The species extensive root system has also been exploited for slope stabilisation and erosion control in a number of industrial and urban environments (Meecham and Bell, 1977; Naidu and Harwood, 1997).

Figure 2-9. *Chloris gayana*. Photo R. Randell (ANBG, 2004).

Figure 2-10. The distribution of *Chloris gayana* Kunth in Australia (ANBG, 2004). The position of the Cannington mine (✕) is included

2.7.3. *Cymbopogon* sp.

Approximately forty species of *Cymbopogon* grasses inhabit, mostly the open savannas, of tropical and subtropical Africa, Asia and Australia. *Cymbopogon ambiguus* (Figure 2.11) grows to 1.8 m in height and prefers rocky red loam soils derived from sands, ironstone, granite, limestone and is found in New South Wales, the Northern Territory, Queensland, South Australia and Western Australia (Figure 2.12) (FloraBase, 2004). In contrast, *Cymbopogon bombycinus* (Figure 2.13) is found in Queensland, the Northern Territory and Western Australia (Figure 2.14) and grows to 1.2 m in height on red-brown soils derived from sands, laterites, granites and sandstone parent materials, in addition to inhabiting swamplands. *Cymbopogon* species been used to revegetate iron ore mine spoils in Western Australia (Jasper *et al.*, 1988). In addition, the concentrations of a range of heavy metals and metalloids has been determined in stands of *Cymbopogon bombycinus* growing on open-cut benches at the Mary Kathleen uranium mine in the Northern Territory (Costelloe, 2003).

Figure 2-11. *Cymbopogon ambiguus*. Photo R. Davis (ANBG, 2004).

Figure 2-12. The distribution of *Cymbopogon ambiguus* in Australia (ANBG, 2004). The position of the Cannington mine (✕) is indicated.

Figure 2-13. *Cymbopogon bombycinus*. Photo L. Wallis (ANBG, 2004)

Figure 2-14. The distribution of *Cymbopogon bombycinus* in Australia (ANBG, 2004). The position of the Cannington mine (✕) is indicated.

2.7.4. *Themeda triandra* (Kangaroo Grass)

Approximately eighteen species of *Themeda* grasses inhabit the warm climate savannah regions of Africa, Asia and Australia (Watson and Dallwitz, 1992). *Themeda triandra* (Figure 2.15) is one of the most widespread members of the gramineae in Australia occurring in all states and territories (Figure 2.16) (Liles, 2004). Kangaroo Grass occurs on a variety of sand, clay, alluvial, lateritic, gravel, granite and basalt soils and is often found growing along roadsides and railway lines to 2 m in height (FloraBase, 2004). *Themeda triandra* is very palatable to stock when young and is moderately drought resistant during the dry season. Overgrazing of *Themeda* grasses may lead to extensive dieback during the dry season leading to major mortalities in plants during the next growth season (Watson and Dallwitz, 1992; Mott *et al.*, 2004).

Figure 2-15. *Themeda triandra*. Photo S.M. Keeling.

Figure 2-16. The distribution of *Themeda triandra* in Australia (ANBG, 2004). The position of the Cannington mine (✕) is indicated.

2.7.5. *Triodia molesta* (Spinifex)

Triodia species are endemic to arid regions of Australia inhabiting open areas on sandy and stony soils (Watson and Dallwitz, 1992; Lazarides, 2004). There are thirty six species of *Triodia* grasses present in Australia distributed across central and western regions of Queensland, most of the Northern Territory and northern Western Australia (Burbridge, 1953, 1960). However, *Triodia molesta* (Figure 2.17) appears to be restricted to a relatively small area in northwestern Queensland and the eastern Northern Territory (Figure 2.18). *Triodia* species have been investigated for their revegetation potential of clay capped mine wastes in the iron ore industry of Western Australia (Jasper *et al.*, 1988; Islam and Adams, 1999).

Figure 2-17. *Triodia molesta*. Photo D.J. Edinger (ANBG, 2004).

Figure 2-18. The distribution of *Triodia molesta* in Australia (ANBG, 2004). The position of the Cannington mine (✕) is included

2.8. Fabaceae

Fabaceae (formally Leguminosae) contains the dicotyledonous *Crotalaria* genus, collectively referred to as the Papillionaceae (FloraBase, 2004). Papillionaceae are members of the pea family and are most notable for their ability to fix rhizospheric nitrogen, distinctive pea-shaped flowers and as a fine accompaniment to a roast (Oliver, 2004). Genus *Crotalaria* contains approximately 600 species worldwide across a range of subtropical to tropical climate zones (ANBG, 2004).

2.8.1. *Crotalaria novae-hollandiae* (New Holland Rattlepod)

Often found in shallow, hard setting, red sandy and loamy soil, *Crotalaria novae-hollandiae* (Figure 2.19) is distributed across inland regions of South Australia and central and northern regions of Queensland, the Northern Territory and Western Australia (Figure 2.20) (FloraBase, 2004). *Crotalaria novae-hollandiae* grows to 2 m in height and although not generally palatable to stock, the plant will be eaten when little else is available (White, 2004). This can result in an accumulation of pyrrolizidine alkaloids in the liver of stock causing Kimberley walkabout disease in horses and emaciation and weakness in cattle and horses (Milson, 2000). The species has been reported to accumulate high concentrations of Pb and Zn indicating its potential for phytoremediation applications (Farago *et al.*, 1977; Catt, 2000).

Figure 2-19. *Crotalaria novae-hollandiae.* Photo L. Wallis (ANBG, 2004).

Figure 2-20. The distribution of *Crotalaria novae-hollandiae* ssp. *novae-hollandiae* in Australia (ANBG, 2004). The position of the Cannington mine (✕) is included

2.9. Cyperaceae

Cyperaceae (sedges) contains the Genus *Cyperus* composed of approximately 4000 species worldwide (FloraBase, 2004). *Cyperus* species inhibit a diverse range of climates zones from frigid to tropical and are characterised by sheathed and closed leaves where stems are solid and often triangular in cross-section (ANBG, 2004). Reproduction is via bisexual flowers and fruit are often triangular in cross-section with the pericarp and testa free from one another.

2.9.1. *Cyperus victoriensis* (Channel Nutgrass)

Approximately 300 species of *Cyperus* inhabit most regions of Australia in low laying open topography adjacent to drainage systems (FloraBase, 2004). *Cyperus victoriensis* (Figure 2.21) is a perennial species growing to 0.6 m in height and characteristically inhabits moist environments along creeks and streams (Milson, 2000). However, aside from the distribution (Figure 2.22) and taxonomy of this species (Hall, 1982), very little is known regarding the plant's ecophysiology.

THE IMAGES ON THIS PAGE HAVE BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS

Figure 2-21. *Cyperus victoriensis*. Photo J.S. Smith (ANBG, 2004).

Figure 2-22. The distribution of *Cyperus victoriensis* in Australia (ANBG, 2004). The position of the Cannington mine (✕) is included

2.10. Amaranthaceae

Amaranthaceae contains approximately 850 different species of the genus *Gomphrena*. *Gomphrena* species are known to inhabit temperate, subtropical and tropical climate zones worldwide (FloraBase, 2004). Plants are characterised as herbaceous perennial species that are self-supporting or occasionally climbing shrubs or herbs with neither basal nor terminal concentrations of leaves (ANBG, 2004).

2.10.1. Gomphrena canescens (Bachelor's Buttons)

Approximately 33 species of the genus *Gomphrena* inhabit Australia preferring woodlands, rocky slopes, coastal dunes and disturbed areas (FloraBase, 2004). *Gomphrena canescens* is an herbaceous forb growing to 0.9 m in height and has characteristic pink, white and purple flowers (Figure 2.23). This species inhabits sandy, clayey or skeletal soils and laterites across Western Australia, the Northern Territory and Queensland (Figure 2.24) (Milson, 2000). The species has been reported to tolerate and accumulate high concentrations of Zn, Cu and Pb indicating potential for phytoremediation applications (Antonovics *et al.*, 1971).

Figure 2-23. *Gomphrena canescens*. Photo G.F. Craig and J. Thomas (ANBG, 2004).

Figure 2-24. The distribution of *Gomphrena canescens* in Australia (ANBG, 2004). The position of the Cannington mine (✕) is indicated.

2.11. Substrate materials

The substrate components used to generate the plant growth media (soil, mine tailings and limestone) were sourced from the BHPBilliton Cannington Ag-Pb-Zn Mine in late 2001. The soil component used in this investigation consisted of a stockpiled soil characterised by the mine as topsoil. Approximately 400 kg of this soil material was sealed inside two new 44-gallon drums and freighted by truck from the Cannington mine to Australian Laboratory Services, Townsville where it was crushed and sieved (<2 mm). Once processed, the soil was returned into the drums and freighted by truck to the JCU Cairns campus. The processed soil remained sealed in the drum until it was required for experimentation.

The mine tailings component was drawn as slurry from an outlet valve on the tailings delivery pipeline to the Cannington mine's Process Residue Facility. Approximately, 250 kg of mine tailings were sealed inside two new 44-gallon drums and frighted by truck directly to the JCU Cairns campus. This material was then exposed to the atmosphere to dry in a subtropical humid environment over a six-month period prior to preparing the various plant growth media.

The limestone component was sourced from a small quarry on pastoral lands (Cannington Station) to the northwest of the Cannington mine. Approximately 100 kg of this material was sealed inside five new 50-litre plastic containers and freighted by truck directly to the JCU Cairns campus.

The limestone was then air dried for approximately one month in a subtropical humid environment prior to preparing the various plant growth media.

2.11.1. Substrate processing

All substrate components (soil, mine tailings and limestone) were air dried in a humid subtropical environment prior to the preparation of the plant growth media. Approximately three times the estimated quantity of each substrate component required to produce the ST and STL mixtures was then processed for each experiment. The soil component used in this study, having already been crushed and sieved, was merely homogenised on a horizontal roller for approximately three hours. The upper 500 mm of air-dried and compacted mine tailings contained in the drums was removed using a geological hammer and stainless steel trowel. The tailings sample was then crushed, sieved (<2 mm) and homogenised on a horizontal roller for approximately three hours. The limestone component used to produce the STL mixtures was jaw crushed, sieved (<2 mm) and then homogenised on a horizontal roller for approximately three hours. Weight percent quantities of each of the processed substrate components were then blended together to produce the various ST and STL mixtures. In addition, the processed substrate components were sampled for chemical and mineralogical analysis. Approximately 100 g of each of the processed substrate components was powdered in a zirconium ring mill for approximately six minutes prior to submission for total element and mineralogical analysis at the AAC. The ring

mill was cleaned before and between samples by crushing approximately 50 g of silica sand for three minutes.

2.11.2. Mineralogical characterisation of the substrate components

Mineralogical analysis of the processed substrate components was performed by X-ray diffraction (XRD) at the AAC. A Siemens D5000 X-ray Diffractometer using a K710H generator (3 kW) operating at 35 kV was used to analyse the substrate components. DiffracPlus software with the search match option was used to interpret the X-ray data (Appendix A, Figure A.1 and Figure A.2). Quantitative XRD analysis was performed on each of the substrate components by the AAC using SIROQUANT (version 2.5) software (Appendix A). The detection limit for quantitative mineral analysis using SIROQUANT software is approximately 2 vol%.

The soil component used in this study was pale yellow (5YR 7/5) in colour and composed of quartz (60 vol%), albite (13 vol%), microcline (8 vol%), kaolin (6 vol%), expanding clays (6 vol%), muscovite (3 vol%) and illite (3 vol%) (Appendix A). Although containing a high proportion of expanding clay minerals, the soil component used in this study cannot be classified as Vertosol soil because by definition a Vertosol soil must contain ≥30 wt% of expanding clay minerals (such as smectite) (Isbell, 1996). Since the Cannington mine is located within a regionally extensive Vertosol soil

association it is likely that the parent material for the soil component used in this study was a Vertosol soil. However, since being removed from land that is now occupied by the Process Residue Facility and stockpiled for approximately ten years, the soil has undergone considerable physical modification. For this reason, the soil component used in this study has been classified as an Anthroposol soil, i.e. a soil resulting from human activity, which has caused profound modification, mixing, truncation or burial (Isbell, 1996).

Assessment of the modified alluvial soil sample used in this study suggests that material was not representative of the soil occurring the mine site. However, in practice the chemically-assisted phytoremediation of heavy metals from contaminated soil would require cultivation of the plant growth medium to optimise the saturation of chemical reagent throughout the soil profile. Furthermore, the oxidation of sulphide minerals would be improved by tillage through higher rates of oxygen ingress. Therefore, significant disruption of the profile of naturally occurring heavy-metal contaminated soil at the Cannington mine can be expected should the technology be applied.

X-ray diffraction analysis of Cannington mine tailings (Appendix A) indicated that the only detectable (>2.0 vol%) sulphide minerals present were pyrrhotite (3%) and galena (2%). The mine tailings used in this investigation were intentionally left to weather in a sheltered tropical environment for approximately 6 months prior to processing for experimentation. However,

this period of time was only sufficient to produce a weathering front approximately 3 cm deep in the tailings surface. This suggests that the mineralogy and geochemistry of the tailings used in this study would not have changed dramatically over the 6 months weathering period. X-ray diffraction analysis of Cannington mine tailings collected in 2004 was also undertaken by Gilfedder (2004) who reported the presence of quartz, K-feldspar, Na-feldspar, magnetite, fluorite, talc, greenalite, chlorite, amphibole and biotite. However, galena was the only ore mineral observed in the oxidised tailings and pyrrhotite was not identified by X-ray diffraction. Gilfedder also reports that surface crusts of oxidised mine tailings contained secondary minerals such as gypsum, anglesite, plumbojarosite, natrojarosite, halite, illite and native sulphur. Due to the period of time the mine tailings had been exposure to a humid tropical environment (6 months), the poor resolution of X-ray diffraction analysis (2 vol%) and the occurrence of secondary minerals in surface crusts found on the mine tailings, it would be prudent to assume that small quantities (<2 vol%) of secondary minerals were also present in the mine tailings used in this research project.

X-ray diffraction analysis of the limestone component used in this study determined that the material was composed of calcite (89 vol%), quartz (6 vol%) and expanding clays (5 vol%).

2.11.3. Substrate preparation

The substrate materials (soil, mine tailings and limestone) were all air-dried, crushed and sieved (<2 mm) prior to preparation of the various plant growth media. The soil-tailings (ST) mixtures (0 wt%, 5 wt%, 12.5 wt%, 15 wt%, 25 wt%, 35 wt% and 50 wt% tailings) were prepared by adding weight percent quantities of processed mine tailings to a base material of processed soil. The components were then blended together overnight (approximately 12 hours) on a horizontal roller and loaded in 280 mL plant growth pots using a spear sampler (N = 4) for each experimentation. Sample homogeneity was determined visually by pouring each drum of blended substrate onto a large plastic sheet prior to loading of the plant propagation pots. Any variation in the textural consistence or colour of the blended substrate resulted in further mixing on the horizontal roller. The roller and all equipment used to transfer the substrate components were thoroughly washed prior to preparation of each substrate mixture.

The soil-tailings-limestone (STL) mixtures were similarly prepared by mixing weight percent quantities of processed mine tailings and processed limestone (0 wt%, 15 wt%, 25 wt% and 50 wt% tailings plus 0 wt%, 10 wt% and 20 wt% limestone) to a base material of processed soil. These mixtures were then blended together overnight (approximately 12 hours) on a horizontal roller and loaded in 280 mL plant growth pots (N = 4) for experimentation. The roller and all equipment used to transfer the substrate components were thoroughly washed prior to preparation of each substrate mixture.

Nutrient deficiencies, particularly N and P, are common characteristics of metal mine tailings, and are major constraints on plant colonisation of these materials (Ye *et al.*, 2000). Fertiliser amendments were therefore investigated as a means of promoting plant growth on undiluted mine tailings. Duplicate samples (2 kg) of fertilised and unfertilised (control) mine-tailings substrates (N = 2) were prepared from air dried and sieved tailings amended with N-rich and N-rich + P-rich fertilisers. The N-rich fertiliser used in this investigation was three-month slow-release Osmocote (Table 2.2) and the P-rich fertiliser was commercial Triphosphate (Incitec Fertilisers Ltd) (Table 2.2). Fertiliser amendments were applied to the surface of tailings at rates equivalent to 300 kg Osmocote ha^{-1} (48 kg N ha^{-1}) or 300 kg Osmocote ha^{-1} (48 kg N ha^{-1}) + 300 kg Triphosphate ha^{-1} (62.1 kg P ha^{-1}). The fertilised and unfertilised mine tailings were prepared for plant propagation in perforated three-litre plastic containers.

Prior to plant cultivation, soil-tailings (ST) and soil-tailings-limestone (STL) mixtures were sampled, oven dried to a constant weight at 65ºC and hand ground with pestle and mortar before being subjected to a number of analytical procedures (Section 2.11.4 to Section 2.11.7). The pestle and mortar were cleaned prior to the processing of each sample by grinding a 20 gram charge of silica sand until both items were clean (usually 2-3 minutes).

Table 2.2. The chemical composition of the fertiliser amendments used in the study (Osmocote Plus, Scotts Australia Pty Ltd; Triphosphate – Incitec Ltd).

Osmocote Plus		Triphosphate	
Element	Conc.	Element	Conc.
N	16 wt%	N	0
P	4.4 wt%	P	20.7 wt%
K	10 wt%	K	0
S	2.5 wt%	S	1.0 wt%
Mg	2.0 wt%	Ag	0.07 $\mu g\ g^{-1}$
B	0.02 wt%	As	2.0 $\mu g\ g^{-1}$
Cu	0.05 wt%	Cd	11.3 $\mu g\ g^{-1}$
Fe	0.4 wt%	Cr	4.0 $\mu g\ g^{-1}$
Mn	0.06 wt%	Cu	8.83 $\mu g\ g^{-1}$
Mo	0.02 wt%	Pb	<0.005 $\mu g\ g^{-1}$
Zn	0.015 wt%	Sb	0.54 $\mu g\ g^{-1}$
		Zn	105 $\mu g\ g^{-1}$

2.11.4. pH determination

The average pH (N = 2) of each ST and STL mixture was determined using a 10 g sample of each prepared substrate and 25 mL of deionised water. The substrate slurries were stirred vigorously using a homogeniser and left to stand overnight (approximately 12 hours). The pH was then determined without further agitation using a Radiometer Analytical (MeterLab) CDM210 instrument. Calibration using standard solutions was performed on the instrument prior to its use.

2.11.5. Electrical conductivity

The average electrical conductivity (EC) (N = 2) of each ST and STL mixture was determined using a 10 g sample of each prepared substrate and 25 mL of deionised water. The substrate slurries were stirred vigorously using a

homogeniser and left to stand overnight (approximately 12 hours). Measurements were taken using a hand-held DiST 3 Conductivity Meter (HANNA, HI 98303). Calibration using standard solutions was performed on the instrument prior to its use.

2.11.6. Estimating heavy metal bioavailability

The extractability of metals and metalloids contained in the tailings-soil (ST) and tailings-soil-limestone (STL) mixtures and the fertilised and unfertilised tailings (Appendix E and I respectively) was estimated using several standard methodologies. Norvell (1984) and Lindsey and Norvell (1978) reported that the extractability of biological trace elements using 0.01 M EDTA and 0.005 M DTPA produced valuable information regarding the nutrient requirements of pasture soils. However, high concentrations of heavy metals in soil may exceed the chelating ability of the extraction (Bell, 1986). The liquid:solid ratio employed for this study was increased from that reported by Norvell (1984) and Lindsey and Norvell (1978) (5:1) to a level of 10:1 in order to account for the wide range of expected heavy metal concentrations in the substrate materials. All pots from each ST and STL mixture were vertically 'spear' sampled, oven dried (65ºC) to a constant weight, and crushed and sieved (<2 mm). The prepared soil samples were then combined (100 grams) to produce a composite sample of each ST and STL mixture. Each composition sample was hand ground to a fine powder with a pestle and mortar prior to extraction. The pestle and mortar was cleaned by grinding a 20 gram charge of silica sand in the device until both pieces were clean

(usually 2-3 minutes). The powdered soils were then sampled (10 gram) and extracted by end-over-end agitation for 24 hours using the methods of Norvell (1984) and Lindsey and Norvell (1978) to assist with estimating the plant availability of heavy metals and metalloids. Since the use of EDDS as a heavy metal chelating agent was intended in the research, a soil extraction using 0.01 M EDDS was also performed on the substrate mixtures for comparison (Vandevivere *et al.*, 2001a).

2.11.7. Estimating metal and metalloid distribution

Duplicate sequential extractions were performed on the 25 wt% ST mixture to operationally define the distribution of elements (Ag, As, Cd, Pb, Sb, and Zn) between various mineral classes in the soil (Appendix D and E). All pots for the 25 wt% ST mixture were sampled and combined to produce a composite sample. The composition sample was then oven dried (65ºC) to a constant weight and hand ground with a pestle and mortar prior to extraction. The pestle and mortar was cleaned by grinding a 20 gram charge of silica sand in the device until both pieces were clean (usually 2-3 minutes). Analyses of the applied sequential extraction procedure are considered an indication of the chemical character of the compounds with which the metals are associated. The sequential extraction proceeded via the scheme:

1. deionised H_2O = water-soluble metal fraction (Gatehouse *et al.*, 1977);
2. 1 M NH_4OAc (pH 7) = ion exchangeable fraction (Robinson, 1997;

Almås *et al.*, 1999);

3. 1 M NH_4OAc. (pH 5) = carbonate (and the majority of the sulphate) fraction (Almås *et al.*, 1999);
4. 0.04 M NH_4.HCl in 25% (v/v) HOAc. = Fe and Mn oxide (and remaining sulphate) fraction (Tessier *et al.*, 1979); and
5. conc. HCl and KCl followed by 4 M HNO_3 = sulphide fraction (Tessier *et al.*, 1979).

Concentrations of metals and metalloids present as silicate phases were not determined in the sequential extraction residues because this fraction is considered unavailable to plants (Alloway, 1995). The sum of extracted elements using dH_2O and 1 M NH_4OAc (pH 7) (extractions 1 and 2) may be considered an estimation of the plant-available (bioavailable) metal or metalloid fraction in the soil (Tessier *et al.*, 1979).

2.12. Plant propagation

All experiments were conducted in shade houses at James Cook University, Cairns, Australia (16° 47' S, 145° 37' E) where the local climate is characterised as humid-wet tropical. Growth lamps were used to maintain illumination similar to the open pasture environment at the Cannington mine site. Initially, plants were hand watered daily until an automated irrigation was implemented.

Seedlings of the various species were propagated in a commercially available germination mix (Yates) before being transplanted into the 280 mL pots containing the various substrate mixtures. Four replicates (N=4) of each plant-substrate mixture were prepared for cultivation and any plants that did not survive the original transplanting (approximately 5%) were replaced with fresh seedlings after two weeks. Periodically during the course of the pot trial experiments, exploratory roots were trimmed from the exterior of pots to ensure maximum root density. At the same time, pots were re-arranged to randomise growth effects inside the shade house. All pot trials were conducted over approximately a 12-week long growth period. The cultivation of *Chloris gayana* on fertilised mine tailings was undertaken over approximately 50 weeks.

2.12.1. Biomass preparation

All plant tissues (leaves, stems, flowers and seed heads) above approximately 20 mm from the soil surface of each pot were harvested for analysis. The whole plant samples were stored in new paper bags throughout the drying and sampling stages of the following procedure. After harvesting, plant samples were immediately rinsed twice with deionised water and then oven-dried to a constant weight at 65° C. Plant samples were then hand ground with a pestle and mortar and accurately weighed into borosilicate test tubes for ashing at 520°C in a muffle furnace (usually over 24 to 48 hours). The resulting ash was digested in near boiling 2M HCl and the digestion was then diluted with deionised water prior to analysis for metals and metalloids.

Elemental concentrations present in the dry weight (DW) of each plant sample (P) was determined by multiplying the element concentration in the analysed digest sample (E) by the sample dilution factor (10) and expressing this concentration as a proportion of the plant biomass (DW) present in the sample. The following equation was used to calculate such DW element concentrations: [P = (E x 10)/DW]. The elemental concentrations contained in the whole plant samples (P) (μg g^{-1} DW) were then used to produce the various graphs and table contained in this report.

2.12.2. Chemical amendments

The various chemical amendments (Table 2.3) were prepared fresh each week from analytical grade reagents and deionised water. Prior to beginning the chemically amended pot trial experiments, plant pots were placed in plastic lined trays to capture any resulting leachate. The chemical amendments were applied in dissolved form directly to the soil surface at concentrations of 0.5 and 2.0 g per kg of soil. Automated irrigation was replaced by hand watering during the CAP pot trial experiments to better control the moisture content of the potted soils. Control plants, propagated for each plant-substrate combination, were set aside prior to beginning the chemical treatments to determine concentrations of metals and metalloids in plants grown on the various substrates without chemical amendments.

Table 2.3. The chemical reagents and their concentrations (in grams of reagent per kg of soil and as molar concentrations) applied to the 12.5 wt% ST mixture (ST) and the fertilised tailings substrates (OSM = 300 kg^{-1} ha^{-1} Osmocote, TPP = 300 kg^{-1} ha^{-1} Osmocote + 300 kg^{-1} ha^{-1} Triphosphate).

Reagent	Conc. (g kg^{-1})	Substrate
EDTA	0.5 (0.001 M)	ST
EDTA	2.0 (0.005 M)	ST, OSM, TPP
DTPA	2.0 (0.005 M)	ST
EDDS	0.5 (0.001 M)	ST
EDDS	2.0 (0.005 M)	ST, OSM, TPP
TSP	0.5 (0.003 M)	ST
TSP	2.0 (0.03 M)	ST
SCN	0.5 (0.006 M)	ST
SCN	2.0 (0.03 M)	ST, OSM, TPP
THIO	0.5 (0.006 M)	ST
THIO	2.0 (0.03 M)	ST

2.13. Hydrometallurgical leaching

Air dried samples of mine tailings were crushed, sieved to <2 mm, and weighed (2 kg) into 0.3 m lengths of transparent 65 mm diameter polycarbonate columns. Silica glass was used as packing at each end of the column to minimise disturbance of the tailings surface. Nylon sieve mesh and a small nylon funnel were attached to one end of the column with sealant and the columns were mounted vertically in clamps. Each column was then flushed with 200 mL of deionised water (dH_2O) and allowed to drain completely (approximately 4 days). Each lixiviant (Table 2.4) was prepared fresh each week and applied as a 200 mL amendment. Collected leachates were measured and analysed periodically over a three-month period. The NaCN column was activated late in the experiment because of Occupational Health and Safety Certification delays and was leached for nine weeks only. Chemical analyses were performed by ICP MS and GFAAS at the AAC, JCU.

Table 2.4. The chemical reagents and their concentrations applied as weekly amendments (200 mL) used in the leaching study. The mass of applied reagent (g kg^{-1} wk^{-1}) is included.

Reagent	Conc. (mol)	Reagent	Conc. (mol)	Reagent	Conc. (mol)
EDTA*	1.0 (74.5g)	TSP	1.0 (29.6g)	SCN	1.0 (15.2g)
EDTA	0.1 (7.5g)	TSP	0.1 (3.0g)	SCN	0.1 (1.5g)
Thiourea	1.0 (15.2g)	NaCN	0.005 wt% (5g)	dH_2O	-

EDTA* = one initial amendment followed by weekly amendments of dH_2O (200 mL).

2.14. Chemical analyses

Most chemical analyses of liquid samples was performed by Inductively Coupled Mass Plasma Spectrometry (ICP MS, Varian UltraMass 700) at the Advanced Analytical Centre (AAC), JCU (Table 2.5). Arsenic analyses were performed at the same facility using Graphite Furnace Atomic Absorption Spectrometry (GFAAS, Varian Spectra AA400). Chemical analysis of liquid samples (Appendix J) were also performed by Australian Laboratory Services (ALS) using ICP MS.

Table 2.5. Analytical methods used to determine the elemental concentrations of various samples resulting from this study.

Sample Type	Method	Technique	Elemental Suite	Results
Substrate Components	Section 2.11.1	ICP MS, GFAAS	Ag, As, Ca, Cu, Cd, Fe, K, Mg, Mn, Na, P, Pb, S, Sb, Zn,	Appendix A
Plant Tissues	Section 2.12.1	ICP MS, GFAAS	Ag, As, Cd, Pb, Sb, Pb, Zn	Appendix B, C, F, G and H
Soil Extractions	Section 2.11.6	ICP MS, GFAAS	Ag, As, Cd, Pb, Sb, Pb, Zn	Appendix E and I
Sequential Extractions	Section 2.11.7	ICP MS, GFAAS	Ag, As, Cd, Pb, Zn	Appendix D
Tailings Leachates	Section 2.13	ICP MS	Ag, Na, Pb, Zn	Appendix J
Reference material (GXR-3)	none	ICP MS	Ag, As, Cd, Pb, Zn	Appendix K

Sample preparation and chemical analysis of solid samples (soil, tailings, limestone and the geochemical reference material) was performed by the AAC. These materials were prepared by open vessel, microwave-assisted acid digestion and analysed using ICP MS and GFAAS. Instrument detection limits for all elements were reported by the AAC and ALS (ICP MS and GFAAS methods) to range from 0.001 $\mu g\ g^{-1}$ to 0.005 $\mu g\ g^{-1}$.

2.15. Quality assurance

Analyses obtained from the AAC were not accompanied by laboratory certification and as a result two internal referencing systems were implemented for quality control. The first system involved sampling the processed substrate components (i.e. soil, mine tailings and limestone) at the beginning of the project to constituent experimental reference materials.

Quality assurance of the data was applied using the repetitive analysis of each processed substrate components (N=4) over the course of the research (approximately every six months) (Appendix A). Statistical analysis of this dataset indicated that replicate analyses of the substrate components deviated by 4% to 18% from the mean for the elements tested (Table 2.6). The data indicates that replicate analyses of the substrate components varied by 6% for As, 2% for Cd, 10% for Zn, and 12% for Pb over the course of the research project.

The second internal reference system that was applied for quality control involved the repetitive analysis of a geochemical reference material (GXR-3), which was submitted for analysis with the two largest sample batches in 2002 and 2003 (Appendix K). Statistical analysis of this dataset indicated that replicate analyses of geochemical reference material deviated by <0.1% to 12% from the mean for the elements tested (Table 2.6). The data indicates that analytical variance for replicate samples of the geochemical reference material were notable for Ag (4.2%) and Cd (11.8%) only.

Table 2.6. Statistical analysis of the replicate sampling performed on the substrate components and the geochemical reference material used in this investigation.

Substrate Component		Ag	As	Cd	Pb	Zn
Soil	Mean (μg g^{-1})	bd	7.0	bd	29.8	71.8
	Standard Deviation	-	0.8		5.4	5.4
	Variance (%)	-	11.7%		18.1%	7.5%
Tailings	Mean (μg g^{-1})	60.0	2039	30.0	11950	4150
	Standard Deviation	8.1	72.8	4.2	1387	561
	Variance (%)	13.5%	3.6%	14.1%	11.6%	13.5%
Limestone	Mean (μg g^{-1})	bd	32.8	7.0	31.5	357
	Standard Deviation	-	1.3	0.8	1.7	64.5
	Variance (%)	-	3.8%	11.7%	5.5%	18.1%
GXR-3	Mean (μg g^{-1})	2.4	3971	1.7	15	208
	Standard Deviation	0.1	0.7	0.2	<0.1	0.7
	Variance (%)	4.2%	<0.1%	11.8%	-	0.3%

2.16. Statistical analysis

The natural uptake of elements by different individuals of one plant species growing on a particular soil type can vary dramatically (Brooks, 1980; Albasel and Cottenie, 1985). This project was designed to evaluate a collection of pasture plant species grown on a range of soils under varying chemical conditions (experimental treatments). In order to obtain robust statistical information for each experimental treatment, it would have been necessary to use replicate values of between 10 and 20 depending on the genetic variability of each native plant species and the combinations of substrate components, chemical amendments and fertilisers used in the experiments. Employing such replicate values at this level would have significantly added to the expense and logistics of evaluating the required collection of plant species. Alternatively, by reducing the number of plant species investigated it would have been impossible to meet the project objectives. Consequently, it was decided to set the pot trial replicate value to four (N=4) for all pot trial

experiments in order to gain information on all of the selected plants species. Plant concentrations of heavy metals and metalloids have been reported as mean values and the variance between samples within individual populations has been reported as the standard deviation (95%) of the mean.

Chapter 3. Phytoextraction of As, Ag, Cd, Pb, and Zn by Subtropical Australian Pasture Plant Species Grown on Soil Contaminated with Mine Tailings

3.1. Introduction

The rehabilitation of lands disturbed by mining in Australia is increasingly being undertaken with native plant species. Species selection for mine rehabilitation programs in regions of high farm productivity is generally made with regard to improving pasture quality (Clem and Hall, 1994; Harwood *et al.*, 1999). The rehabilitation and revegetation of less productive pastoral lands disturbed by mining activities, particularly those in central and northern regions of Australia, is generally undertaken with native plant species in order to preserve biological diversity. The EMOS for the Cannington mine reflects this approach to land rehabilitation by stipulating that 'only native, or tenement hosted, flora should be used in revegetation programs' (BHPBilliton, 2002). However, at present very little is known about the revegetation potential of subtropical pasture plant species in Australia and at the Cannington mine.

The revegetation of contaminated lands and mine waste repositories at many base metal mining operations is often further complicated by elevated chemical toxicities and/or poor soil-nutrient status (Carroll *et al.*, 2000; Ye *et*

al., 2000). The identification of plant species capable of tolerating elevated levels of metals and metalloids in soil (i.e. hypertolerance) is of critical importance to the success of EMOS controlled revegetation programs. Of equal importance is the identification of plants that can exclude soil contaminants from uptake, since elevated levels of contaminants in plants will lead to contamination of higher trophic levels of the food chain (Lodge *et al.*, 2003). Some types of contaminants, such as Cd and As, are relatively mobile at soil-pH conditions favoured by plants and may bioaccumulate in the food chain. In addition, some plant species (e.g. *Berkheya coddii*, *Alyssum bertolonii*) are predisposed to accumulating specific metals or metalloids (Brooks, 1987; Hamon *et al.*, 1997) and may be exploited to 'phytoremediate' contaminated soil. The solubility of a contaminant may be reduced in soil using amendments, such as limestone, phosphate rock, or organic matter (Ma *et al.*, 1997; Bernal *et al.*, 2002; Catalan *et al.*, 2002; Hamon *et al.*, 2002). However, most techniques for the immobilisation of heavy metal contaminants in soil (Li *et al.*, 2000) are not permanent and require periodic re-application. Yet, the effects of limestone amendments (reduced metal solubility, increased soil pH) would be of sufficient duration for a vegetation cover to temporarily contain contamination while a permanent solution was devised.

The enforcement of an Environmental Management Overview Strategy now limits plant selection for mine site revegetation to tenement-bound plant species in order to maintain a biological diversity that existed prior to mining. Therefore, this study was conducted to determine the revegetation potential

of native subtropical pasture plant species that occur near the BHPBilliton Cannington Ag-Pb-Zn mine in northwest Queensland. Substrate tolerance, biomass production and the uptake of Ag, As, Cd, Pb and Zn were determined for plants cultivated on a range of soil-tailings (ST) and soil-tailings-limestone (STL) mixtures. Results of this study will contribute to the selection of native plant species for the revegetation of the Cannington mine site. In addition, the distribution of some of the plant species investigated (Chapter 2, Section 2.5) indicates that this study may be relevant to the rehabilitation of metalliferous soils in temperate to tropical regions of Australia, North and South America, India and Africa.

3.2. Materials and methods

Bulk mineralogical characterisation of the substrate components (Chapter 2, Section 2.10.2) was determined by X-ray diffraction (Appendix A). The geochemical and chemical properties of the substrate materials (total and extractable element concentrations, pH and EC) were also determined (Chapter 2, Section 2.11). Plant growth media were then prepared by blending weight percent quantities of soil and tailings (ST mixtures) and soil, tailings and limestone (STL mixtures) (Chapter 2, Section 2.10.3). Seedlings were transplanted for cultivation (Chapter 2, Section 2.11) and allowed to mature for approximately 12 weeks. After twelve weeks of cultivation, above ground plant tissues were sampled (Chapter 2, Section 2.12.1) and analysed (Chapter 2, Section 2.14) from ST mixtures (Appendix B) and STL mixtures (Appendix C) for their elemental abundances.

3.3. Results

3.3.1. Substrate characterisation

Cannington tailings contain minor quantities of sulphide minerals and hence elevated total S concentrations (max. 2.1 wt%) (Appendix K). Much of this total S is present as sulphidic S in the form of galena (Chapter 2, Section 2.11.2), as determined by XRD analysis. Galena is a sulphide mineral that does not generate acid whereas pyrrhotite is an acid-generating phase (e.g. Williams, 1990). The tailings used in this study reduced the substrate pH values by only 0.5-1.0 units in the ST mixtures. Therefore, the Cannington mine tailings contain trace quantities of acid-generating minerals such as pyrrhotite whose oxidation and acid production reduced the pH of the investigated substrates. The addition of 10 wt% limestone to the ST mixtures (the STL mixtures) raised the pH by an equivalent amount. Substrates containing ≥15 wt% tailings exceeded the Australian and New Zealand Environment and Conservation Council (ANZECC) Investigation Limits for Pb contaminated industrial and commercial sites (1500 μg Pb g^{-1}), and substrates containing >25 wt% tailings exceeded the As Investigation Limit (500 μg As g^{-1}) (ANZECC, 1999).

The total concentration of Zn in substrates containing >15 wt% tailings (>683 μg Zn g^{-1}) indicates the potential for Zn toxicity to occur in plants grown on these substrates (Table 3.1). Glendinning (2000) reports that total Zn concentrations in soil exceeding approximately 600 μg Zn g^{-1} can induce

Zn toxicity symptoms in common agricultural crop species. Alloway (1995), on the other hand, indicates that the critical soil concentration for Zn, above which toxicity symptoms in plants could be expected, is 400 μg Zn g^{-1}. Considering the variation in reportedly phytotoxic concentrations of Zn in soil, the general trend towards metal exclusion by grasses (Islam and Adams, 1999), and the reported Zn tolerance in Vetiver grass (Truong, 1998), I decided that a total Zn concentration exceeding 650 μg Zn g^{-1} to be potentially phytotoxic to the plant species investigated. This concentration was selected based upon the data of Glendinning (2000) plus a modest quantity to allow for a margin of error when comparing datasets.

Table 3.1. Total element concentrations (μg g^{-1}) and pH of the plant growth medium used in this study (e.g., S5T10L = Soil amended with 5 wt% Tailings and 10 wt% Limestone). ANZECC Investigation Limits for contaminated industrial and commercial sites (ANZECC, 1999) are also listed.

Substrate	pH	Total Element Concentrations (μg g^{-1})				
		Ag	As	Cd	Pb	Zn
Soil	7.7	<0.001	7	<0.001	30	72
5T	7.6	3	109	2	626	276
15T	7.8	8	261	4	1818	683
25T	7.7	9	312	5	3010	1091
35T	7.4	15	515	8	4202	1499
50T	7.5	21	718	11	5990	2111
Tailings	5.4	30	1023	15	11950	4150
S10L	9.2	<0.001	10	1	30	100
S5T10L	8.9	3	111	2	626	304
S15T10L	8.4	9	314	5	1818	712
S25T10L	8.2	15	518	8	3010	1120
S50T10L	8.2	30	1026	16	5990	2139
S20L	9.2	<0.001	10	1	30	100
S5T20L	8.8	3	114	3	626	333
S15T20L	8.4	9	317	6	1818	741
S25T20L	8.6	15	520	9	3010	1148
S50T20L	8.4	30	1028	16	5990	2168
ANZECC Investigation Limits			500	100	1500	35000

3.3.2. Plant growth for the soil-tailings mixtures

All plant species were found to grown in plant growth media that exceeded the ANZECC Investigation Limits for Pb and As contaminated industrial and commercial sites (Figure 3.1) (ANZECC, 1999). *Cymbopogon ambiguus* and *Triodia molesta* tolerated ST mixtures containing ≤15 wt% tailings (1818 μg Pb g^{-1}), whereas *Gomphrena canescens* tolerated substrates containing ≤25 wt% tailings (3010 μg Pb g^{-1}). *Cymbopogon bombycinus*, *Crotalaria novae-hollandiae* and *Cyperus victoriensis* tolerated the entire range of tailings proportions investigated (30 to 5990 μg Pb g^{-1}).

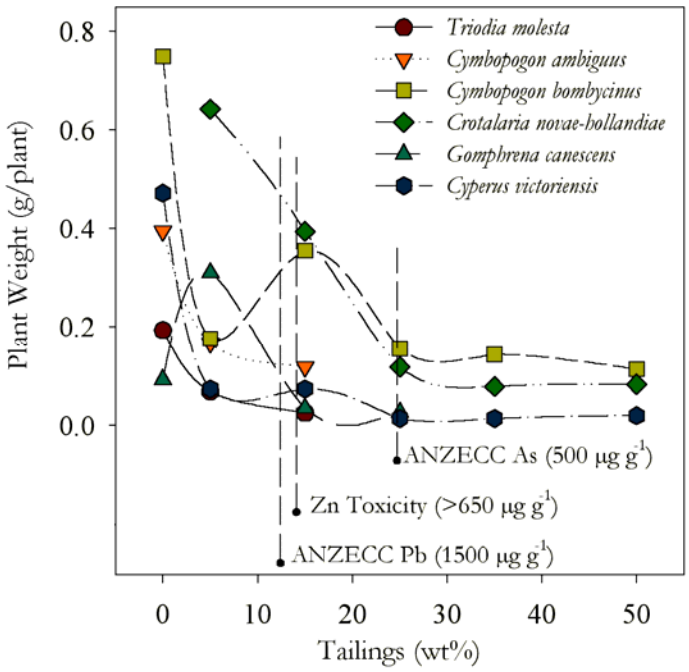

Figure 3-1. Average (N=4) biomass production (g $plant^{-1}$) of selected subtropical pasture plant species cultivated on a range of soil contaminated with mine tailings (ST mixtures). ANZECC Investigation Limits for Pb (1500 μg g^{-1}) and As (500 μg g^{-1}) contaminated industrial sites (ANZECC, 1999), and the total concentration of Zn deemed to be potentially phytotoxic (>650 μg g^{-1}) to the plants are indicated.

Leaf-tip necrosis, chlorotic foliage and reduced plant mass were visible evidence that metal induced toxicity was reached in all plant species grown on the ST and STL mixtures. Continual and vigorous growth of exploratory roots occurred from pots containing ST mixtures and *Triodia molesta* (<15 wt% tailings), *Cymbopogon ambiguous* (<15 wt% tailings), and *Cymbopogon bombycinus* (<35 wt% tailings). Exploratory root growth was pronounced from pots containing STL mixtures of 10 wt% limestone and *Cymbopogon ambiguus* (<25 wt% tailings) and *Cymbopogon bombycinus* (<25 wt% tailings). *Cymbopogon ambiguus* and *Cymbopogon bombycinus* grown on STL mixtures containing 20 wt% limestone also developed exploratory root from pots containing <35 wt% tailings. Exploratory root growth diminished with increasing tailings content (≥25 wt% to 35 wt% tailings) in the substrate and was absent from pots containing visibly unhealthy plants (≤25 wt% to 35 wt% tailings).

The average DW (N=4) of plants grown on ST mixtures containing mine tailings (Figure 3.1) was generally lower than the weights of plants grown on uncontaminated soil (Appendix B). Plant weights decreased by 45-85% for substrates containing Pb concentrations in excess of the ANZECC Investigation Limit (15 wt%; 1818 μg Pb g^{-1}). Further reductions in plant weights occurred in all plant species grown on ST mixtures containing <25 wt% mine tailings. The weight of plants grown on ST mixtures containing >25 wt% mine tailings was relatively constant and approximately 95% lower than the weight of plants grown on unamended soil.

3.3.3. Plant uptake of metals and arsenic from soil-tailings mixtures

Silver concentrations in the plant species increased with increasing tailings proportions (>5 wt%) in the ST mixtures. Analyses of aboveground biomass (Appendix B) determined that *Cyperus victoriensis* accumulated the greatest concentration of Ag (1.6 ± 0.4 μg Ag g^{-1}) compared to the other plant species(Figure 3.2). *Triodia molesta* accumulated high concentrations of Ag (0.8 ± 0.06 μg g^{-1}), in addition to As, Cd, Pb and Zn, from substrates containing low concentrations of tailings (5 and 15 wt. %). Silver concentrations in the remaining plant species were low (<0.7 μg Ag g^{-1}) over the range of tailings concentrations investigated.

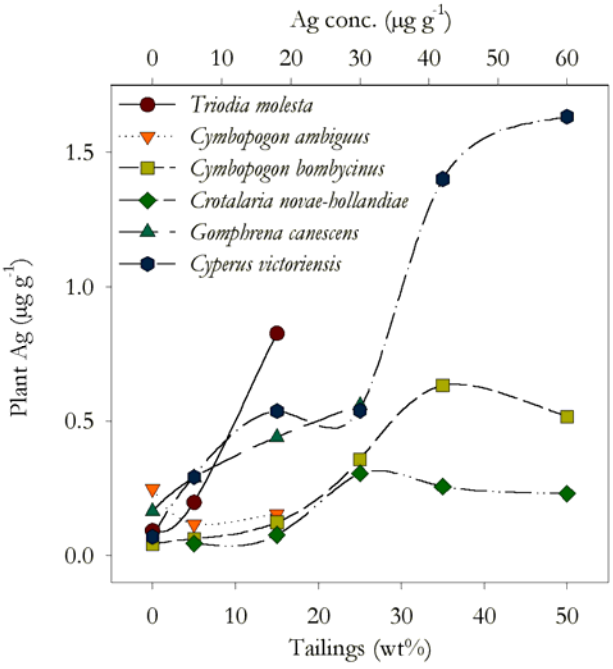

Figure 3-2. Average (N=4) plant concentrations of Ag (μg g^{-1}) in selected subtropical pasture plant species cultivated on soil contaminated with mine tailings (ST mixtures).

Maximum uptake of As (Figure 3.3) occurred in *Crotalaria novae-hollandiae* (64 ± 13.1 μg g^{-1}) grown on substrates containing 25 wt% and 35 wt% tailings (515 and 718 μg As g^{-1}, respectively). Above a level of 35 wt% tailings in the soil, the As concentrations in *Crotalaria novae-hollandiae* decreased with increasing tailings proportions in the ST mixture. The concentration of As in *Cyperus victoriensis* increased dramatically (49.0 ± 13.5 μg As g^{-1}) when grown on ST mixtures containing >35 wt% tailings compared to ST mixtures containing lesser tailings proportions. Arsenic concentrations in the remaining species (*Triodia molesta*, *Cymbopogon ambiguus*, *Cymbopogon bombycinus* and *Gomphrena canescens*) increased with increasing tailings proportions in the ST mixture, maximum plant concentrations in these species was <30 μg g^{-1} (dry wt.).

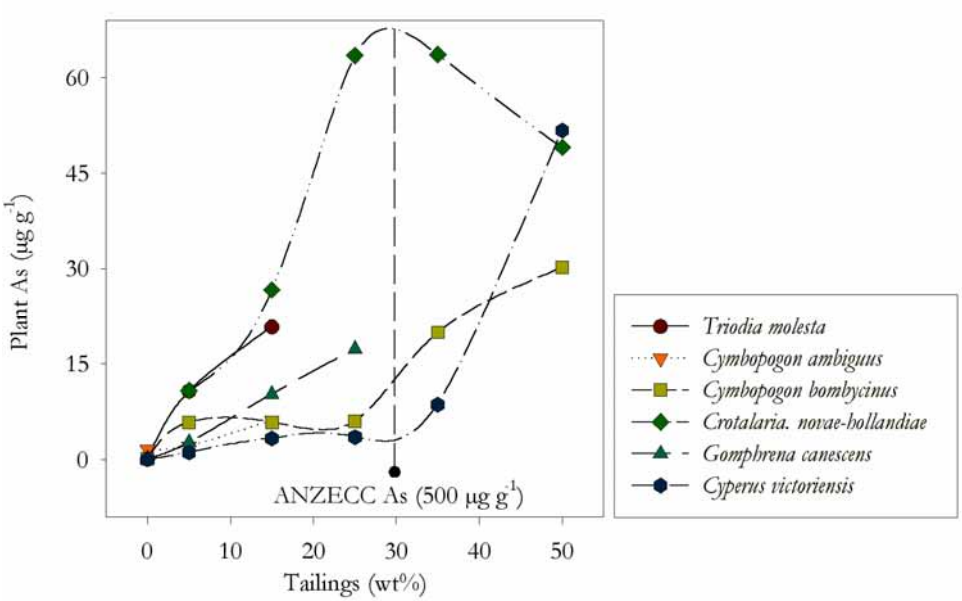

Figure 3-3. Average (N=4) plant concentrations of As (μg g^{-1}) in selected subtropical pasture plants cultivated on soil contaminated with mine tailings (ST mixtures). ANZECC Investigation Limit for As contaminated industrial soil (500 μg g^{-1}) is included (ANZECC, 1999).

Cadmium uptake was highest in *Gomphrena canescens* (14.1 μg g^{-1}, Appendix B) and its accumulation increased with increasing concentrations of tailings (Figure 3.4). *Cyperus victoriensis* accumulated a maximum concentration of Cd (9.5 ± 3.7 μg g^{-1}) from ST mixtures containing 35 wt% mine tailings. Maximum Cd uptake by *Crotalaria novae-hollandiae* (5.1 ± 1.4 μg g^{-1}) occurred on ST mixtures containing 25 wt% mine tailings. Cadmium concentrations in these species increased with increasing proportions of mine tailings in the ST mixtures, plant concentrations of Cd were <4 μg g^{-1} (dry wt.).

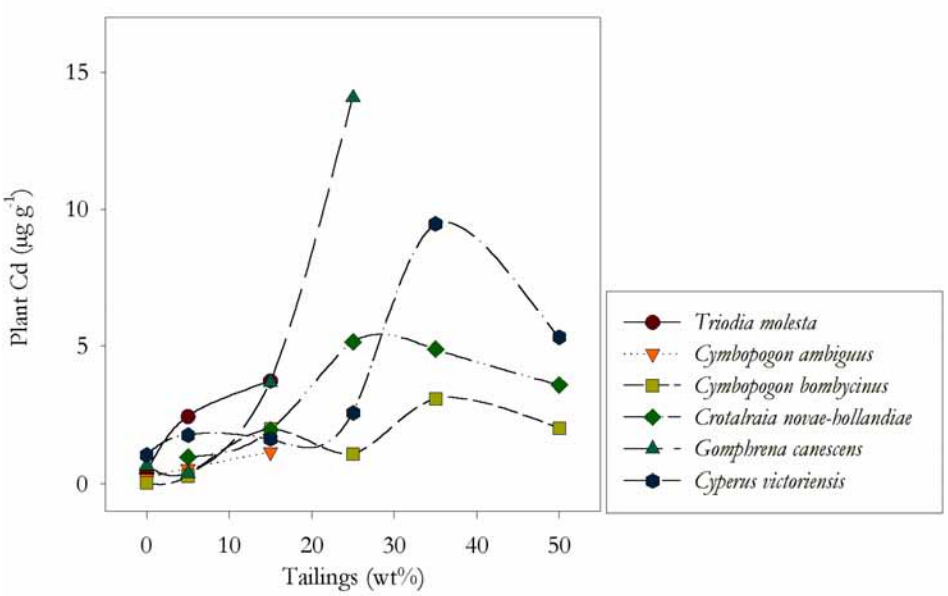

Figure 3-4. Average (N=4) plant concentrations of Cd (μg g^{-1}) in selected subtropical pasture plant species cultivated on soil contaminated with mine tailings (ST mixtures).

Cyperus victoriensis accumulated the greatest concentration of Pb (377 ± 194 μg g^{-1}), compared to the other plant species (Figure 3.5), from ST mixtures containing 35 wt% tailings (4202 μg Pb g^{-1}). Lead concentrations then decreased to 276 ± 23 μg Pb g^{-1} for ST mixtures containing 50 wt% tailings (5990 μg Pb g^{-1}). The concentrations of Pb in *Cymbopogon bombycinus* were low (<100 μg Pb g^{-1}) for ST mixtures containing <35 wt% mine tailings. However, Pb uptake increased to 239 ± 197 μg g^{-1} when the plants were grown on ST mixtures containing 50 wt% mine tailings. In contrast, *Triodia molesta* accumulated a high concentration of Pb (174 ± 150 μg g^{-1}) from an ST mixture containing 15 wt% mine tailings. The remaining plant species (*Triodia molesta*, *Cymbopogon ambiguus*, *Crotalaria novae-hollandiae* and *Gomphrena canescens*) also accumulated low concentrations of Pb from the ST mixtures, Pb uptake

increased with increasing tailings proportions in the ST mixtures.

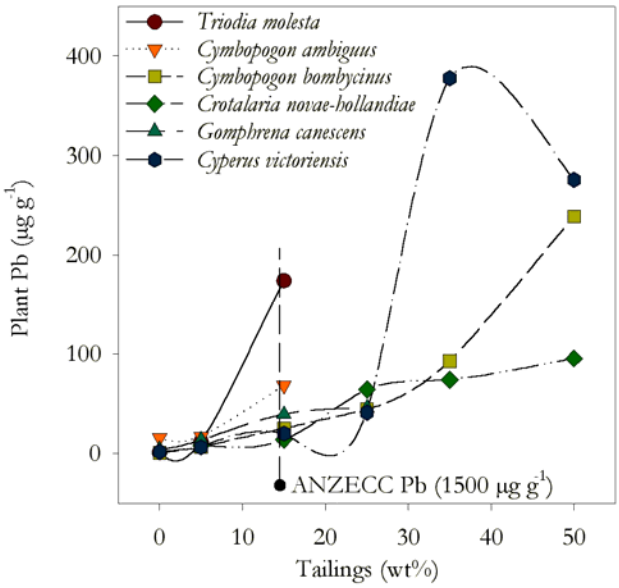

Figure 3-5. Average (N=4) plant concentrations of Pb (µg g^{-1}) in selected subtropical pasture plant species cultivated on soil contaminated with mine tailings (ST mixtures). ANZECC Investigation Limit for Pb contaminated industrial soil (1500 µg g^{-1}) is included (ANZECC, 1999).

Plant uptake of the essential trace element Zn (Figure 3.6) increased progressively with increasing tailings proportions for all the plant species (except *Cyperus victoriensis* and *Crotalaria novae-hollandiae*), Zn accumulation in the plants was greatest in *Cymbopogon bombycinus* (786.9 ± 341 µg Zn g^{-1}). The maximum concentrations of Zn in *Cyperus victoriensis* and *Crotalaria novae-hollandiae* (754 ± 315 and 414 ± 99 µg g^{-1}, respectively) occurred on ST mixtures containing 35 wt% tailings (1499 µg Zn g^{-1}) and then decreased with higher proportions of tailings in the ST mixtures. All plant species tolerated total Zn concentrations in the ST mixtures that may be considered

phytotoxic (>15 wt%; >650 μg g^{-1}), although the growth of *Triodia molesta* was significantly reduced at this level of tailings content.

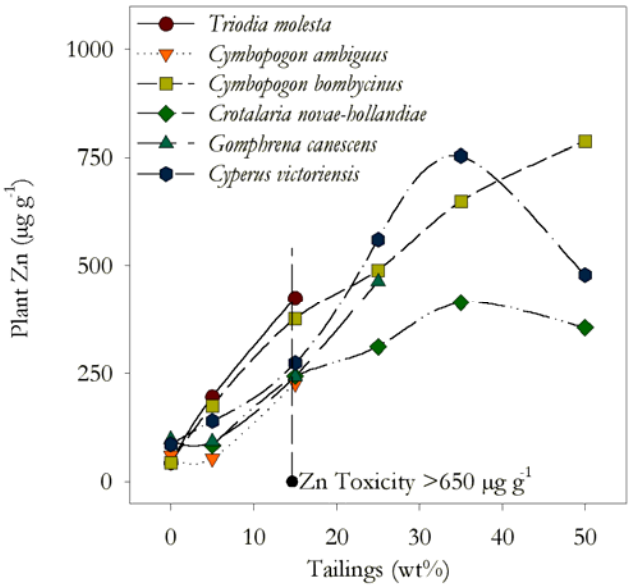

Figure 3-6. Average (N=4) plant concentrations of Zn (μg g^{-1}) in selected subtropical pasture plant species cultivated on soil contaminated with mine tailings (ST mixtures). The total Zn concentration deemed to be potentially phytotoxic (>650 μg g^{-1}) is also shown.

3.3.4. Plant growth on limestone amended soil-tailings mixtures

The three pasture plant species (*Cymbopogon ambiguus*, *Cymbopogon bombycinus*, and *Crotalaria novae-hollandiae*) that had the highest biomass production values when grown on ST mixtures were cultivated on STL substrates containing 10 wt% and 20 wt% limestone. Biomass production of the plants decreased with increasing concentrations of tailings in the STL mixtures for both levels of limestone addition (Figure 3.7) (Appendix C). The growth of *Cymbopogon*

ambiguus was relatively unaffected by the addition of 10 wt% and 20 wt% limestone to the ST mixtures. Both limestone amendments improved substrate tolerance in *Cymbopogon ambiguus* from 15 wt% tailings (1818 µg Pb g^{-1}) to 50 wt% mine tailings (5990 µg Pb g^{-1}).

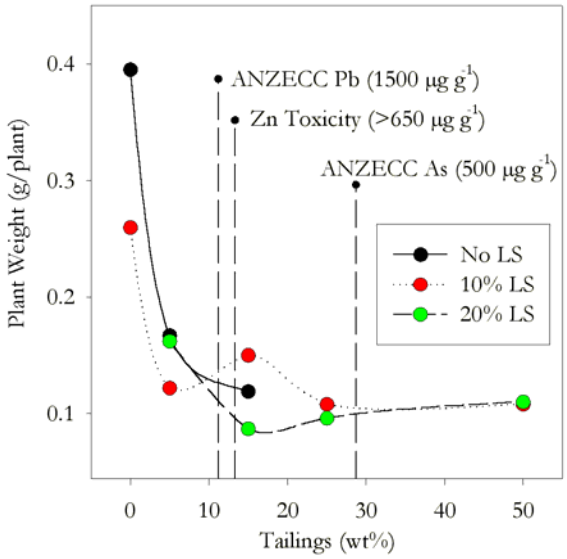

Figure 3-7. Average (N=4) biomass production (g $plant^{-1}$) of *Cymbopogon ambiguus* cultivated on a range of soil contaminated with mine tailings and amended with 0 wt%, 10 wt% and 20 wt% limestone (LS) (STL mixtures). The ANZECC Investigation Limits for Pb (1500 µg g^{-1}) and As (500 µg g^{-1}) contaminated industrial sites and the Zn concentration deemed to be potentially phytotoxic to the plants (>650 µg g^{-1}) are included (ANZECC, 1999).

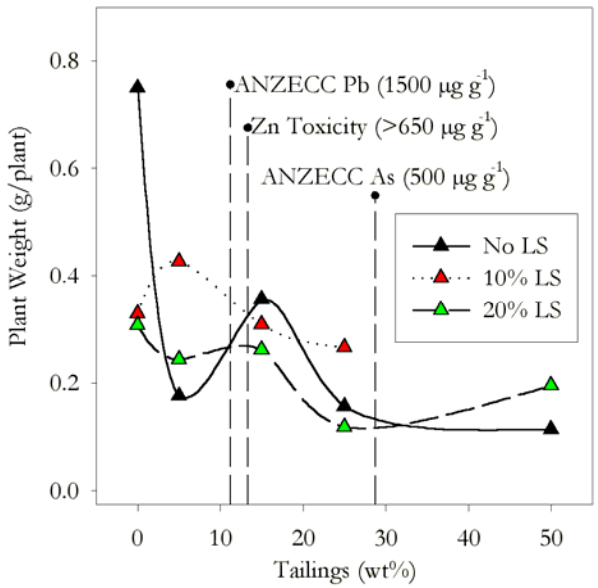

Figure 3-8. Average (N=4) biomass production (g plant-1) of *Cymbopogon bombycinus* cultivated on a range of soil contaminated with mine tailings and amended with 0 wt%, 10 wt% and 20 wt% limestone (LS) (STL mixtures). The ANZECC Investigation Limits for Pb (1500 µg g^{-1}) and As (500 µg g^{-1}) contaminated industrial sites and the Zn concentration deemed to be potentially phytotoxic to the plants (>650 µg g^{-1}) are included (ANZECC, 1999).

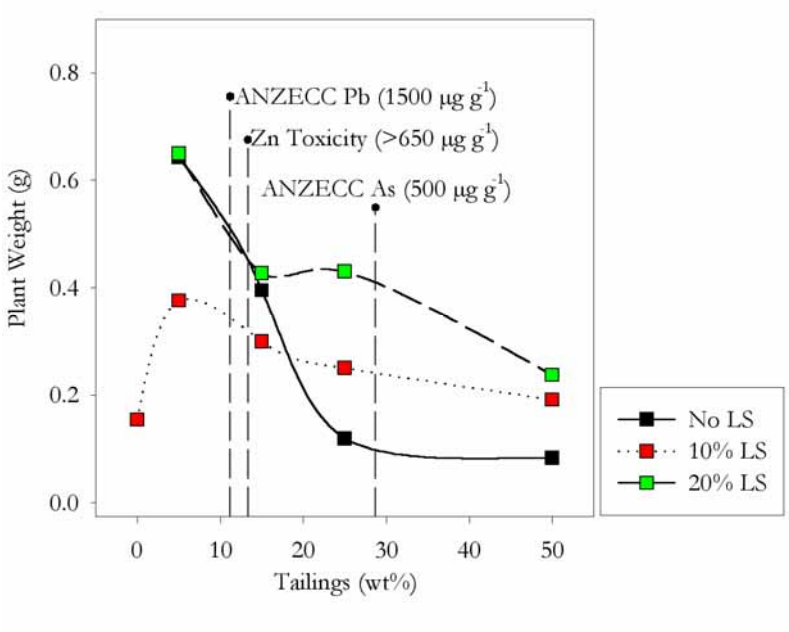

Figure 3-9. Average (N=4) biomass production (g plant-1) of *Crotalaria novae-hollandiae* cultivated on a range of soil contaminated with mine tailings and amended with 0 wt%, 10 wt% and 20 wt% limestone (LS) (STL mixtures). The ANZECC Investigation Limits for Pb (1500 µg g^{-1}) and As (500 µg g^{-1}) contaminated industrial sites and the Zn concentration deemed to be potentially phytotoxic to the plants (>650 µg g^{-1}) are included (ANZECC, 1999).

The growth of *Cymbopogon bombycinus*, by comparison, was reduced at low tailings concentrations (<15 wt%) for both limestone amendments. The application of 10 wt% limestone to ST mixtures containing >15 wt% mine tailings improved the biomass production of *Cymbopogon bombycinus* by approximately 100%, compared to ST mixtures containing 20 wt% limestone. The average weight of *Crotalaria novae-hollandiae* increased with increasing proportions of limestone in STL mixtures containing >15 wt% mine tailings. The average plant weight of *Crotalaria novae-hollandiae* increased by 100% for substrates containing 10 wt% limestone and by 320% for substrates

containing 20 wt% limestone compared to unamended ST mixtures.

3.3.5. Plant uptake of metals and arsenic from limestone amended soil-tailings mixtures

The addition of both quantities of limestone (10 wt% and 20 wt%) to the ST mixtures caused a general 10-fold decrease in the uptake of Ag, As, Cd, Pb and Zn by *Cymbopogon ambiguus*, *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae* compared to ST mixtures that did not receive limestone amendments. Both limestone amendments resulted in relatively constant concentrations of Ag (0.003 μg g^{-1}) and Zn (1 μg g^{-1}) in the leaves of *Cymbopogon ambiguus* over the range of tailings proportions in the STL mixtures (Figures 3.8 and 3.9). Arsenic, Cd and Pb concentrations in *Cymbopogon ambiguus* decreased with increasing proportions of limestone in STL mixtures containing >15 wt% tailings (Figures 3.10, 3.11 and 3.12). Silver, As, Cd and Zn concentrations in *Cymbopogon bombycinus* decreased with increasing proportions of limestone in STL mixtures containing low proportions of mine tailings (≤5 wt%). However, with higher proportions of mine tailings in the STL mixtures (≥5 wt%) *Cymbopogon bombycinus* accumulated slightly higher concentration of Ag. The average concentrations of Pb in *Cymbopogon bombycinus* (≈0.9 μg g^{-1}) were approximately equivalent for both levels of limestone addition over the range of tailings concentrations investigated. By comparison, *Crotalaria novae-hollandiae* accumulated a higher average concentration of Ag (0.02 μg g^{-1}), As (4.2 μg g^{-1}), Cd (0.2 μg g^{-1}), Pb (1.4 μg g^{-1}) and Zn (29.0 μg g^{-1}) from ST mixtures amended with 20 wt%

limestone compared to ST mixtures receiving a 10 wt% limestone amendment (0.01 μg Ag g^{-1}, 1.9 μg As g^{-1}, 0.08 μg Cd g^{-1}, 0.6 μg Pb g^{-1} and 12.2 μg Zn g^{-1}).

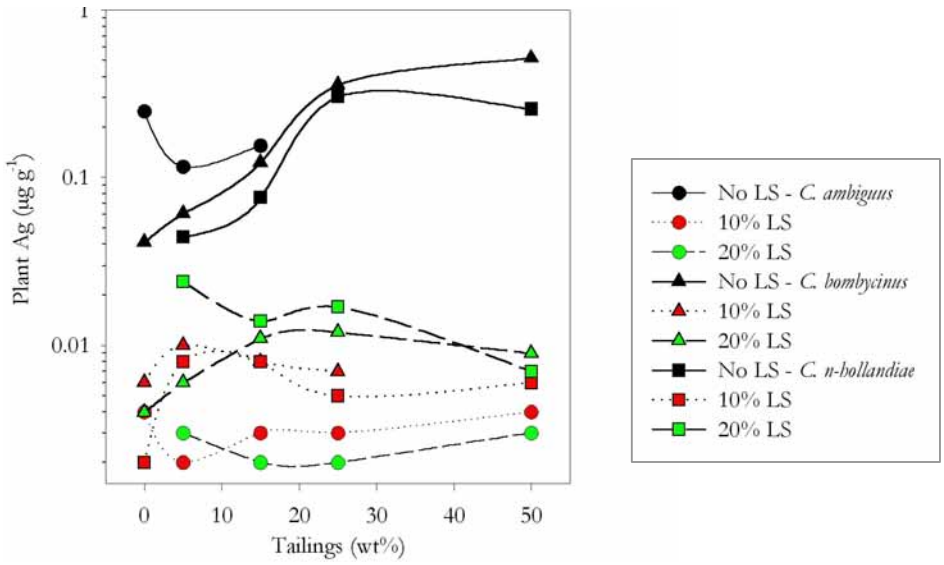

Figure 3-10. Average (N=4) plant concentrations of Ag (μg g^{-1}) in three pasture plant species cultivated on soil contaminated with mine tailings and amended with 0 wt%, 10 wt% and 20 wt% limestone (LS) (STL mixtures).

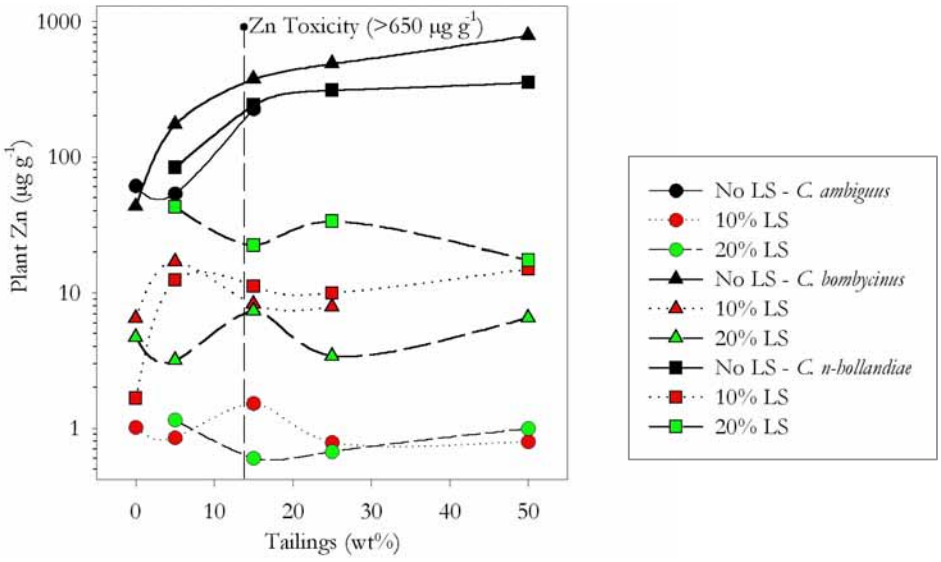

Figure 3-11. Average (N=4) plant concentrations of Zn (µg g^{-1}) in three pasture plant species cultivated on soil contaminated with mine tailings and amended with 0 wt%, 10 wt% and 20 wt% limestone (LS) (STL mixtures). The total Zn concentration deemed to be potentially phytotoxic to the plants (>650 µg g^{-1}) is included

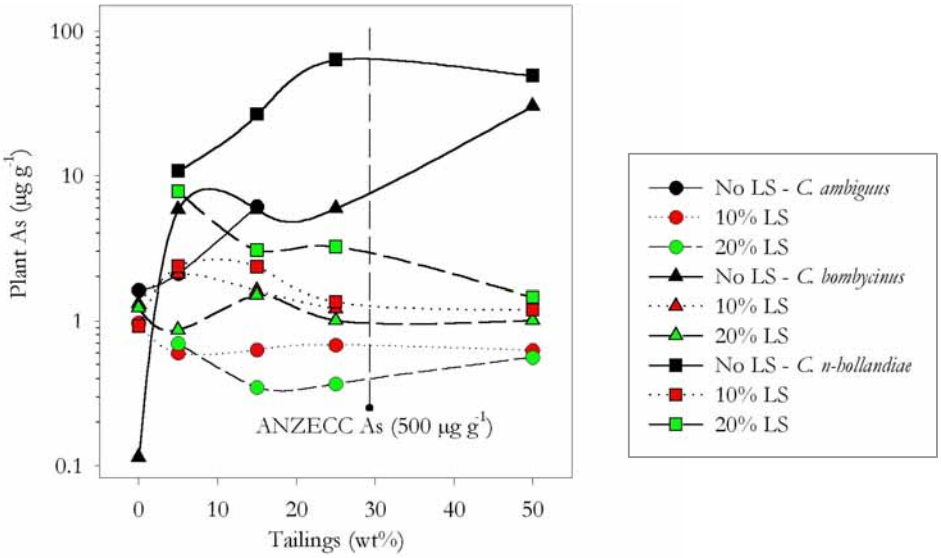

Figure 3-12. Average (N=4) plant concentrations of As (µg g^{-1}) in three pasture plant species cultivated on soil contaminated with mine tailings and amended with 0 wt%, 10 wt% and 20 wt% limestone (LS) (STL mixtures). The ANZECC Investigation Limit for As contaminated industrial sites (500 µg g^{-1}) is included (ANZECC, 1999).

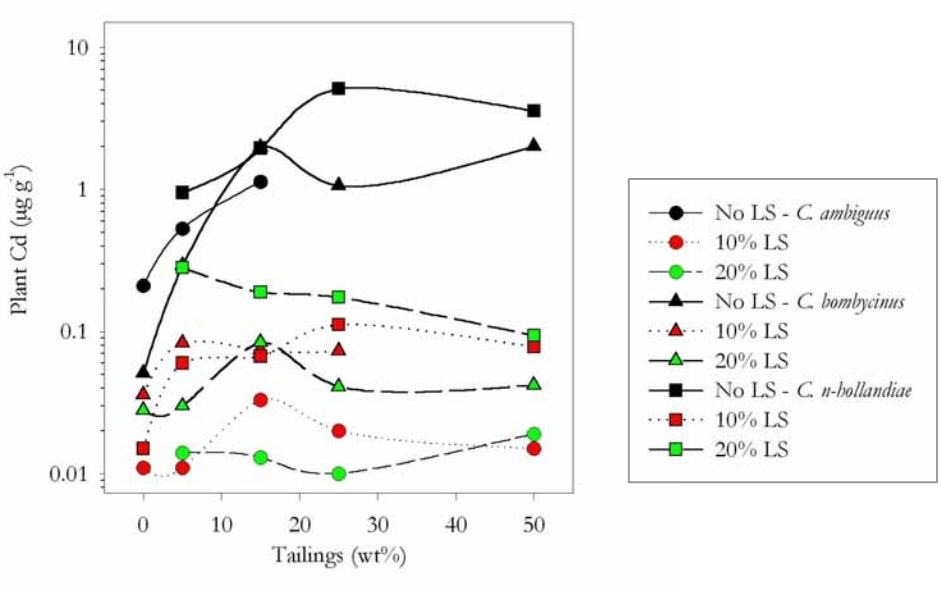

Figure 3-13. Average (N=4) plant concentrations of Cd (µg g^{-1}) in three pasture plant species cultivated on soil contaminated with mine tailings and amended with 0 wt%, 10 wt% and 20 wt% limestone (LS) (STL mixtures).

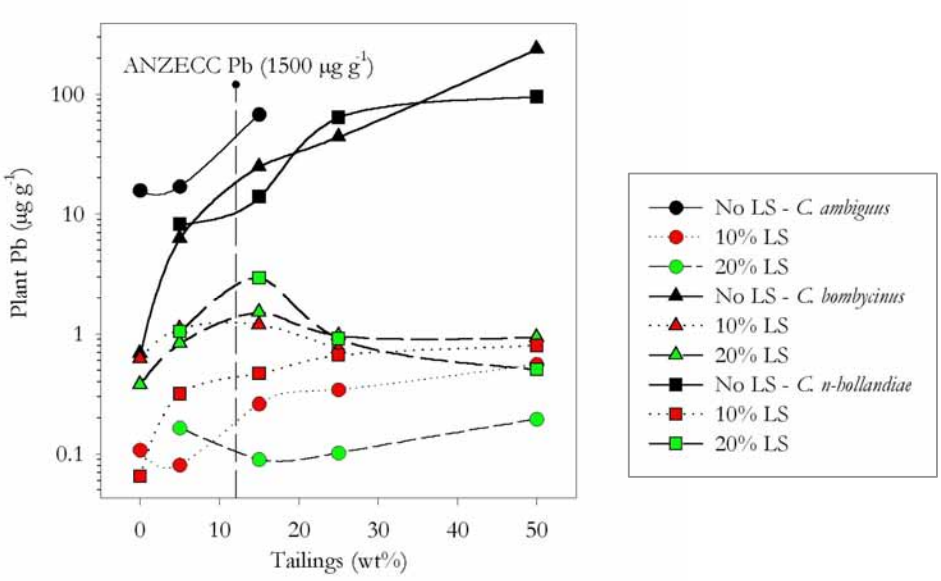

Figure 3-14. Average (N=4) plant concentrations of Pb (µg g^{-1}) in three pasture plant species cultivated on soil contaminated with mine tailings and amended with 0 wt%, 10 wt% and 20 wt% limestone (LS) (STL mixtures). The ANZECC Investigation Limit for Pb contaminated industrial sites (1500 µg g^{-1}) is included (ANZECC, 1999).

3.4. Discussion

3.4.1. Plant tolerance and biomass production

All plant species tolerated total Pb and As concentrations in plant growth medium (0 wt%, 10 wt% and 20 wt% limestone amendments) that exceeded the ANZECC Investigation Limits for Pb (1500 μg g^{-1}) and As (500 μg g^{-1}) contaminated industrial sites (ANZECC, 1999). *Cymbopogon ambiguus* and *Triodia molesta* survived on ST mixtures containing ≤15 wt% mine tailings indicating As, Pb and Zn tolerances in soil of ≤261 μg As g^{-1}, ≤1818 μg Pb g^{-1} and ≤582 μg Zn g^{-1} respectively. *Gomphrena canescens* survived on ST mixtures containing ≤25 wt% tailings indicating As, Pb and Zn tolerances in soil of ≤515 μg As g^{-1}, ≤3010 μg Pb g^{-1} and ≤1091 μg Zn g^{-1} respectively. In contrast, *Cymbopogon bombycinus*, *Crotalaria novae-hollandiae* and *Cyperus victoriensis* survived on all soils contaminated with mine tailings indicating As, Pb and Zn tolerances in soil of ≤1023 μg As g^{-1}, ≤5990 μg Pb g^{-1} and ≤2111 μg Zn g^{-1} respectively. All plant species tested are therefore considered suitable for the revegetation of soil contaminated with base-metal mine tailings that exceed the ANZECC Investigation Limits for As and Pb contamination. In addition, *Cymbopogon bombycinus*, *Crotalaria novae-hollandiae*, *Cyperus victoriensis* and *Gomphrena canescens* tolerated total Zn concentrations in soil considered to be toxic to common agricultural crop species (>650 μg Zn g^{-1}).

Limestone amendments of 20 wt% to ST mixtures containing <25 wt% tailings reduced the biomass production of *Cymbopogon ambiguus* and

Cymbopogon bombycinus by 11% and 56% respectively, compared to ST mixtures containing 10 wt% limestone. The addition of 10 wt% and 20 wt% amendments of limestone to ST mixtures containing >25 wt% mine tailings (equivalent to 3010 μg Pb g^{-1}) enabled *Cymbopogon ambiguus* to survive on soil containing an otherwise toxic concentration of mine tailings. Biomass production for *Cymbopogon ambiguus* grown on substrates containing >25 wt% tailings were relatively constant for both the ST and STL mixtures. By comparison, plant weights for *Cymbopogon bombycinus* decreased with increasing proportions of limestone in STL mixtures containing >25 wt% tailings. Limestone amendments improved the biomass production of *Crotalaria novae-hollandiae* (100% and 320%, respectively) compared to plant growth on ST mixtures not receiving limestone amendments. The data indicate that a 10% (wt/wt) amendment of limestone to ST mixtures containing tailings proportions that exceed the ANZECC Investigation Limits for Pb and As contamination (equivalent to ≥15 wt% mine tailings) would be sufficient to counteract tailings induced toxicity in *Cymbopogon ambiguus*. Limestone amendments to ST mixtures containing 25 wt% and 50 wt% tailings (3010 and 5990 μg Pb g^{-1} respectively) had little effect on the biomass production of *Cymbopogon ambiguus* and *Cymbopogon bombycinus* compared to unamended ST mixtures. In contrast, the biomass production of *Crotalaria novae-hollandiae* greatly improved with the addition of limestone (10 wt% and 20 wt%) to ST mixtures containing <50 wt% tailings (<5990 μg Pb g^{-1}).

3.4.2. Plant uptake of metals and arsenic from ST and STL mixtures

The accumulation of Ag, As, Cd, Pb and Zn in aboveground tissues of all plant species generally increased with increasing tailings proportions in the ST mixtures. *Triodia molesta* indicated a high potential to accumulate Ag, As, Cd, Pb and Zn from ST mixtures containing a low proportion (≤15 wt%) of mine tailings (equivalent to 1818 μg Pb g^{-1}). *Cyperus victoriensis*, *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae* accumulated high concentrations of Ag, As, Pb and Zn from ST mixtures containing 15 wt%-35 wt% mine tailings (equivalent to 1818-4202 μg Pb g^{-1}). The uptake of Cd, Pb and Zn by *Cyperus victoriensis* then decreased for substrates containing >35 wt% mine tailings in the ST mixtures. The uptake of Ag and Cd by *Crotalaria novae-hollandiae* also decreased for ST mixtures containing <25 wt% tailings (equivalent to 3010 μg Pb g^{-1}). In contrast, the accumulation of As and Zn in *Crotalaria novae-hollandiae* was reduced for ST mixtures containing <35 wt% mine tailings (equivalent to 4202 μg Pb g^{-1}). Reduced biomass production and a decrease in the uptake of As and Zn by *Crotalaria novae-hollandiae* cultivated on ST mixtures containing >25-35 wt% tailings may indicate the occurrence of tailings-induced element deficiencies in the plant (e.g. reduced Fe uptake resulting from elevated Zn concentrations in soils). Cadmium and Zn accumulation in *Gomphrena canescens* grown on the ST mixtures was also high (14.1 and 463 μg g^{-1} respectively) and notably from ST mixtures containing only 25 wt% mine tailings (equivalent to 3010 μg Pb g^{-1}).

The uptake of Ag, As, Cd, Pb and Zn from the ST mixtures by *Cymbopogon ambiguus*, *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae* decreased significantly with the addition of 10 wt% limestone. Arsenic, Cd, Pb and Zn uptake by *Cymbopogon ambiguus* and *Cymbopogon bombycinus* decreased further by a small amount when 20 wt% limestone was added to the ST mixtures. The reduction in heavy metal uptake by these species resulting from the addition of limestone indicates that limestone amendments can reduce the bioavailability of heavy metals in soil. The geochemical behaviour of Pb indicates that sufficient quantities of phosphate will reduce Pb solubility, and possibly bioavailability, in soil (Nriagu, 1974; Ruby *et al.*, 1994). Bernal *et al.* (Bernal *et al.*, 2002) attributed a reduction in the concentration of heavy metals in the biomass of *Brassica juncea* following the application of limestone to an increase of soil pH and the precipitation of heavy metals with inorganic anions such as phosphate, sulphate, carbonate and chloride. The alkalinity of ST mixtures used in this study that received limestone amendments increased from approximately pH 7.5 to approximately pH 8.5 indicating that pH dependant metal solubility in the substrates was responsible for reduced metal uptake.

The maximum concentration of Pb accumulated by *Gomphrena canescens* (46 μg Pb g^{-1}) from the ST mixtures compares favourably with Pb concentrations found in native stands of the same species by Cole *et al.* (1968). However, the level of Zn accumulation by *Gomphrena canescens* (max. 463 μg Zn g^{-1}) is significantly lower by comparison (1500-2220 μg g^{-1}). In addition, the

concentrations of Zn accumulated by *Crotalaria novae-hollandiae* (max. 417.7 μg Zn g^{-1}) from the same ST mixture did not compare favourably with foliar Zn concentrations reported for native stands of *Crotalaria novae-hollandiae* (8975 μg Zn g^{-1}) (Farago *et al.*, 1977). Sample preparation described by Cole *et al.* (1968) and Farago *et al.* (1977) indicate that plant materials were not washed prior to analysis suggesting that metallic contaminants (e.g. metallic dust) may have been present on the vegetation samples. Whereas, the preparation of samples for this research project involved the double rinsing of vegetation prior to thermal reduction, acid digestion and analysis by ICP MS (Chapter 2, Section 2.11.1) indicating that sample contamination may have produced spurious results. Physiological variation between the species collected for this study and those reported by Farago *et al.* (1977) and Cole *et al.* (1968) may also be responsible for the lack in correlation between the studies. Furthermore, the addition of heavy metals and metalloids contained in the mine tailings to the soil may have resulted in elemental interference of natural Pb and Zn uptake by *Crotalaria novae-hollandiae* and *Gomphrena canescens*. In addition, the geochemistry and microbial content of soil sampled by (Bulman-Waimuna Springs) and by Farago *et al.* (1977) (Dugald River lode) under native stands of *Gomphrena canescens* and *Crotalaria novae-hollandiae* are likely to have been different to the ST mixtures used in this study, which may have produced the variations in Zn uptake by these species.

3.4.3. A note on the classification of Crotalaria novae-hollandiae

Reports by Farago *et al.*, (1977) and Catt (2000) do not specify which of the

subspecies of *Crotalaria novae-hollandiae* were collected from the Dugald River lode, Western Queensland, as described by Lee (1978). The subspecies classification for seed of *Crotalaria novae-hollandiae* supplied by Kimseed Ltd. (WA) could not be provided. The distribution of *Crotalaria novae-hollandiae* subspecies in Australia (Lee, 1978) indicates that Western Australia is dominated by *Crotalaria novae-hollandiae* subsp. *lasiophylla* Benth.. In contrast, specimens collected from the Dugald River lode could be one of three eastern subspecies of *Crotalaria novae-hollandiae* (*C. crassipes* Hook., *Crotalaria novae-hollandiae* f. *paviflora* Benth., or *Crotalaria novae-hollandiae* f. *lasiophylla* Benth.). It is therefore likely that the seed supplied by Kimseed Ltd was *Crotalaria novae-hollandiae* subsp. *lasiophylla*. This omission in reporting the subspecies of *Crotalaria novae-hollandiae* with respect to Zn accumulation in the species requires clarification in the literature.

3.4.4. Species selection for revegetation

The selection of plant species to revegetate a soil contaminated with mine tailings must be based upon the healthy growth of plants and their continued survival on the contaminated soil. In addition, the plants selected should not accumulate concentrations of metals and metalloids in aboveground plant tissues that might pose a risk to native fauna and livestock. In general, the biomass production of all plant species was reduced when grown on soils containing progressively higher proportions of mine tailings. However, all plants survived on soil containing 15 wt% tailings (equivalent to 1818 μg Pb g^{-1}) and would be suitable for the revegetation of soils exceeding the

ANZECC Investigation Limits for Pb contaminated industrial and commercial sites (1500 μg g^{-1}). Of the plant species investigated, *Triodia molesta*, *Crotalaria novae-hollandiae*, and *Cyperus victoriensis* indicated the potential to accumulate high concentrations of As, Cd and Pb from soil containing ≤15 wt% tailings suggesting that these species should be avoided in situations where the vegetation may pose a risk to local stock and wildlife.

3.5. Conclusions

This study determined that the plant species investigated (*Triodia molesta*, *Cyperus victoriensis*, *Cymbopogon ambiguus*, *Cymbopogon bombycinus*, *Crotalaria novae-hollandiae* and *Gomphrena canescens*) were tolerant to total As and Pb concentrations in plant growth media that exceed the ANZECC Investigation Limits for industrial and commercial sites (ANZECC, 1999). All species exhibited typical exclusion behaviour for the uptake of non-essential elements (Baker, 1981). However, as the tailings concentration of the substrate increased, so too did the concentrations of metals and arsenic in the plants. The application of limestone (10 wt. %) was found to effectively inhibit the accumulation of all elements (Ag, As, Cd, Pb and Zn) in *Cymbopogon ambiguus*, *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae* from soil contaminated with mine tailings. Limestone amendments also promoted the growth of *Cymbopogon ambiguus* on otherwise phytotoxic levels of mine tailings in the contaminated soils. Revegetation of soils containing modest levels of base metal mine tailings (e.g. ≤3000 μg Pb g^{-1}) would best be carried out using a variety of the plant species investigated and a small quantity of

limestone (e.g. 10 wt. %). Highly contaminated soil (e.g. ≥3000 μg Pb g^{-1}), on the other hand, could be revegetated using *Cymbopogon ambiguus* and *Cymbopogon bombycinus* following the application of a greater mass of limestone (e.g. >10 wt. %).

Chapter 4. The Uptake of Metals and Metalloids by Rhodes grass (*Chloris gayana* Kunth cv. 'Pioneer') Grown on Mine Tailings and Soil Contaminated with Mine Tailings

4.1. Introduction

Pasture-based systems, using exotic tussock-forming grasses such as Buffel grass (*Cenchrus ciliaris*), Vetiver grass (*Vetiveria zizaniodes*) and Rhodes grass (*Chloris gayana* Kunth cv. 'Pioneer') are commonly used in mined land revegetation programs in Australia (Truong, 1998; Menzies and Mulligan, 2000). The coal industry utilises pasture-based systems for the revegetation of non-hazardous over-burden and inter-burden waste materials in addition to hazardous mine wastes that have been capped with topsoil (Truong, 1998; Harwood *et al.*, 1999). Pasture-based systems are also becoming increasingly important for the sustainable revegetation of capping materials used to isolate metalliferous mine wastes, particularly in arid and semi-arid regions of Australia (BHPBilliton, 2002; O'Kane and Waters, 2003). Vetiver grass, used to directly revegetate metalliferous coal mine spoils, exhibits high tolerances to, and low accumulation potential for, the heavy metals and metalloids contained in the waste (Truong, 2000). In most situations, revegetated sites have been returned to pastoral land use (Grigg *et al.*, 2000) because Buffel grass and Vetiver grass are comparatively high in nutritional value for stock (MacKenzie *et al.*, 1982).

Rhodes grass - another hardy exotic pasture species in tropical and subtropical environments ('t Mannetje and Kersten, 1992) - is less palatable to livestock than Vetiver grass (Graham and Lambert, 1996). However, the tolerance of Rhodes grass to soils of poor nutrient status, high salinity or low pH, in addition to an extensive root system, has been exploited for slope stabilisation and erosion control in a number of industrial and urban environments (Meecham and Bell, 1977; Naidu and Harwood, 1997; Carroll *et al.*, 2000; Grigg *et al.*, 2000). Furthermore, Rhodes grass has been used to revegetate a multi-layer capping system over metalliferous mine wastes at the Rum Jungle U-Cu mine, but with limited success (Menzies and Mulligan, 2000). After several years, die-back of the vegetation cover resulted from high concentrations of Cu (4540 μM) in the soil solution and acidification in the root zone (pH 3.6). This was attributed to compaction and failure of the capillary break layer within the capping system itself which allowed contaminants contained in the underlaying pyritic mine waste to migrate upwards. To date, however, little research has been conducted on the use of Rhodes grass to directly revegetate metalliferous soils and mine waste materials.

Northern Australia hosts an array of metallic mining operations, exploiting predominantly sulphidic base-metal mineralisations. Many mining tenements are surrounded by pastoral leases and, following mine closure, are required to support some form of agricultural land use capability. Excavated non-hazardous mine waste is usually revegetated directly or after a thin topsoil

layer of plant growth media has been laid down. Hazardous mine wastes are usually isolated from the environment in large tailings dam repositories, which are later capped at considerable expense with various geologic and synthetic materials before being revegetated. The extensive root systems of some vegetation cover systems may penetrate these capping materials thus exposing plants to the underlaying wastes contained metals and metalloids (French, 2001; Gilfedder, 2004). This may lead to accumulation of heavy metals and metalloids in above ground tissues and subsequent contamination of the food chain (Nicholas and Egan, 1975), in addition to their upward migration into the capping materials. This study was conducted to determine two critical aspects of the use of Rhodes grass for metalliferous mined land rehabilitation in northern Australia. Firstly, the study sought to determine the species' heavy metal tolerance and biomass production potential when grown on soils contaminated with varying levels of base metal mine tailings. In addition, the study determined the natural levels of Ag, As, Cd, Pb and Zn accumulation by Rhodes grass grown on these substrates to consider potential fodder toxicity risks to livestock when grazing on such capped mine waste repositories.

4.2. Materials and methods

This pot trial experiment (Figure 4.1) involved the cultivation of Rhodes grass in uncontaminated soil, undiluted mine tailings, and a range of weight percent mixtures of soil and mine tailings (5 wt%, 15 wt%, 25 wt%, 35 wt% and 50 wt%) over a 12-week period (Chapter 2, Section 2.11.2 and Section

2.11.3). Plant samples were collected (Chapter 2, Section 2.12.1) and analysed (Chapter 2, Section 2.14) to determine rates of biomass production and the uptake of Ag, As, Cd, Pb and Zn by Rhodes grass from the soil-tailings mixtures (Appendix B). Post-harvest soil samples were collected and analysed to determine the pH (Chapter 2, Section 2.11.4) of the substrates resulting from the addition of mine tailings and plant cultivation. A sequential extraction was also conducted on plant growth media containing 25 wt% mine tailings (Chapter 2, Section 2.11.7) to determine the distribution and plant availability of metals and As contained in the substrate (Appendix D).

Figure 4-1. The growth of Rhodes grass (*Chloris gayana* Kunth cv. 'Pioneer') after three months of cultivation on a range of soil contaminated with mine tailings (5T = 5 wt% Cannington mine tailings + 95 wt% anthroposol soil).

4.3. Results

4.3.1. Substrate characterisation

Mineralogical analysis of the materials used to prepare the tailings contaminated plant growth media (Appendix A) indicated the soil component was an anthroposol soil (<35% clay). The pH range of the soil-tailings mixtures was slightly alkaline (Figure 4.2) and the low proportion of acid-generating sulphides in the tailings caused the pH of the soil-tailings mixtures to decline by just 0.6 units over the experimental range. Substrates containing ≥15 wt% tailings exceeded the Australian and New Zealand Environment and Conservation Council (ANZECC) Investigation Limit for Pb contamination (1500 μg g^{-1}) of industrial and commercial sites (ANZECC, 1999). Substrates containing ≥25 wt% tailings also exceeded the As Investigation Limit (500 μg g^{-1}), in addition to having Zn concentrations (>650 μg g^{-1}) that would be toxic to common agricultural crop species (Glendinning, 2000).

4.3.2. Plant growth

The growth of Rhodes grass on uncontaminated anthroposol soil was significantly lower than plant growth on soil-tailings mixtures containing 5 wt% to 25 wt% tailings (276 to 3010 μg Pb g^{-1}). Plants grown on soil-tailings mixtures containing 5 wt% to 25 wt% tailings did not exhibit toxicity symptoms (such as leaf tip necrosis, noticeably pale or spotted foliage) after three months of plant growth. In contrast, plants grown on soils containing

>25 wt% tailings (>3010 μg Pb g^{-1}) indicated increasing levels of stunting and leaf tip necrosis, in addition to a reduction in the number of leaves per plant. Exploratory root growth was vigorous from pots containing between 5 wt% and 35 wt% tailings (276 to 4202 μg Pb g^{-1}). A reduction in root mass was also noted for substrates containing ≥50% tailings (5990-11950 μg Pb g^{-1}).

The biomass production (g $plant^{-1}$) of Rhodes grass grown on uncontaminated soil was very low (0.1 ± 0.01 g $plant^{-1}$) over the 12-week growth period (Figure 4.2). The addition of 5 wt% and 15 wt% mine tailings to soil (626 and 1818 μg Pb g^{-1}, respectively) significantly increased the biomass production of Rhodes grass (3.2 ± 1.6 and 3.9 ± 1.1 g $plant^{-1}$, respectively) compared to uncontaminated soil. The biomass production of Rhodes grass was reduced by approximately 60% (1.4 ± 0.3 g $plant^{-1}$) for soil-tailings mixtures containing 25 wt% tailings, compared to the weight of plant grown on the 5 wt% ST mixture. Substrates containing 35 wt% tailings reduced the biomass production of Rhodes grass by 75% (0.9 ± 0.7 g $plant^{-1}$) and those containing 50 wt% tailings reduced the biomass production by a further 20% (0.2 ± 0.1 g $plant^{-1}$), compared to the 5 wt% ST mixture.

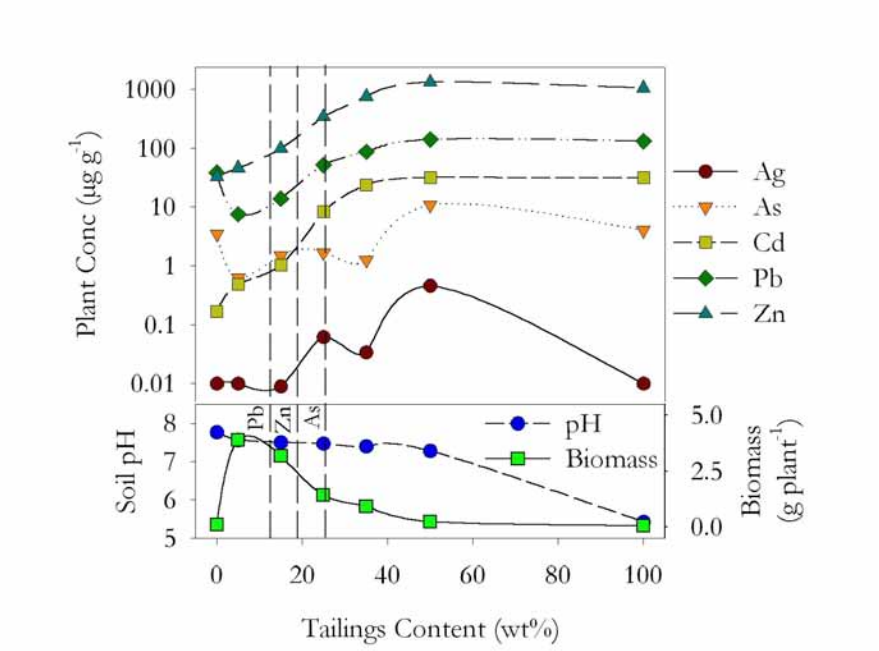

Figure 4-2. Average (N=4) concentrations of Ag, As, Cd, Pb and Zn (µg g^{-1}) and biomass production (g $plant^{-1}$) for *Chloris gayana* grown on uncontaminated soil, undiluted mine tailings and a range of soils contaminated with mine tailings (ST mixtures). The figure includes (a) ANZECC Investigation Limits for Pb (1500 µg g^{-1}) and As (500 µg g^{-1}) contaminated industrial sites (ANZECC, 1999), (b) total concentration of Zn deemed to be potentially to be phytotoxic (650 µg g^{-1}) to the plants and (c) the pH of the various plant growth media.

4.3.3. Sequential extraction

The growth response of plants to elevated concentrations of non-essential metals in soils can be characterised as either tolerance or toxicity (Reichman, 2000). The definition of a critical threshold concentration of heavy metals in soils that cause significant growth decreases are a relatively simple means of estimating a substrate's toxicity to plants (Davies *et al.*, 1993). A 50% decrease in biomass production was reported by Zhang *et al.* (1998) as a useful estimate of Zn toxicity in soil. Consequently, a sequential extraction of the 25

wt% ST mixture was conducted to determine the distribution of metals and arsenic at this threshold concentration of tailings (Chapter 2, Section 2.11.7).

Very low proportions (0.1%) of all the elements were extracted from the 25 wt% ST mixture in a water-soluble form (Table 4.1). Further sequential extractions of this substrate indicated that Ag and As were predominantly contained in the sulphide (87.3% and 64.0%, respectively) and silicate fractions (7.5% and 22.9%, respectively). Silver was also present in small proportion in the oxide extractant (4.9%), while small proportions of As were extracted as oxide (7.6%), carbonate (4.6%) and ion exchangeable (0.8%) fractions. High proportions of Cd were extracted in ion exchangeable (30.1%), sulphide (33.9) and silicate (22.5%) fractions. In addition, smaller quantities of Cd were dissolved by the carbonate (11.5%) and oxide (1.9%) extractants. Lead extraction indicated that the carbonate (45.5%) fraction contained the majority of the metal along with broadly similar quantities of Cd extracted as oxide (16.6%), sulphide (11.7%) and ion exchangeable (8.0%) fractions. By comparison, Zn extractability indicated that the sulphide (54.7%) and silicate (22.0%) fraction contained the majority of the metal. Zinc was also present in high proportion in the carbonate fraction (14.6%) in addition to smaller amounts extracted as oxide (5.4%) and ion exchangeable (3.3%) forms.

Table 4.1. The distribution of sequentially extracted elemental fractions (% of total conc.) in the 25 wt% ST mixture (Appendix D).

Extract.	Element Extractability (% of total element conc.)				
	Ag	As	Cd	Pb	Zn
Water Soluble	0.1%	0.1%	0.1%	0.1%	0.1%
Ion Exchangeable	0.1%	0.8%	30.1%	8.0%	3.3%
Carbonate	0.1%	4.6%	11.5%	45.5%	14.6%
Oxide	4.9%	7.6%	1.9%	16.6%	5.4%
Sulphide	43.6%	32.0%	17.0%	5.8%	27.3%
Residual/Silicate	51.1%	54.9%	39.4%	23.9%	49.3%
Total Conc. ($\mu g\ g^{-1}$)	15	515	8	3010	1091

Table 4.2. Water-soluble (dH_2O) and ammonium-acetate-extractable (1 M NH_4OAc) Ag, As, Cd, Pb and Zn ($\mu g\ g^{-1}$) concentrations that were used to estimate total and proportional plant availability of metals and arsenic in the 25 wt% ST mixture.

Parameter.	Extract Concentration ($\mu g\ g^{-1}$)				
	Ag	As	Cd	Pb	Zn
Deionised water	0.016	0.311	0.008	1.980	0.947
1 M NH_4OAc (pH 7)	0.019	4.360	2.405	241.5	36.20
Plant Available ($\mu g\ g^{-1}$)	0.035	4.671	2.413	243.480	37.147
Plant Available (%)	0.2%	0.9%	30.2%	8.1%	3.4%

Heavy metals and As dissolved by the first two sequential extraction steps (water soluble, ion exchangeable) may be considered the more plant-available elemental fractions (cf. Gatehouse *et al.*, 1977; Almås *et al.*, 1999) (Table 4.2). Silver, As and Cd extracted from the substrate using deionised water and ammonium acetate was low (0.04 $\mu g\ g^{-1}$, 4.7 $\mu g\ g^{-1}$ and 2.4 $\mu g\ g^{-1}$, respectively). In contrast, the concentration of Pb and Zn extracted by these same solutions was significantly higher (243.5 μg Pb g^{-1} and 37.1 μg Zn g^{-1}). On a proportional basis, significantly higher concentrations of Cd (30.2%) were extracted in a plant available form from the substrate compared to Pb

(8.1%), Zn (3.4%), As (0.9%) and Ag (0.2%).

4.3.4. Phytextraction of As, Cd, Pb, and Zn by Rhodes grass

Compared to ST mixtures containing 5 wt% tailings, Rhodes grass grown on uncontaminated soil accumulated relatively high concentrations of As and Pb (3.5 μg g^{-1} and 38.5 μg g^{-1}, respectively) (Figure 4.2). For substrates containing mine tailings, the maximum accumulations of Ag (0.5 ± 0.2 μg g^{-1}), As (10.8 ± 7.6 μg g^{-1}), Cd (31.7 ± 6.0 μg g^{-1}), Pb (142 ± 63 μg g^{-1}) and Zn (1329 ± 700 μg g^{-1}) in Rhodes grass occurred on substrates containing 50 wt% mine tailings (equivalent to 5990 μg Pb g^{-1}) (Appendix B). Silver uptake increased with increasing proportions of tailings for soil-tailings mixtures containing <50 wt% tailings and then decreased significantly above this level of tailings content in the substrate. Arsenic uptake was relatively constant (≈1.4 μg g^{-1}) for substrates containing between 5 wt% and 35 wt% mine tailings. For substrates containing >50 wt% mine tailings, the uptake of As by Rhodes grass decreased to 4.0 μg g^{-1}. In contrast, Cd, Pb and Zn uptake by Rhodes grass increased with increasing proportions of tailings in the substrate between 5 wt% and 50 wt% tailings, and remained relatively constant thereafter (31.4 ± 16.0 μg Cd g^{-1}, 131 ± 81 μg Pb g^{-1} and 1051 ± 809 μg Zn g^{-1}) (Figure 4.2).

4.4. Discussion

4.4.1. Plant growth

Reduced plant growth on uncontaminated anthroposol soil indicated that factors other than soil contamination by mine tailings may negatively influence plant growth. High proportions of kaolin (6 vol%), expanding clays (6 vol%), muscovite (3 vol%) and illite (3 vol%), a slightly alkaline pH (7.7), and relatively low P (270 μg g^{-1}) and K (612 μg g^{-1}) concentrations indicated the uncontaminated soil had a low cation exchange capacity and was nutrient deficient (Appendix A). In addition, pots containing uncontaminated soil were noted to retain a high level of soil moisture over the experimental period, which was attributed to the materials high clay content. This suggests that Rhodes grass may not tolerate nutrient deficient soil or a high level of soil moisture for prolonged periods of time.

The biomass production of Rhodes grass (Figure 4.2) increased between 200% and 3600% when grown on soil contaminated with 15 wt% and 5 wt% mine tailings, compared to uncontaminated soil and undiluted tailings. In addition to containing modest concentrations of heavy metals, the 5 wt% amendment of mine tailings contained small quantities of a range of plant nutrients and essential trace elements (3405 μg Ca g^{-1}, 7028 μg Fe g^{-1}, 329 μg Mg g^{-1}, 1074 μg Mn g^{-1}, 119 μg P g^{-1}, 1029 μg S g^{-1}, and 199 μg Zn g^{-1}) (Appendix A). Application of the 5 wt% amendment of mine tailings to anthroposol soil has therefore provided a source of nutrients for effective

plant growth by *Chloris gayana*. This indicates that the addition of small quantities of mine tailings may act as a fertiliser to the improved biomass production of *Chloris gayana*.

Biomass production was greatly reduced (>50%) above a tailings content of 25 wt% (>3010 μg Pb g^{-1} and >1091 μg Zn g^{-1}, respectively) compared to the maximum growth of Rhodes grass on substrates containing 5 wt% tailings (626 μg Pb g^{-1} and 276 μg Zn g^{-1}, respectively). The data indicates that the critical threshold concentrations of mine tailings for effective plant growth of Rhodes grass is approximately 25 wt%. This level of contamination indicates that Rhodes grass tolerated higher total concentrations of As (2039 μg g^{-1}) and Cd (30 μg g^{-1}), in addition to Pb and Zn, in the substrates compared to Vetiver grass (250 μg As g^{-1}, 20 μg Cd g^{-1}, >1500μg Pb g^{-1} and 240 μg Zn g^{-1}) (Truong, 1998). Reduced biomass production in Rhodes grass grown on soil contaminated with ≥25 wt% mine tailings may have also resulted from induced nutrient deficiency (e.g. Zn) by the addition of Fe and P contained in the mine tailings.

4.4.2. Metals and arsenic uptake by Rhodes grass

Problems associated with sequential extraction procedures, i.e. non-selectivity of reagents, reabsorption and mineral disassociation, are well documented (e.g. Tessier *et al.*, 1979; Rendell *et al.*, 1980; Tipping *et al.*, 1985). Nevertheless, information on the partitioning of metals provided by

sequential extractions is useful in assessing metal distribution and mobility in contaminated soils and the bioavailability of metals to plants (cf. Papp *et al.*, 1991; Basta and Gradwohl, 2000). Of particular importance is the assessment of metal distributions between secondary minerals, organic compounds and clay particles in contaminated soils because these materials are most likely to exchange metals with pore fluids and thus influence the bioavailability of metals to plants (Miller *et al.*, 1987; Mortvedt *et al.*, 1991; Pampura *et al.*, 1993). These metal-bearing phases are usually extracted with weakly reactive solutions (e.g. H_2O, 1 M NH_4OAc [pH 7 and 5] and 0.04 M NH_4.HCl) at the beginning of a sequential extraction procedure to model natural solvents and plant exudates (Tessier *et al.*, 1979; Haq *et al.*, 1980; Singh and Narwal, 1984; McGrath *et al.*, 1997). These reagents would not cause appreciable dissolution of sulphide minerals, such as those contained in Cannington mine tailings and therefore extractions with these reagents would be relatively useful for estimating the bioavailable and potentially bioavailable metal pools in soils. However, the sequential extraction of the soil-tailings mixture containing the critical growth concentration of tailings (25 wt%) indicated that Pb bioavailability (243.5 μg g^{-1}) was significantly higher than that of Zn (37.1 μg g^{-1}) (Table 4.2). Whereas, plant analyses indicated that Rhodes grass accumulated significantly higher concentrations of Zn (344 ± 201 μg g^{-1}) compared to that of Pb (51.8 ± 32.9 μg g^{-1}), and the other elements, from this substrate. This indicates that the sequential extraction procedure was not predictive of heavy metal uptake by *Chloris gayana*.

Rhodes grass was capable of naturally accumulating a modest quantity of Zn from soil contaminated with mine tailings. The uptake of Ag, As, Cd, and Pb by Rhodes grass significantly lower by comparison indicating a strong mechanism for excluding heavy metals and arsenic from uptake. (Figure 4.2). This demonstrates the suitability of Rhodes grass for the revegetation of mine tailings and soil contaminated with mine tailings in the Australian tropics and subtropics. However, it would be remiss not to cite some precautionary ecological principles and to consider the potential impact of using this exotic pasture grass. All of the grass species mentioned here are introduced exotic species. Many species are currently used as high utility plants to increase stock production in pastoral systems. Most introduced pasture species are inherently aggressive competitors with native species and can rapidly invade native ecosystems. Buffel grass is a well recognised weed of the Burdekin Rangelands in north central Queensland (Grice, 2001) and considerable attention is given to the management of this weed in various parts of Australia (Pitts and Albrecht, 2000). It is also considered a threat to the biological integrity of places such as the Sonoran Desert of north-western Mexico and south-western United States (James, 1995) and an agent of change in the grasslands and shrublands of Texas, Hawaii and Australia (Tix, 2000). On the other hand, recent research (Woolnough and Foley, 2002) has documented its role in providing an important nutritional source for Australia's most critically endangered mammal, the northern hairy-nosed wombat (*Lasiorhinus krefftii*). Rhodes grass is itself also officially documented as an environmental weed (Queensland Government, 2002). It is therefore recommended that caution be applied in the selection of even demonstrably

suitable species for phytoremediation when they are not native. This would be particularly important in or near areas where the conservation of native biodiversity is a desired land rehabilitation outcome.

4.5. Conclusion

The study has shown Rhodes grass to be an extremely hardy species capable of surviving in undiluted base-metal mine tailings and soil contaminated with mine tailings containing up to 0.2 wt% As, 1.2 wt% Pb and 0.4 wt% Zn. The addition of small quantities of mine tailings (≈5 wt%) contained modest levels of a range of plant nutrients and appeared to act as a fertiliser by dramatically improving the species biomass production. Plant growth was significantly reduced (>50%) on substrates containing ≥25 wt% tailings indicating a critical threshold concentration for the revegetation of soil contaminated with base-metal mine tailings. Furthermore, any positive nutritive effects associated with the addition of small amounts of mine tailings to uncontaminated soil appears to be outweighed at higher quantities by the introduction of phytotoxic concentrations of heavy metals. The accumulation of As, Cd and Pb in Rhodes grass was low over the experimental range and the exclusion of As, Cd and Pb appears more efficient in Rhodes grass compared to Vetiver grass (Truong, April 1999).

This study concludes that Rhodes grass is well suited to the revegetation of soil contaminated with mine tailings and at low levels of contamination, mine

tailings may improve the species growth potential. Low levels of metal and metalloid accumulation in aboveground tissues indicate a low fodder toxicity risk and suggest that the species may be better suited to revegetation of metalliferous soils than Vetiver grass and Buffel grass. However, it is likely that Zn toxicity (>650 $\mu g\ g^{-1}$) would significantly affect plant growth. Rhodes grass is recommended as a species for mine tailings rehabilitation where there are no major issues for biodiversity conservation and where pastoral land use is a desired rehabilitation outcome.

Chapter 5. Chemically-Assisted Phytoremediation of Soil Contaminated with Mine Tailings using Australian Subtropical Pasture Plant Species

5.1. Introduction

Phytoremediation is an environmental tool for the *in situ* rehabilitation of heavy metal contaminated soil (Begonia *et al.*, 2002; Greman *et al.*, 2003; Kos and Lestan, 2003). It is one of a burgeoning number of *phyto*-technologies (Chapter 1, Section 1.2) that employ the biochemical properties of plants for land rehabilitation (Chaney *et al.*, 1997; Glass, 2000). Phytotechnologies have the capacity to preserve valuable physical, chemical and biological properties of the soil while rehabilitation is performed. As such, environmental specialists often favour these practices because soils are readily returned to agricultural land uses once rehabilitated.

Naturally occurring heavy metal and metalloid accumulating plants are known (Reeves *et al.*, 1995; Brooks, 1998) and have been studied for their environmental applications (Baker *et al.*, 1994; McGrath *et al.*, 1999). Unfortunately, there are very few examples of heavy metal accumulating plant species in the Australian botanical record (Blissett, 1966; Cole, 1973; Correll and Taylor, 1974; Farago *et al.*, 1977; Farago and Mahmoud, 1983; Bidwell *et al.*, 2002). Soil amendments of chelating agents (EDTA and DTPA) have

been demonstrated to promote heavy metal phytoextraction by a variety of agricultural plant species (*Brassica juncea*, *B. napus*, *B. rapa*, *Zea mays* and *Pisum sativum*) (Blaylock *et al.*, 1997; Ebbs *et al.*, 1997; Huang *et al.*, 1997a; Huang *et al.*, 1997b; Wu *et al.*, 1999). The technology has been termed 'chemically-assisted' or 'induced' phytoextraction (CAP) (Huang and Cunningham, 1996). However, the persistence of some chelates, such as EDTA, in soil (Means *et al.*, 1980) may lead to the leaching of heavy metal contaminants into groundwater (Yordanov and Roundhill, 1998). EDDS has recently been shown to be effective in promoting base metal uptake in plants (Vandevivere *et al.*, 2001a; Greman *et al.*, 2003). The biological half-life of EDDS is relatively short (2.5 days) compared to that of EDTA (180 days) (Vandevivere *et al.*, 2001b), which significantly reduces the environmental risk of leaching and groundwater contamination. Thio-compounds such as SCN and TSP have also been shown to effect metal uptake in some plant species, particularly Au accumulation (Anderson *et al.*, 1999a; Keeling, 2001). Ammonium thiocyanate is relatively selective towards Au and Ag complexation, particularly under slightly acid conditions (pH 6.4) (Keeling, 2000). In contrast, TSP shows non-specificity to metal complexation under slightly alkaline (pH 7.2) conditions (Anderson, 2000).

The Cannington Ag-Pb-Zn mine is surrounded by pastoral leases and, following mine closure, is required to support some form of agricultural land use capability. The mine's Environmental Management and Operations System (EMOS) stipulates that only native flora can be used in revegetation

programs (BHPBilliton, 2002) therefore, only native plant species were available for the phytoremediation of contaminated soil. The study of heavy metal tolerance and natural metal and metalloid uptake in the dominant pasture species on the Cannington mine tenement (Chapter 3) indicated that *Cyperus victoriensis*, *Crotalaria novae-hollandiae*, and *Cymbopogon bombycinus* exhibited growth potentials (high biomass production and heavy metal tolerance) on soil contaminated with mine tailings that were suitable for further phytoextraction analysis. In addition, the periodic leaching of mine tailings with the EDTA, TSP, SCN and THIO was found to effectively dissolve high levels of various combinations of metals and metalloids (ref. Chapter 7) and hence might be suitable for promoting heavy metal uptake in the above plant species.

Chemically-assisted phytoremediation of heavy metals from contaminated soil involves undertaking several sequential steps in achieving a reduction in the contaminant load of the soil. Firstly, plant selection must be made based upon each available species abilities to tolerate elevated concentrations of heavy metals in soil. Secondly, high biomass producing plant species must be used because metal accumulation in plants is proportional to plant weight (Brooks, 1980). Thirdly, revegetation of the contaminated soil is undertaken until the cultivated plants have reached their maximum level of biomass production. At that point, the soil is irrigated with a chemical reagent to dissolve the contaminants thereby making them available for plant uptake. Finally, after the plants have accumulated the contaminants from interstitial

pore fluids in the soil and sequestered them in aboveground plant tissues, these tissues are harvested and removed from site thus reducing the soils contaminant load. For the sake of clarity in documenting the use of chemically-assisted phytoremediation, the technology will be referred to in the text by its acronym; CAP.

The present study was conducted to determine the potential of *Cyperus victoriensis*, *Crotalaria novae-hollandiae*, and *Cymbopogon bombycinus* to accumulate Ag, As, Cd, Pb, Sb and Zn from soil contaminated with Cannington mine tailings using chemical amendments. The primary aim of the study was to identify efficient plant-chemical combinations for the rapid depletion of metals and metalloids from the contaminated soil. In addition, plant mortality resulting from the chemical amendments was used to identify whether or not any potentially sustainable CAP technique could phytoremediate soil without resulting in the death of the pasture plant species.

5.2. Materials and methods

This pot trial experiment (Figure 5.1) involved the cultivation of *Cyperus victoriensis*, *Crotalaria novae-hollandiae*, and *Cymbopogon bombycinus* on an anthroposol soil contaminated with 12.5 wt% Cannington mine tailings (ST mixture) (Chapter 2, Section 2.11.3). This level of tailings contamination was selected because the mass of tailings added to the soil was sufficient to elevate the Pb concentration to 1520 μg Pb g^{-1}. This total Pb concentration is

slightly above the Australian and New Zealand Environment and Conservation Council (ANZECC) Investigation Limit for Pb contaminated industrial and commercial sites (1500 μg Pb g^{-1}) and was therefore a useful benchmark concentration upon which to base the investigation (ANZECC, 1999).

Figure 5-1. Pot trial experiment of *Cyperus victoriensis* (foreground) and *Crotalaria novae-hollandiae* (background) cultivated in a soil contaminated with 12.5 wt% mine tailings after one application of the various phytoextractive chemical amendments.

The plants were cultivated on the contaminated soil for twelve weeks (Chapter 2, Section 2.12) and then the soils were amended with weekly applications of chemical reagents (EDTA, DTPA, EDDS, TSP, SCN and THIO) over a five-week period (Chapter 2, Section 2.12.2). Plant samples were collected (Chapter 2, Section 2.12.1) three days after application of the chemical amendments and prepared for Ag, As, Cd, Pb, Sb, and Zn analysis (Appendix F). Soil samples were extracted with 0.01 M EDTA, 0.005 M

DTPA and 0.01 M EDDS (Chapter 2, Section 2.11.6) to estimate the plant available fraction of metals and metalloids in the contaminated soil (Appendix E). In addition, the soil was sequentially extracted (Chapter 2, Section 2.11.7) to determine the mobility and distribution of metals and metalloids contained in the plant growth medium (Appendix E).

5.3. Results

5.3.1. Partial and sequential extractions

Deionised water (dH_2O) dissolved low proportions of the total element concentrations (TEC) of As, Cd, Pb, Sb and Zn from the ST mixture (Table 5.1). The aqueous extractability of Ag was below detection limit (Appendix E). The dissolution of metals and metalloids from the ST mixture using 0.01 M EDTA, 0.005 M DTPA and 0.01 M EDDS was broadly similar in proportion for all elements (Table 5.1). Extraction of the ST mixture with 0.01 M EDTA removed higher proportions of As, Cd, Pb and Zn compared to extractions with 0.005 M DTPA and 0.01 M EDDS. Antimony was extracted in equal proportion from the ST mixture using 0.01 M EDTA and 0.01 M EDDS.

Soil extractions using 0.01 M EDTA and 0.005 M DTPA removed similar proportions of Cd and Zn from the ST mixture. While, extractions with 0.005M DTPA and 0.01 M EDDS removed similar proportions of Pb and As from the ST mixture, which were lower than the concentrations of Pb and As

extracted using 0.01 M EDTA (Table 5.1). Small proportions of Ag and Sb were extracted from the ST mixture using 0.01 M EDTA, 0.005 M DTPA and 0.01 M EDDS. This indicates that the proportions of extractable element in the ST mixture decreased in the order Pb > Zn = Cd >> As > Sb > Ag. In addition, the average concentrations of metals and metalloids extracted from the ST mixture by EDTA, DTPA and EDDS were 0.01 μg Ag g^{-1}, 16.6 μg As g^{-1}, 2.3 μg Cd g^{-1}, 1366 μg Pb g^{-1}, 2.1 μg Sb g^{-1} and 355 μg Zn g^{-1} (Appendix E). This indicates that the concentrations of plant available metals and metalloids in the ST mixture decreased in the order Pb >> Zn >> As > Cd = Sb > Ag.

Table 5.1. Total metal and metalloid concentrations of the soil, mine tailings and a soil contaminated with 12.5 wt% mine tailings (μg g^{-1}). Below are the proportions (% of the total element concentration – TEC) of metals and metalloids extracted from the contaminated soil using deionised water (dH_2O), 0.01M EDTA, 0.005M DTPA and 0.01M EDDS.

Substrate	Total Element Concentrations (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn
Soil	<0.1%	7	<0.1%	30	<0.1%	72
Tailings	60	2039	30	11950	335	4150
ST mixture	8	261	4	1520	42	582

Extract	Elemental Extractability (TEC) of the ST mixture					
	Ag	As	Cd	Pb	Sb	Zn
dH_2O	<0.1%	0.3%	2.0%	<0.1%	0.8%	0.4%
0.01M EDTA	0.1%	11.7%	57.7%	98.8%	5.9%	61.5%
0.005M DTPA	0.1%	4.7%	56.9%	84.4%	3.5%	60.5%
0.01M EDDS	0.1%	2.7%	44.5%	86.3%	5.9%	42.0%

Table 5.2. The proportions of metals and metalloids (% of total concentration - TEC) that were sequentially extracted from a soil contaminated with 12.5 wt% mine tailings (Appendix E).

Extract Fraction	Element Extractability (% of total element conc.)					
	Ag	As	Cd	Pb	Sb	Zn
Water Soluble	<0.1%	<0.1%	2.5%	<0.1%	0.1%	0.6%
Ion Exchangeable	<0.1%	0.6%	31.2%	11.4%	5.0%	6.9%
Carbonate	0.6%	2.1%	16.2%	27.4%	5.7%	16.5%
Oxide	1.1%	4.0%	16.2%	33.6%	0.6%	30.9%
Sulphide	89.2%	91.6%	27.3%	25.8%	80.9%	42.1%
Residual/Silicate	9.2%	1.6%	6.5%	1.9%	6.9%	3.0%

Sequential extraction of the ST mixture (Table 5.2) indicated that the majority of Ag, As and Sb was contained in the sulphide fraction. Silver and As were also present in low proportions in the carbonate, oxide and residual/silicate fractions. In addition, a trace amount of As was extracted from the ST mixture in the ion exchangeable fraction. Small quantities of Sb were extracted from the ST mixture as soluble, ion exchangeable, carbonate and oxide fractions. In contrast, the sequential extraction procedure removed high proportions of Cd, Pb and Zn from a range of elemental fractions in the ST mixture. Cadmium was extracted in high proportion from the ST mixture as ion exchangeable, sulphide, carbonate and oxide fractions. Small quantities of Cd were also extracted as water soluble and residual/silicate fractions. In comparison, large quantities of Pb were extracted as oxide, carbonate and sulphide fractions, in addition to smaller proportions extracted as ion exchangeable and residual/silicate fractions.

Sequentially extractable Zn indicated that large proportions of the metal were

present as sulphide and oxide fractions in the ST mixture. Smaller quantities of Zn were extracted from the ST mixture as carbonate, ion exchangeable, residual/silicate, and water soluble fractions. Heavy metals and metalloids extracted as water soluble and ion exchangeable fractions of the sequential extraction scheme may be considered the more plant available elemental fractions in the ST mixture (cf. Gatehouse *et al.*, 1977; Tessier *et al.*, 1979). This suggests that the proportions of liable elements in the ST mixture, estimated by sequential extraction, decreased in the order Cd >> Pb > Zn > Sb > As > Ag. Furthermore, the concentrations of metals and metalloids removed by these stages of the sequential extraction procedure were 173 μg Pb g^{-1}, 44 μg Zn g^{-1}, 3 μg Sb g^{-1}, 2 μg As g^{-1}, 1 μg Cd g^{-1} and <0.01 μg Ag g^{-1} (Appendix E). This indicates that the sequential extraction procedure estimated the plant availability of metals and metalloids in the ST mixture to decrease in the order Pb >> Zn >> Sb > As > Cd > Ag.

5.3.2. Chemical amendment toxicity

Chemical amendments applied to the soil produced markedly different growth responses in the plants over the five-week treatment period (Figure 5.2). An estimation of the phytotoxic effect of each of the chemical amendments was based upon visibly identifiable toxicity symptoms such as wilting, leaf tip necrosis and plant death. Chemical amendments were deemed to have had a toxic effect on plant health when ≥50% of the plants in a treatment group had died. Weekly applications of THIO, for example, resulted in rapid plant death indicating the chemical amendments produced

highly toxic conditions in the soil. Amendments of EDTA to the ST mixture produced a gradual increase in visible toxicity symptoms in *Cymbopogon bombycinus*, such as yellowing and gradual necrosis of leaves. In contrast, the same chemical amendments did not cause toxicity symptoms to appear in *Cyperus victoriensis* over the five week treatment period (5 weeks).

All species died within several days following one application of 0.5 and 2.0 g kg^{-1} SCN and thiourea, or 2.0 g kg^{-1} TSP to the ST mixtures. Rapid plant death was also observed in *Cymbopogon bombycinus* after a single treatment of 2 g kg^{-1} EDDS. In contrast, *Cymbopogon bombycinus* survived two weekly amendments of 2 g kg^{-1} TSP and four weekly amendments of either 0.5 or 2.0 g kg^{-1} EDTA. Furthermore, the 0.5 g kg^{-1} TSP and EDDS amendments did not adversely affect the health of *Cymbopogon bombycinus* over the five week treatment period. Four weekly amendments of 2.0 g kg^{-1} DTPA, TSP, or EDDS were possible before toxicity symptoms quickly appeared in *Cyperus victoriensis* and the plant died. However, amendments of 0.5 and 2.0 g kg^{-1} EDTA, 0.5 TSP, and 0.5 EDDS did not cause toxicity symptoms to appear in *Cyperus victoriensis* over the five week treatment period.

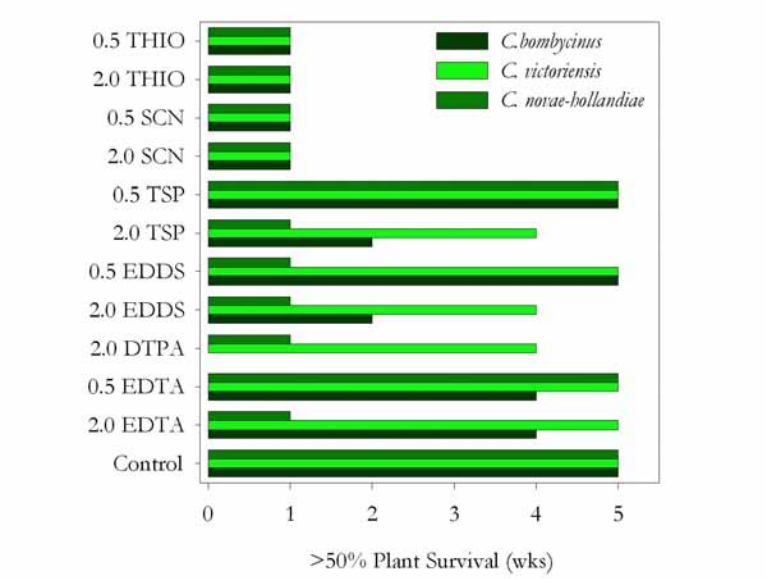

Figure 5-2. Plant survival (i.e. >50% of plants) (weeks) for *Crotalaria novae-hollandiae*, *Cymbopogon bombycinus* and *Cyperus victoriensis* grown on a soil contaminated with 12.5 wt% mine tailings and amended with weekly application of the various chemical amendments (e.g. 2.0 EDTA = 2.0 g EDTA per kg soil).

One application of all the chemical amendments, except for 0.5 g kg^{-1} EDTA and TSP, caused rapid necrosis in *Crotalaria novae-hollandiae*, which was preceded by complete leaf excision. Amendments of 0.5 g kg^{-1} EDTA and TSP did not result in *Crotalaria novae-hollandiae* excising leaves, however, after five weeks of treatment the leaves were noticeably yellow compared to control plants. Plant survival and the development of visible toxicity symptoms in response to weekly applications of the chemical amendments indicated that *Cyperus victoriensis* is tolerant to prolonged application of EDTA, DTPA, EDDS and TSP at both concentrations (0.5 and 2.0 g kg^{-1}). *Cymbopogon bombycinus*, on the other hand, tolerated ongoing amendments of 0.5 and 2.0 g kg^{-1} EDTA but only the 0.5 g kg^{-1} amendments of EDDS and

TSP. In contrast, *Crotalaria novae-hollandiae* exhibited extremely low tolerance to all the chemical amendments except 0.5 g kg^{-1} amendments of EDTA and TSP. Although, prolonged application of these treatments caused toxicity symptoms to appear in the plant.

5.3.3. Plant chemistry

Vegetation samples were analysed to determine plant concentrations of metals and metalloids taken up from the contaminated soil following weekly applications of the chemical amendments. Where possible, leaf samples were collected weekly for analysis (Appendix F), however this was not feasible for *Crotalaria novae-hollandiae* or *Cyperus victoriensis*. Both species grew a small number of large leaves and it was felt that weekly vegetation sampling might interfere with the plant's normal growth response on the contaminated soil. The average plant concentrations of metals and metalloids for each chemical treatment were compared and the highest weekly average plant concentration for each element has been plotted below for discussion.

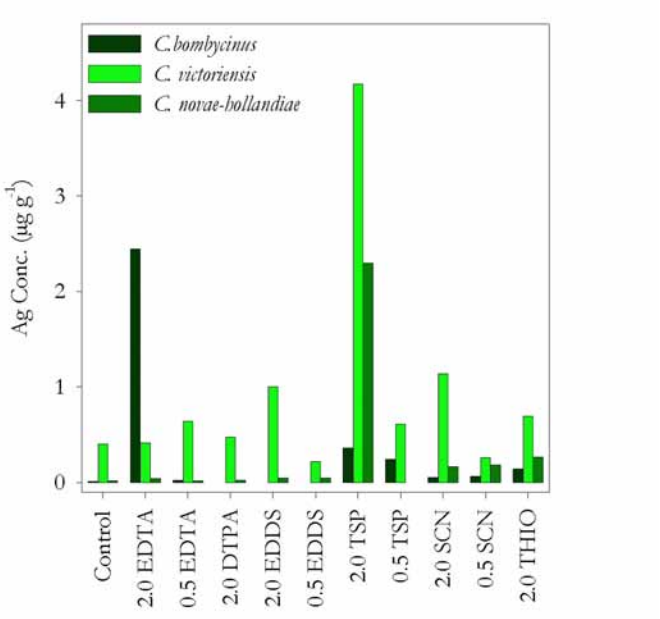

Figure 5-3. The maximum concentration (N=4) of Ag (μg g^{-1} DW) accumulated by plants grown on a soil contaminated with 12.5 wt% mine tailings and amended with various chemical reagents (e.g. 2.0 EDTA = 2 g EDTA per kg soil).

Plant tissue analyses indicated that four weekly treatments of 2.0 g kg^{-1} EDTA promoted Ag uptake in *Cymbopogon bombycinus* of 2.4 ± 1.0 μg g^{-1}. In contrast, the application of 2.0 g kg^{-1} TSP promoted Ag up of 4.2 ± 1.9 μg g^{-1} in *Cyperus victoriensis* after four weekly treatments and 2.3 ± 0.9 μg Ag g^{-1} in *Crotalaria novae-hollandiae* after one application. None of the remaining chemical amendments promoted Ag uptake in any plant species over 2.0 μg g^{-1}. However, *Cyperus victoriensis* accumulated >1.0 μg Ag g^{-1} in aboveground plant tissues after one application of 2.0 g kg^{-1} SCN and after four applications of 2.0 g kg^{-1} EDDS. All remaining chemical amendments promoted higher levels of Ag uptake in *Cyperus victoriensis* compared to *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae*.

Cadmium biomagnification (plant conc. > soil conc.) occurred in *Cyperus victoriensis* (Figure 5.4) for all chemical amendments and in the control (8.6 ± 2.1 μg Cd g^{-1}). Four applications of the 2 g kg^{-1} EDDS and TSP amendments and one application of the 0.5 g kg^{-1} THIO amendments caused Cd accumulation in *Cyperus victoriensis* of 22.4 ± 5.8, 22.4 ± 4.8 and 21.9 ± 2.2 μg Cd g^{-1}, respectively. This species also accumulated high levels of Cd after four applications of 2 g kg^{-1} DTPA (16.7 ± 4.3 μg Cd g^{-1}) and after one application of 2 g kg^{-1} SCN (15.9 ± 2.6 μg Cd g^{-1}). Treatments with 2.0 g kg^{-1} EDTA and 0.5 g kg^{-1} TSP and SCN reduced Cd uptake in *Cyperus victoriensis* (7.4 ± 2.8, 5.9 ± 3.3 and 5.3 ± 1.8 μg Cd g^{-1}, respectively) compared to the control (8.6 ± 2.1 μg g^{-1}).

All chemical treatments resulted in Cd biomagnification (>4.0 μg g^{-1}) in *Cymbopogon bombycinus* and no amendment reduced Cd uptake compared to the control (1.9 ± 0.5 μg Cd g^{-1}). Cadmium uptake in *Cymbopogon bombycinus* was greatest following amendments of 0.5 and 2 g kg^{-1} EDTA (10.3 ± 4.0 and 11.0 ± 7.4 μg Cd g^{-1}) and 2.0 g kg^{-1} TSP (10.2 ± 6.6 μg Cd g^{-1}), SCN (9.4 ± 7.2 μg Cd g^{-1}) and THIO (15.2 ± 5.3 μg Cd g^{-1}). In comparison, *Crotalaria novae-hollandiae* biomagnified Cd subsequent to all chemical amendments to the ST mixture, except for the 0.5 g kg^{-1} TSP amendment (2.7 ± 1.0 μg Cd g^{-1}). The accumulation of Cd in *Crotalaria novae-hollandiae* was low compared to *Cyperus victoriensis* and *Cymbopogon bombycinus*. Cadmium uptake by *Crotalaria novae-hollandiae* was elevated using soil amendments of 0.5 g and 2 g kg^{-1} EDTA (4.2 ± 1.4 and 7.3 ± 4.6 μg Cd g^{-1}) and EDDS (4.8 ± 1.3 and 4.1 ±

1.0 μg Cd g^{-1}) compared to the control (3.1 ± 1.3 μg Cd g^{-1}) (Figure 5.4). Elevated Cd concentrations also occurred in *Crotalaria novae-hollandiae* following the application of 2 g kg^{-1} DTPA (5.2 ± 1.6 μg Cd g^{-1}) and SCN (5.6 ± 0.2 μg Cd g^{-1}), and 0.5 g kg^{-1} THIO (5.3 ± 1.6 μg Cd g^{-1}) and EDTA (4.2 ± 1.4 μg Cd g^{-1}) compared to the control.

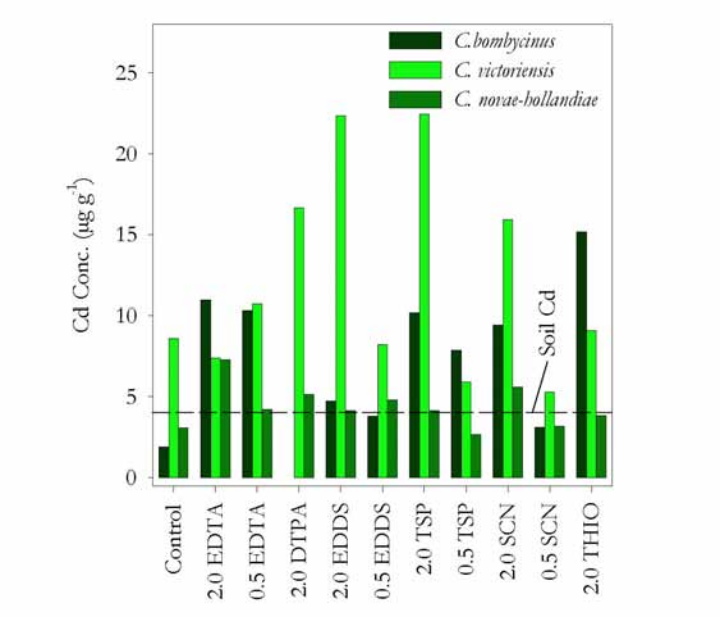

Figure 5-4. The maximum concentration (N=4) of Cd (μg g^{-1} DW) accumulated by plants grown on a soil contaminated with 12.5 wt% mine tailings and amended with various chemical reagents (e.g. 2.0 EDTA = 2 g EDTA per kg soil). The total concentration of Cd in the ST mixture is also shown (4.0 μg Cd g^{-1}).

Crotalaria novae-hollandiae was the only plant species to biomagnify Pb (>1520 μg g^{-1}) and only when treated with 2 g kg^{-1} EDTA (1908.7 ± 686 μg Pb g^{-1}) (Figure 5.5). A high concentration of Pb in *Crotalaria novae-hollandiae* also occurred following soil treatment with 0.5 g kg^{-1} EDTA (902.8 ± 246 μg Pb g^{-1}). Elevated Pb concentrations were detected in *Cyperus victoriensis* following

soil amendments of 0.5 and 2 g kg^{-1} EDTA (1299.8 ± 419 and 607.2 ± 162 μg Pb g^{-1}), 2 g kg^{-1} DTPA (705.7 ± 434 μg Pb g^{-1}) and EDDS (1200.8 ± 677 μg Pb g^{-1}) compared to the control (6.8 ± 1.0 μg Pb g^{-1}). The 0.5 and 2.0 g kg^{-1} EDTA treatments were the only amendments to produce elevated Pb concentrations in *Cymbopogon bombycinus* (412 ± 388 and 996.2 ± 145 μg Pb g^{-1}) compared to the control (4.9 ± 0.4 μg Pb g^{-1}) (Figure 5.4). With the exception of the 2.0 g kg^{-1} TSP treatments to ST mixtures containing *Cyperus victoriensis* (209.4 ± 41 μg Pb g^{-1}), the remaining thio-compound treatments (0.5 and 2.0 g kg^{-1} SCN and thiourea, and 0.5 g kg^{-1} TSP) did not elevate the Pb concentrations of the plants compared to the concentration of Pb in control plants.

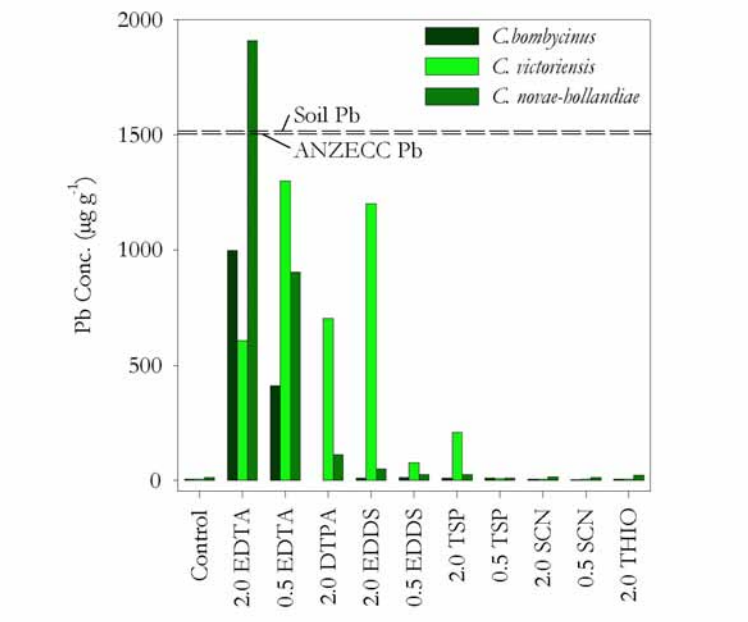

Figure 5-5. The maximum concentration (N=4) of Pb (μg g^{-1} DW) accumulated by plants grown on a soil contaminated with 12.5 wt% mine tailings and amended with various chemical reagents (e.g. 2.0 EDTA = 2 g EDTA per kg soil). The total concentration of Pb in the ST mixture (1520 μg Pb g^{-1}) and the ANZECC Investigation Limit for Pb contaminated industrial and commercial sites (1500 μg Pb g^{-1}) are included (ANZECC, 1999).

Biomagnification of growth essential Zn (>582 μg Zn g^{-1}) by the plant species (Figure 5.6) was confined to soil treatment with chelate preparations. *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae* biomagnified Zn following soil treatment with 0.5 g kg^{-1} EDTA (909 ± 748 and 1956 ± 366 μg Zn g^{-1}, respectively) and 2.0 g kg^{-1} EDTA (962 ± 145 and 790 ± 608 μg Zn g^{-1}, respectively). The 0.5 g kg^{-1} THIO treatment reduced the concentration of Zn in *Cymbopogon bombycinus* by 24% (71.2 ± 12.9 μg Zn g^{-1}) compared to the control (93.3 ± 43.8 μg Zn g^{-1}) (Figure 5.6). Biomagnification of Zn in *Cyperus victoriensis* was observed for soil treatments of 0.5 and 2.0 g kg^{-1} EDTA (1448 ± 410 and 725 ± 192 μg Zn g^{-1}), 0.5 and 2.0 g kg^{-1} EDDS (786 ± 260 and 1390 ± 661 μg Zn g^{-1}) and 2.0 g kg^{-1} DTPA (1078 ± 424 μg Zn g^{-1}). Zinc uptake by *Cyperus victoriensis* was reduced following soil amendments of 0.5 g kg^{-1} TSP (301 ± 40.2 μg Zn g^{-1}) and 2.0 SCN (338 ± 98.2 μg Zn g^{-1}) compared to the control (362 ± 61.9 μg Zn g^{-1}).

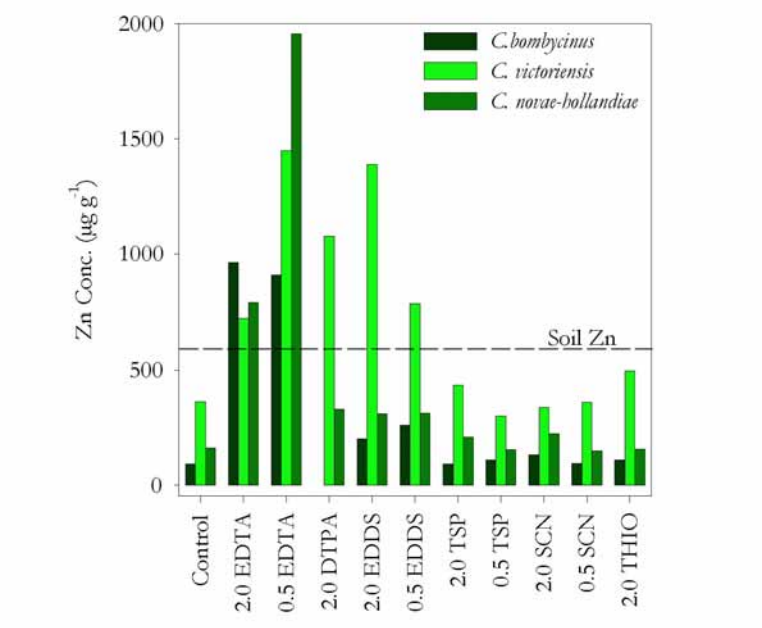

Figure 5-6. The maximum concentration (N=4) of Zn (μg g^{-1} DW) accumulated by plants grown on a soil contaminated with 12.5 wt% mine tailings and amended with various chemical reagents (e.g. 2.0 EDTA = 2 g EDTA per kg soil). The total concentration of Zn in the ST mixture is also shown (582 μg Zn g^{-1}).

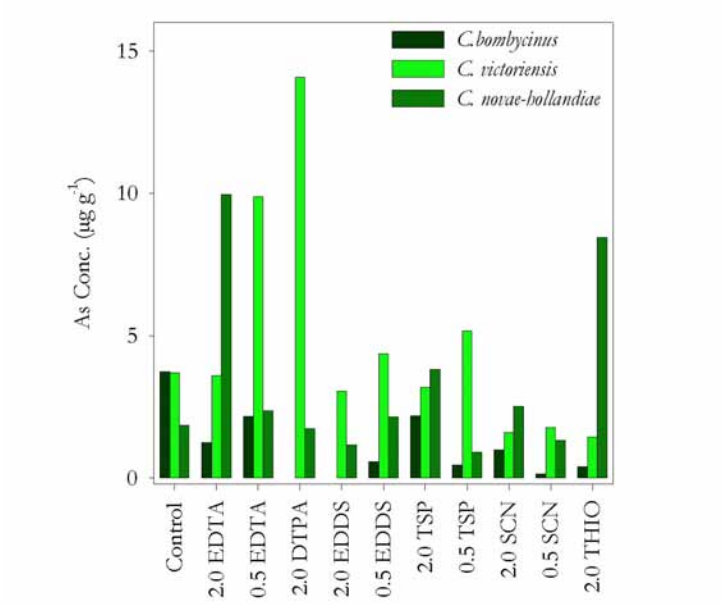

Figure 5-7. The maximum concentration (N=4) of As (μg g^{-1} DW) accumulated by *Cymbopogon bombycinus*, *Cyperus victoriensis* and *Crotalaria novae-hollandiae* when grown on a soil contaminated with 12.5 wt% mine tailings and amended with various chemical amendments (e.g. 2.0 EDTA = 2 g EDTA per kg soil).

None of the chemical amendments applied to the ST mixture caused As biomagnification (>261 μg g^{-1}) in any plant species (Figure 5.7). *Cyperus victoriensis* accumulated maximum As concentrations after soil treatment with 2 g kg^{-1} DTPA (14.1 ± 7.1 μg As g^{-1}) and 0.5 g kg^{-1} EDTA (9.9 ± 6.4 μg As g^{-1}). Soil amendments of 0.5 g kg^{-1} EDDS or TSP, and 2.0 g kg^{-1} EDDS or EDTA also produced elevated concentrations of As in *Cyperus victoriensis* (5.8 ± 0.2, 5.2 ± 1.1, 4.1 ± 1.4 and 3.6 ± 1.1 μg g^{-1} As, respectively) compared to the control (3.7 ± 1.9 μg g^{-1}) (Figure 5.7). All other chemical amendments failed to promote the uptake of As in *Cyperus victoriensis* compared to the control. Soil amendments of 2 g kg^{-1} EDTA and THIO resulted in maximum As accumulation in *Crotalaria novae-hollandiae* (10.0 ± 9.0 and 8.4 ± 2.0 μg g^{-1} As, respectively). Elevated concentrations of As in *Crotalaria novae-hollandiae* were also detected after the application of 2 g kg^{-1} TSP and SCN (3.8 ± 1.8 and 2.5 ± 1.0 μg As g^{-1}, respectively), and 0.5 g kg^{-1} EDTA, EDDS and THIO (2.4 ± 0.5, 2.2 ± 1.7 and 2.9 ± 1.5 μg As g^{-1}, respectively) compared to the control (1.8 ± 0.9 μg As g^{-1}). All chemical amendments to the ST mixture caused a decrease in the As concentration in *Cymbopogon bombycinus* compared to the control (3.7 ± 1.4 μg As g^{-1}).

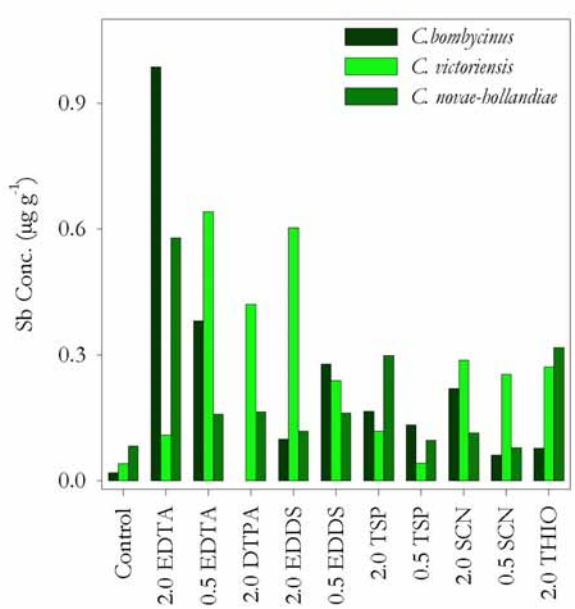

Figure 5-8. The maximum concentration (N=4) of Sb (µg g^{-1} DW) accumulated plants grown on a soil contaminated with 12.5 wt% mine tailings and amended with various chemical reagents (e.g. 2.0 EDTA = 2 g EDTA per kg soil).

Cymbopogon bombycinus accumulated maximum concentrations of Sb (Figure 5.8) following soil amendments of 0.5 and 2 g kg^{-1} EDTA (0.2 ± 0.1 and 1.0 ± 0.12 µg Sb g^{-1}, respectively) and 2 g kg^{-1} SCN (0.2 ± 0.2 µg Sb g^{-1}) compared to the control (0.02 µg g^{-1}). All chemical amendments, except 0.5 g kg^{-1} TSP (0.04 ± 0.2 µg Sb g^{-1}), elevated the concentration of Sb in *Cyperus victoriensis* compared to the control (0.04 ± 0.03 µg Sb g^{-1}). Maximum uptake of Sb occurred after soil amendments of 0.5 g kg^{-1} EDTA (0.6 ± 0.5 µg Sb g^{-1}) and 2 g kg^{-1} DTPA and EDDS (0.4 ± 0.1 and 0.6 ± 0.2 µg Sb g^{-1}, respectively). In contrast, the uptake Sb by *Crotalaria novae-hollandiae* was greatest after soil amendments with 2 g kg^{-1} EDTA (0.6 ± 0.5 µg Sb g^{-1}) and to a lesser extent with 2.0 g kg^{-1} TSP and THIO (0.3 ± 0.2 and 0.3 ± 0.1 µg

Sb g^{-1}, respectively).

5.4. Discussion

5.4.1. Metal and metalloid availability in the ST mixture

Estimating the plant availability of micronutrients and heavy metals in soil is commonly performed by extraction with chelating agents such as EDTA and DTPA (e.g. Lindsay and Norvell, 1978; Papp *et al.*, 1991; Hayes *et al.*, 2003; Kos and Lestan, 2003). Soil extractions with these reagents are useful in determining micronutrient deficiencies that adversely affect plant growth in soil. However, they may not relate well to observed metal toxicity in plants (Reichman, 2000). Soil properties such as pH and Ca:Mg ratio will directly affect metal solubility and the concentration of heavy metals in interstitial pore fluids in soil (Alloway, 1995). These factors will also influence the ability of chelating agents to dissolve heavy metals in soil (Cooper *et al.*, 1999; Reichman, 2000; Bernal *et al.*, 2002). Furthermore, the complexing capacity of the chelate used in these soil extraction procedures may become saturated by an overabundance of heavy metals in the sample leading to underestimations of heavy metal availability to plants in soil (Bell, 1986). Strong correlations have, however, been made between plant growth and heavy metal extractability using EDTA and DTPA for plants grown on similar soil types (Plenderleith, 1990). Therefore, soil extractions can be useful for estimating the plant availability and heavy metal toxicity of a contaminated soil under different geochemical conditions. However, soil extractions from different

soil types cannot be compared with accuracy.

The plant availability of metals and metalloids in the ST mixture, determined by soil extraction (Table 5.1) was Pb (1367 μg g^{-1}) >> Zn (318 μg g^{-1}) >> As (17 μg g^{-1}) > Cd = Sb (≈2 μg g^{-1}) >Ag (0.1 μg g^{-1}) (Appendix E). EDDS dissolved lower concentrations of Cd, Pb and Zn from the ST mixture compared to the EDTA soil extraction as reported by Greman *et al.* (2003). in comparing the use of EDTA and EDDS to extract Cd, Pb and Zn from a Pb-Zn smelter contaminated soil. Sequential extraction of the ST mixture (Table 5.2) indicated that Cd, Pb and Zn were distributed between a range of operationally defined mineral fractions (oxides, carbonates, ion exchangeable, water soluble), whereas Ag, As and Sb were present mainly in the sulphide and silicate/residual fractions. Plant availability of the elements (water soluble + ion exchangeable fractions) in the ST mixture, as determined by sequential extraction, was estimated to be Pb (173 μg g^{-1}) > Zn (44 μg g^{-1}) > Sb (3 μg g^{-1}) > As (2 μg g^{-1}) > Cd (1 μg g^{-1}) > Ag (<0.01 μg g^{-1}) (Appendix E). The data indicate that soil extractions using chelating agents (EDTA, DTPA and EDDS) dissolved significantly higher quantities of As, Cd, Pb and Zn from the ST mixture compared to the sequentially extracted plant available fractions (dH_2O + 1M NH_4OAc). By proportion however, both extraction methods indicated that the ST mixture contained high concentrations of plant available Pb and Zn, moderate concentrations of plant available As and Cd and low concentrations of plant available Sb and Ag.

5.4.2. Chemical amendment toxicity

The toxicity of weekly soil amendments, as determined by plant survival (Figure 5.2), indicated that SCN and THIO were highly toxic (100% plant mortality after one/two weekly chemical amendments) to all plant species. Elevated concentrations of metals or metalloids in plant tissues were not associated with the observed toxic response of the plants to these reagents. This indicates that chemical toxicity to SCN and THIO was responsible for plant death, rather than a lethal accumulation of metals or metalloids. Further reductions in the strength of THIO treatments (<0.5 g kg^{-1}) to *Cyperus victoriensis* may eliminate the risk of necrosis while still promoting high levels of Cd uptake (≤21.9 μg g^{-1}). The 0.5 g kg^{-1} TSP amendment was the only thio-compound treatment found to be non-toxic to all plant species over the five week treatment period. In addition, *Cyperus victoriensis* survived four weekly amendments of 2.0 g kg^{-1} TSP indicating that a small reduction in reagent concentration may perpetuate plant survival while maintaining a high level of Ag (≤4.2 μg g^{-1}) and Cd (≤24.4 μg g^{-1}) uptake. The data also suggests that a greater reduction in the concentration of applied TSP (<2.0 g kg^{-1}) would be required to promote long-term plant survival of *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae* growing on the ST mixture.

Soil amendments of EDDS (0.5 and 2.0 g kg^{-1}), in addition to 2.0 g kg^{-1} DTPA and EDTA, were highly toxic to *Crotalaria novae-hollandiae*. In contrast, weekly amendments of 0.5 g kg^{-1} EDTA did not cause plant death, although toxicity symptoms such as yellowed leaves and wilting were seen to develop

over time. This suggests that the plant would not have survived further sustained applications of the reagent. In contrast, *Cymbopogon bombycinus* survived four weekly applications of EDTA (0.5 and 2.0 g kg^{-1}) and two weekly applications of 0.5 g kg^{-1} EDDS. *Cymbopogon bombycinus* also tolerated five weekly applications of 0.5 g kg^{-1} EDDS. However, plant concentrations of metals and metalloids were low using this reagent. By comparison, *Cyperus victoriensis* survived longer soil treatment periods with the chelating agents than *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae*. *Cyperus victoriensis* survived four weekly amendments of 2.0 g kg^{-1} EDDS and DTPA. In addition, 2.0 g kg^{-1} EDDS amendments promoted high accumulation of Ag (1 μg g^{-1}), Cd (22 μg g^{-1}), Pb (1201 μg g^{-1}), Sb (0.6 μg g^{-1}) and Zn (1390 μg g^{-1}) in *Cyperus victoriensis*. In contrast, high accumulations of As (14 μg g^{-1}), Cd (17 μg g^{-1}), Pb (706 μg g^{-1}), Sb (0.4 μg g^{-1}) and Zn (1798 μg g^{-1}) by this species were associated with the 2.0 g kg^{-1} DTPA soil amendment. This suggests that a small reduction in the concentration of both applied reagents (EDTA and DTPA) may prolong plant growth on the ST mixture while maintaining high plant concentrations of metals and metalloids. *Cyperus victoriensis* survived five weekly amendments of EDTA at both application strengths (0.5 g and 2.0 g kg^{-1}) and high plant concentrations of As (10 μg g^{-1}), Cd (11 μg g^{-1}), Pb (1300 μg g^{-1}), Sb (0.6 μg g^{-1}) and Zn (1448 μg g^{-1}) were associated with the low strength amendment. This suggests that the weaker strength amendments of EDTA (<0.5 g kg^{-1}) applied to the ST mixture may promote higher concentrations of metals and metalloids to accumulate in the leaves of *Cyperus victoriensis* over a prolonged treatment period.

5.4.3. Phytoremediation potential of the plant-chemical combinations

The potential of a plant species to recover heavy metals and metalloids from a contaminated soil (i.e. phytoremediation potential, g of element ha^{-1} $crop^{-1}$) can be estimated by multiplying the plant concentration (μg g^{-1}) of an accumulated element by the plants dry matter production rate (t ha^{-1}) (McGrath *et al.*, 1993). To date, there are no published estimates of the dry matter production potential of the test species on different soil types. However, an estimate of the combustible pasture fuel load for the mine site in June 2004 was 5 t ha^{-1} (Malone, 2004). As a result, a dry matter production rate of 5 t ha^{-1} has been used to estimate the chemically-assisted phytoremediation potential (g ha^{-1} $crop^{-1}$) for each plant species grown on the ST mixture (Tables 5.3, 5.4 and 5.5).

The 2.0 g kg^{-1} DTPA soil treatment was only investigated for *Cyperus victoriensis* and *Crotalaria novae-hollandiae* due to a shortfall in the number of seedlings of *Cymbopogon bombycinus* at the beginning of the experiment. One DTPA amendment to the ST mixture resulted in rapid necrosis of *Crotalaria novae-hollandiae* and was associated with a modest phytoremediation potential for Cd (26 g ha^{-1} $crop^{-1}$) (Table 5.3). In contrast, the data indicate that a slight reduction in the concentration of applied DTPA (<2.0 g kg^{-1}) may perpetuate the growth of *Cyperus victoriensis* on the ST mixture while maintaining a high phytoremediation potential for As (≤70 g ha^{-1}), Cd (≤83 g ha^{-1}), Pb (≤3528 g ha^{-1}) and Zn (≤5388 g ha^{-1}) (Table 5.4).

Table 5.3. The estimated phytoremediation potential of *Crotalaria novae-hollandiae* grown on soil contaminated with 12.5 wt% mine tailings that has been treated with various chemical reagents (e.g. 2.0 EDTA = 2.0 g EDTA kg^{-1} soil, 0.5 EDTA = 0.5 g EDTA kg^{-1} soil) over a five week period.

Treatment	Phytoextraction potential (g ha^{-1} $crop^{-1}$)					
	Ag	As	Cd	Pb	Sb	Zn
Control	<1	9	15	67	<1	811
2.0 EDTA	<1	50	37	9543	3	3951
0.5 EDTA	<1	12	21	4514	1	9782
2.0 DTPA	<1	9	26	568	1	1649
2.0 EDDS	<1	6	21	252	1	1552
0.5 EDDS	<1	11	24	131	1	1562
2.0 TSP	12	19	21	130	2	1046
0.5 TSP	1	5	13	50	1	772
2.0 SCN	1	13	28	78	1	1123
0.5 SCN	1	7	16	67	<1	748
2.0 THIO	1	42	19	109	2	791
0.5 THIO	<1	15	27	151	1	1139

Table 5.4. The estimated phytoremediation potential of *Cyperus victoriensis* grown on soil contaminated with 12.5 wt% mine tailings that has been treated with various chemical reagents (e.g. 2.0 EDTA = 2.0 g EDTA kg^{-1} soil, 0.5 EDTA = 0.5 g EDTA kg^{-1} soil) over a five week period.

Treatment	Phytoremediation potential (g ha^{-1} $crop^{-1}$)					
	Ag	As	Cd	Pb	Sb	Zn
Control	2	19	43	34	<1	1812
2.0 EDTA	2	18	37	3036	1	3625
0.5 EDTA	3	49	54	6499	3	7240
2.0 DTPA	2	70	83	3528	2	5388
2.0 EDDS	5	15	112	6004	3	6950
0.5 EDDS	1	22	41	385	1	3930
2.0 TSP	21	16	112	1047	1	2169
0.5 TSP	3	26	30	37	<1	1503
2.0 SCN	6	8	80	30	1	1690
0.5 SCN	1	9	26	24	1	1802
2.0 THIO	3	7	45	33	1	2477
0.5 THIO	3	2	110	70	1	2478

Table 5.5. The estimated phytoremediation potential of *Cymbopogon bombycinus* grown on soil contaminated with 12.5 wt% mine tailings that has been treated with various chemical reagents (e.g. 2.0 EDTA = 2.0 g EDTA kg^{-1} soil, 0.5 EDTA = 0.5 g EDTA kg^{-1} soil) over a five week period.

Treatment	Phytoextraction potential (g ha^{-1} $crop^{-1}$)					
	Ag	As	Cd	Pb	Sb	Zn
Control	<1	19	10	24	<1	467
2.0 EDTA	12	6	55	4981	5	4808
0.5 EDTA	<1	11	52	2058	2	4544
2.0 EDDS	<1	<1	24	58	<1	1008
0.5 EDDS	<1	3	19	65	1	1305
2.0 TSP	2	11	51	48	1	465
0.5 TSP	1	2	39	49	1	548
2.0 SCN	<1	5	47	25	1	656
0.5 SCN	<1	1	15	11	<1	476
2.0 THIO	1	2	76	28	<1	547
0.5 THIO	<1	<1	29	45	1	356

Soil amendments of EDTA produced the highest tissue concentrations of Pb in the three plant species. Both EDTA treatments (0.5 g and 2.0 g kg^{-1}) were non-toxic to *Cyperus victoriensis* and the 0.5 g kg^{-1} treatment produced higher estimated phytoremediation potentials for As (49 g ha^{-1}), Cd (54 g ha^{-1}), Pb (6499 g ha^{-1}), Sb (3 g ha^{-1}) and Zn (7240 kg ha^{-1}) compared to the 2.0 g kg^{-1} EDTA treatment (Table 5.4). However, the As phytoremediation potential of *Cyperus victoriensis* was estimated to be higher using a 2.0 g kg^{-1} DTPA soil amendment (70 g As ha^{-1} $crop^{-1}$). In addition, *Cyperus victoriensis* was estimated to phytoremediate higher levels of Cd from the ST mixture using 2.0 g kg^{-1} EDDS (112 g Cd ha^{-1} $crop^{-1}$) and TSP (112 g Cd ha^{-1} $crop^{-1}$) and with 0.5 g kg^{-1} THIO (110 g Cd ha^{-1} $crop^{-1}$) compared to the 2.0 g kg^{-1} EDTA amendment (37 g Cd ha^{-1} $crop^{-1}$). The 2.0 g kg^{-1} EDDS soil amendments also promoted higher estimated levels for Pb and Zn phytoremediation by *Cyperus victoriensis* (6004 g Pb and 6950 g Zn ha^{-1} $crop^{-1}$) compared to soil

amendments of 0.5 g kg^{-1} EDDS (385 g Pb and 3930 g Zn ha^{-1} $crop^{-1}$). *Crotalaria novae-hollandiae* was estimated to phytoremediate maximum levels of As (50 g ha^{-1} $crop^{-1}$), Cd (37 g ha^{-1} $crop^{-1}$), Pb (9543 kg ha^{-1} $crop^{-1}$), and Sb (3 g ha^{-1} $crop^{-1}$) from the ST mixture after one phytotoxic amendment of 2.0 g kg^{-1} EDTA (Table 5.3). In contrast, five non-toxic weekly amendments of 0.5 g kg^{-1} EDTA reduced the estimated phytoremediation potential of *Crotalaria novae-hollandiae* to 12 g As ha^{-1} $crop^{-1}$, 21 g Cd ha^{-1} $crop^{-1}$, 4514 g Pb ha^{-1} $crop^{-1}$ and 1 g Sb ha^{-1} $crop^{-1}$. The 0.5 g kg^{-1} EDTA amendment raised the estimated Zn phytoremediation potential of *Crotalaria novae-hollandiae* by 60% (9782 g Zn ha^{-1} $crop^{-1}$) compared to the 2.0 g kg^{-1} EDTA soil amendment (3951 g Zn ha^{-1} $crop^{-1}$). However, *Crotalaria novae-hollandiae* tended to rapidly excise leaves in response to toxic applications of the chemical reagents, which would hamper the harvesting of the metal-rich plant biomass essential to the phytoremediation process. *Cymbopogon bombycinus* displayed equal tolerance to both concentrations of EDTA amendments (0.5 g and 2.0 g kg^{-1}) and accumulated greater levels of Ag (12 g ha^{-1} $crop^{-1}$), Cd (55 g ha^{-1} $crop^{-1}$), Pb (4981 kg ha^{-1} $crop^{-1}$), Sb (5 g ha^{-1} $crop^{-1}$) and Zn (4808 kg ha^{-1} $crop^{-1}$) using the 2.0 g kg^{-1} EDTA amendments (Table 5.5). Although, the estimated Cd and Zn phytoremediation potential of *Cymbopogon bombycinus* were very similar (52 g Cd and 4588 g Zn ha^{-1} $crop^{-1}$) using 0.5 g kg^{-1} EDTA amendments compared to the 2.0 g kg^{-1} EDTA amendments. *Cymbopogon bombycinus* was estimated to phytoremediate a maximum quantity of Cd from the ST mixture using one phytotoxic amendment of 2.0 g kg^{-1} THIO (76 g ha^{-1} $crop^{-1}$).

Both applications of short range biodegradable EDDS (0.5 and 2.0 g kg^{-1}) were highly toxic to *Crotalaria novae-hollandiae* (Figure 5.2). The 0.5 g kg^{-1} EDDS treatment promoted slightly higher phytoremediation estimates for As (11 g ha^{-1} $crop^{-1}$) and Cd (24 g ha^{-1} $crop^{-1}$) compared to the 2.0 g kg^{-1} treatment (6 g As and 21 g Cd ha^{-1} $crop^{-1}$) (Table 5.3). The estimated Cd and Zn phytoremediation potential of *Crotalaria novae-hollandiae* were relatively unaffected by the concentration of applied EDDS (≈24 g Cd ha^{-1} $crop^{-1}$ and ≈1560 g Zn ha^{-1} $crop^{-1}$). Whereas the Pb phytoremediation potential of *Crotalaria novae-hollandiae* was estimated to be low using soil amendments of 0.5 g kg^{-1} EDDS (131 g Pb ha^{-1} $crop^{-1}$) and increased slightly using the 2.0 g kg^{-1} EDDS amendments (252 g Pb ha^{-1} $crop^{-1}$). *Cyperus victoriensis*, by comparison, was estimated to phytoremediate high levels of Ag (5 g ha^{-1} $crop^{-1}$), Cd (112 g ha^{-1} $crop^{-1}$), Pb (6004 g ha^{-1} $crop^{-1}$), Sb (3 g ha^{-1} $crop^{-1}$) and Zn (6950 g ha^{-1} $crop^{-1}$) from the ST mixture using the mildly toxic 2.0 g kg^{-1} EDDS amendments.

The data indicate that each plant species accumulated varying amounts of metals and metalloids in response to the various chemical reagents and application strengths. Therefore, the phytoremediation of individual metals and metalloids from the contaminated soil must be prioritised to optimise plant selection. For example, the chelate treatments had variable phytotoxicities and caused significant accumulations of metal and metalloids in all three species. It was estimated that ongoing applications of non-toxic 0.5 g kg^{-1} EDTA to managed pastures of *Crotalaria novae-hollandiae* could

phytoremediate 4514 g Pb ha^{-1} $crop^{-1}$ and 9782 g Zn ha^{-1} $crop^{-1}$ (Table 5.3). In contrast, pastures of *Crotalaria novae-hollandiae* treated with a phytotoxic amendment of 2.0 g kg^{-1} EDTA were estimated to phytoremediate 9543 g Pb ha^{-1} $crop^{-1}$ and 3951 g Zn ha^{-1} $crop^{-1}$ from the contaminated soil. The thio-compound treatments were highly phytotoxic (Figure 5.2) and failed to promote high levels of estimated base metal and metalloid recovery by the plants (cf. Tables 5.3, 5.4 and 5.5). However, the estimated phytoremediation potential for Ag was highest in all plant species using soil amendments of 2.0 g kg^{-1} TSP (Figure 5.3). *Crotalaria novae-hollandiae* was estimated to phytoremediate 12 g Ag ha^{-1} $crop^{-1}$ after one application of 2.0 g kg^{-1} TSP whereas *Cyperus victoriensis* was estimated to phytoremediate 21 g Ag ha^{-1} $crop^{-1}$ using the same chemical amendment. Therefore, the CAP operator could choose to remediate base metals and metalloids from a soil contaminated with mine tailings using applications of EDTA to pastures of *Crotalaria novae-hollandiae* and then target Ag for phytoextraction using TSP amendments to pastures of *Cyperus victoriensis* cultivated on the same ground. However, these soil amendments were found to cause toxicity symptoms and reduced plant growth in all species grown on the contaminated soil. Alternatively, lesser quantities of all the elements could be phytoremediated from the contaminated soil using ongoing amendments of 2.0 g kg^{-1} EDDS to pastures of *Cyperus victoriensis*, which were much less toxic to the plant. This would favour a reduction in the concentration of all heavy metals and metalloids in the contaminated soil while maintaining a relatively healthy vegetation cover.

Finally, the mean concentrations of Sb accumulated by the three test species (Figure 5.8) from the ST mixture were low (<1 μg Sb g^{-1}) using all the chemical amendments (Appendix F). Alloway (1995) reports that the normal range of Sb concentrations in plants is 0.0001-0.2 μg Sb g^{-1}, in addition to indicating that the critical concentration of Sb in plant tissues is 1-2 μg Sb g^{-1}. The data from this study indicate that a soil amendment of 2.0 g kg^{-1} EDTA was the only treatment to promote Sb concentrations in the plants that were within this critical concentration range and only in *Cymbopogon bombycinus* (Appendix F). This indicates that the potential to phytoremediate Sb from the tailings-contaminated soil using the plant species and chemical reagents investigated is very low. In addition, the concentrations of Sb accumulated by *Cymbopogon bombycinus* appear to support the critical plant concentration for Sb defined by Alloway (1995).

This study indicates that large quantities of Cd, Pb and Zn can be phytoremediated from soil contaminated with mine tailings using soil amendments of 0.5 g and 2.0 g kg^{-1} EDTA to pastures of *Cyperus victoriensis*, *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae*. However, the persistence of EDTA in the soil profile is known to effect the leaching of contained heavy metals leading to groundwater contamination (Means *et al.*, 1980). The estimated Pb and Zn phytoremediation potential of *Cyperus victoriensis* using 2.0 g kg^{-1} soil amendments of short-range biodegradable EDDS (Vandevivere *et al.*, 2001b) were comparable to estimates for the 2.0 g kg^{-1} EDTA treatment. However, the estimated phytoremediation potential of *Cyperus*

victoriensis to recover Cd and Sb from the ST mixture using amendments of 2.0 g kg^{-1} EDDS were 70% and 65% higher respectively, compared to the 2.0 g kg^{-1} EDTA treatment. This would suggest that in areas where heavy metal leaching may pose a risk to the local environment, the chemically-assisted phytoremediation of Cd, Pb, Sb and Zn from soil contaminated with mine tailings could be undertaken using EDDS amendments to pastures of *Cyperus victoriensis*.

5.5. Conclusion

This study concludes that the chelates (EDTA, DTPA and EDDS) investigated are capable of promoting significant accumulations of base metals and metalloids in Australian pasture plant species (*Crotalaria novae-hollandiae*, *Cyperus victoriensis* and *Cymbopogon bombycinus*). Soil amendments with the thio-compounds (TSP, SCN and thiourea) generally caused rapid plant death and did not promote high levels of base-metal and metalloid uptake in the plants. However, 2.0 g kg^{-1} TSP did promote high levels of Ag uptake in *Cyperus victoriensis* (4.2 μg g^{-1}) and *Crotalaria novae-hollandiae* (2.3 μg g^{-1}) before chemical toxicity killed the plants. *Crotalaria novae-hollandiae* showed the highest potential to phytoremediate Pb (9543 g ha^{-1} $crop^{-1}$) and Zn (9782 g ha^{-1} $crop^{-1}$) from the contaminated soil using 5.0 g kg^{-1} EDTA amendments. However, toxicity-induced leaf excision caused by both chemical amendments limits this plants use for phytoremediation. *Cymbopogon bombycinus* was estimated to have a low phytoremediation potential for all elements and chemical amendments, except perhaps for Pb and Zn recovery

using 0.5 and 2.0 g kg^{-1} EDTA amendments. It should be noted that *Cymbopogon bombycinus* had the fastest growth rate of the species investigated indicating the potential for higher rates of dry matter production and subsequent metal and metalloid yield by this species. *Cyperus victoriensis* treated with 2.0 g kg^{-1} amendments of EDTA and EDDS was estimated to phytoremediate large quantities of Pb (6499 g and 6004 g ha^{-1} $crop^{-1}$, respectively) and Zn (7240 g and 6950 g ha^{-1} $crop^{-1}$, respectively). In addition, this species was estimated to phytoremediate 112 g Cd ha^{-1} $crop^{-1}$ using soil amendments of 2.0 g kg^{-1} EDDS and TSP as well as 110 g Cd ha^{-1} $crop^{-1}$ using amendments of 0.5 g kg^{-1} THIO to the soil contaminated with 12.5 wt% mine tailings.

Chapter 6. Chemically-Assisted Phytoextraction of Metals and Metalloids from Mine Tailings and Soil Contaminated with Mine Tailings using Rhodes grass (*Chloris gayana* Kunth cv. Pioneer)

6.1. Introduction

The use of monocotyledon pasture systems for the revegetation of degraded lands and mine waste repositories has become an integral part of modern minesite rehabilitation programs (Russell and Roberts, 1986; Harwood *et al.*, 1999). Tussock-forming grasses such as Buffel grass (*Cenchrus ciliaris*), Vetiver grass (*Vetiveria zizaniodes*) and Rhodes grass (*Chloris gayana* Kunth cv. Pioneer) are commonly used in the revegetation of coal mine wastes (Truong and Baker, 1998; Grigg *et al.*, 2000; Mentis, 2001). However, the use of these vegetative covers to perform remedial work on soil contaminated with heavy metals, beyond erosion control and moderating saline runoff (Truong, 1998), is largely unexplored. In particular, the application of pasture grasses to phytoremediate heavy metal contaminants from soils using chemical amendments requires evaluation.

Phytoextraction is a plant-based environmental tool for the *in situ* removal of contaminants from soil (Chaney *et al.*, 1999). The technology preserves valuable physical and chemical soil properties and as a result, industry often

favour these rehabilitation practices because operational and environmental costs are comparatively low (Glass, 2000). Several field applications have demonstrated the efficiency of the technology using agricultural crops (e.g. *Zea mays*, *Brassica juncea*) and chelate amendments to decontaminate metal laden soils (Banuelos, 1993; Blaylock, 1999, 2000). However, very little published information exists on the use of grass species to perform phytoextraction.

The success of a phytoextraction operation is largely dependent upon the selection of plant species. Certain plant species are known to *hyper*accumulate metals and metalloids (Brooks, 1998) and have been studied for their environmental applications (e.g. Baker *et al.*, 1994; Brown *et al.*, 1994; McGrath *et al.*, 1999). However, heavy metal hyperaccumulation by plants is generally limited to single or narrow groups of elements (e.g. Ni and Co, Cd and Zn) (Brooks, 1980; Reeves and Baker, 2000; Keeling *et al.*, 2003). In addition, heavy metal hyperaccumulating plant species are extremely rare in the botanical record and often exhibit growth behaviours (e.g. low biomass production, potential to be highly invasive) that make them unsuitable for effective phytoextraction (Brooks, 1998).

To overcome this problem, various chemical amendments have been employed to dissolve heavy metals in contaminated soils and to promote their uptake by agricultural crop species (*Brassica juncea*, *B. napus*) (e.g. Blaylock *et al.*, 1997; Cooper *et al.*, 1999; Begonia *et al.*, 2002; Rossi *et al.*, 2002).

Dicotyledonous plants, such as those listed above, were chosen for phytoextraction studies because considerable knowledge exists concerning their cultivation and management; in addition, some species display heavy-metal-accumulating potential (Dushenkov *et al.*, 1995; Ebbs *et al.*, 1997). Chelating agents, such as EDTA and DTPA, are highly effective at promoting Pb uptake by these species (Huang *et al.*, 1997a; Wu *et al.*, 1999). However, the persistence of chelates in the soil (Means *et al.*, 1980) may lead to the leaching of contaminants into groundwater (Yordanov and Roundhill, 1998). The short range biodegradable chelate EDDS may represent a means of limiting potential leaching while still promoting heavy metal uptake by plants (Vandevivere *et al.*, 2001b; Greman *et al.*, 2003). While soil amendments of EDTA, DTPA and EDDS have been used to chemically assist the uptake of metals by plants, the use of thio-compounds to promote phytoextraction of metals from soil remains largely unexplored (Anderson *et al.*, 1999b; Keeling, 2001). Thiosulphate and thiocyanate act as complexing agents for precious metals (PGE, Au, Ag) (Mann, 1984; Plimer and Williams, 1987) and, in addition to thiourea, have been used to leach Au and Ag from geologic materials (Akretche *et al.*, 1995; Feng and Van Deventer, 2002). It was thought that these reagents might provide mechanisms for the dissolution and phytoextraction of Ag (as well as other metals and metalloids) from soil contaminated with mine tailings and from directly vegetated mine tailings.

This study was conducted to evaluate the environmental applications of

Chloris gayana at the Cannington mine, NW Queensland. The CAP of Ag, As, Cd, Pb, Sb and Zn by *Chloris gayana* from soils contaminated with mine tailings was facilitated using amendments of EDTA, DTPA, EDDS, TSP, SCN and THIO. The uptake of metals by the plants was used to evaluate the species potential to phytoremediate heavy metals and metalloids from the contaminated soil. The long-term revegetation potential of *Chloris gayana* was also determined using fertiliser amendments to undiluted mine tailings. Phytoextraction of Ag, Pb and Zn from fertilised mine tailings was then investigated using chemical amendments of EDTA, EDDS and SCN to determine the species potential to recover valuable metals from revegetated fertilised mine tailings.

6.2. Materials and methods

Two pot trial experiments were conducted involving the cultivation of Rhodes grass on an anthroposol soil contaminated with 12.5 wt% mine tailings and on fertilised and unfertilised mine tailings (Chapter 2, Section 2.11.3). A soil contamination level of 12.5 wt% mine tailings was selected because the mass of tailings added to the soil was sufficient to elevate the Pb concentration to 1520 μg Pb g^{-1}. This total Pb concentration is slightly above the Australian and New Zealand Environment and Conservation Council (ANZECC) Investigation Limit for Pb contaminated industrial and commercial sites (1500 μg Pb g^{-1}) and was therefore a useful benchmark concentration upon which to base the investigation (ANZECC, 1999). Rhodes grass was cultivated on the contaminated soil for twelve weeks and

then the soil was amended with weekly applications (0.5 and 2.0 g kg^{-1} soil) of various chemical reagents (EDTA, EDDS, DTPA, TSP, SCN and THIO) for a five-week period (Chapter 2, Section 2.12.2). Rhodes grass was also cultivated on undiluted mine tailings amended with 300 kg ha^{-1} of N-rich fertiliser (Osmocote - OSM) in addition to 300 kg ha^{-1} of N-rich fertiliser plus 300 kg ha^{-1} of a P-rich fertiliser (Triphosphate - TPP) (Chapter 2, Section 2.11.3). The plants were allowed to mature for twelve weeks prior to receiving the first of four applications of the chemical amendments (2.0 g EDTA, EDDS or SCN kg^{-1} tailings) (Chapter 2, Section 2.12.2), applied every ten weeks over a thirty-week period. Control pots for each fertiliser treatment (OSM-Cont. and TPP-Cont.) were set aside prior to commencement of the chemical treatments and were not amended with the reagents over the 42-week experiment period. Control plants were sampled at the same time as plant samples were collected for the first chemical amendment to the fertilised mine tailings. The remaining vegetation samples from each of the chemically amended and fertilised mine tailings (OSM-EDTA, OSM-EDDS, OSM-SCN, TPP-EDTA, TPP-EDDS, TPP-SCN) were also collected (Chapter 2, Section 2.12.1) one week after application of the chemical amendments. Vegetation samples collected from the chemically amended and mine-tailings contaminated soil were analysed (Chapter 2, Section 2.14) for Ag, As, Cd, Pb, Sb and Zn (Appendix G) whereas vegetation samples from the chemically amended and fertilised mine tailings were analysed for Ag, Pb and Zn (Appendix H). Post-harvest soil extractions (Chapter 2, Section 2.11.6) were performed on the soil contaminated with 12.5 wt% mine tailings (Appendix E) and the fertilised and unfertilised mine tailings (Appendix I) to

estimate the availability of metals and metalloids to the plants in the substrates.

6.3. Results

6.3.1. Metals and metalloids extractability

The total concentration of metals and metalloids contained in the soil contaminated with 12.5 wt% mine tailings was 8 μg Ag g^{-1}, 261 μg As g^{-1}, 4 μg Cd g^{-1}, 1520 μg Pb g^{-1}, 335 μg Sb g^{-1} and 582 μg Zn g^{-1} (Appendix A), whereas the fertilised and unfertilised mine tailings contained 60 μg Ag g^{-1}, 2039 μg As g^{-1}, 30 μg Cd g^{-1}, 11950 μg Pb g^{-1} and 4150 μg Zn g^{-1}. This indicates that the total Pb concentration of the contaminated soil exceeded the ANZECC Investigation Limits for industrial and commercial sites (1500 μg Pb g^{-1}), whereas the mine tailings exceeded these same Investigation Limits for Pb in addition to As (500 μg Pb g^{-1}) (ANZECC, 1999).

Table 6.1. Total (μg g^{-1}) and proportional (% TEC) metal and metalloid concentrations in solvent extractions of the soil contaminated with 12.5 wt% mine tailings. Total metal and metalloid concentrations are also shown.

Extract	Metal and Metalloid Extractability [μg g^{-1} (% TEC)]					
	Ag	As	Cd	Pb	Sb	Zn
deionised H_2O	<0.01 (<0.1%)	0.8 (0.3%)	0.1 (2.5%)	0.1 (<0.1%)	0.3 (0.1%)	2.1 (0.4%)
0.01 M EDTA	0.01 (0.1%)	30.4 (11.6%)	2.3 (57.5%)	1502 (98.8%)	2.5 (0.7%)	358 (61.5%)
0.005 M DTPA	0.01 (0.1%)	12.3 (4.7%)	2.3 (57.5%)	1283 (84.4%)	1.5 (0.4%)	352 (60.4%)
0.01 M EDDS	0.01 (0.1%)	7.0 (2.7%)	1.8 (45%)	1311 (86.3%)	2.5 (0.7%)	244 (41.9%)
Total Conc.	8	261	4	1520	335	582

Solvent extractions were performed on the soil contaminated with 12.5 wt% mine tailings (Table 6.1) and the fertilised and unfertilised mine tailings (Table 6.2) using deionised water, 0.01 M EDTA, 0.005 M DTPA and 0.01 M EDDS. The water soluble concentrations of As (0.8 μg g^{-1}) and Cd (0.1 μg g^{-1}) in the contaminated soil (Table 6.1) exceeded the ANZECC Groundwater Investigation Limits (0.1 μg As g^{-1}, 0.01 μg Cd g^{-1}) (ANZECC, 1999). The concentration of Pb extracted from the contaminated soil by deionised water was equivalent to the ANZECC Groundwater Investigation Limit (0.1 μg Pb g^{-1}). This indicates that the contaminated soil would pose a potential risk to the environment from leaching and groundwater contamination. The proportions of Ag, As, and Sb extracted from the contaminated soil (Table 6.1) using 0.01M EDTA and 0.005M DTPA were low (<12 % of the Total Element Concentration, TEC). In contrast, the proportions of Cd, Pb and Zn extracted by 0.01 M EDTA and 0.005 M DTPA from the contaminated soil were much higher (≈57% Cd, 84%-99% Pb and ≈61% Zn TEC).

The 0.01 M EDTA extraction removed the highest concentration of As (30.4 μg g^{-1}), Pb (1502 μg g^{-1}) and Zn (358 μg g^{-1}) from the contaminated soil compared to extraction with 0.005 M DTPA and 0.01 M EDDS (Table 6.1). The 0.005 M DTPA extraction removed a similar concentration of Cd (2.3 μg g^{-1}) from the contaminated soil and 0.01 M EDDS removed a similar concentration of Sb (2.5 μg g^{-1}). In addition, 0.01 M EDTA extracted higher concentrations of As and Pb from the contaminated soil compared to 0.005 M DTPA, and the 0.005 M DTPA extraction removed higher concentrations

of these elements compared to the 0.01 M EDDS extraction (Table 6.1). In contrast, soil extractions with 0.01 M EDTA and 0.005 M DTPA contained approximately equivalent concentrations of Zn (≈355 μg g^{-1}), whereas extractions with 0.01 M EDDS contained 244 μg Zn g^{-1}. Soil extractions with 0.01 M EDTA, 0.005 M DTPA and 0.01 M EDDS removed equally low concentrations of Ag, Cd and Sb from the soil contaminated with 12.5 wt% mine tailings. Overall, the three reagents extracted similar concentrations of metals and metalloids from the soil contaminated with 12.5 wt% mine tailings. The data indicate that the average relative concentrations of plant-available metals and metalloids extracted by 0.01 M EDTA, 0.005 M DTPA and 0.01 M EDDS from the soil contaminated with 12.5 wt% mine tailings were Pb>>Zn>>Sb=Cd>Ag (Table 6.1).

Table 6.2. Chemical properties (pH, EC) and the total (μg g^{-1}) and proportional (% of total metal concentration) Pb and Zn extractability for the unfertilised and fertilised (OSM = 300 kg ha^{-1} equiv. Osmocote, TPP = OSM + 300 kg ha^{-1} equiv. Triphosphate) mine tailings (11950 μg Pb g^{-1}, 4150 μg Zn g^{-1} and 60 μg Ag g^{-1}). Silver concentrations in extract solutions were below the detection limit (0.005 μg g^{-1}) and are not reported.

Parameter	Unfertilised Tailings	OSM Fertilised Tailings	TPP Fertilised Tailing
pH	5.4	5.4	5.9
EC (μS cm^{-1})	5760	5870	6080
		Extractable Pb (μg g^{-1})	
deionised H_2O	0.3 (<0.1%)	1.9 (<0.1%)	0.1 (<0.1%)
0.01 M EDTA	892 (7.5%)	843 (7.1%)	101 (0.8%)
0.005 M DTPA	208 (1.7%)	36 (0.3%)	749 (6.3%)
0.01 M EDDS	902 (7.5%)	551 (4.6%)	346 (2.9%)
		Extractable Zn (μg g^{-1})	
deionised H_2O	1.7 (<0.1%)	33.1 (0.8%)	2.8 (0.1%)
0.01 M EDTA	157 (3.8%)	106 (2.6%)	97.2 (2.3%)
0.005 M DTPA	135 (3.3%)	98.0 (2.4%)	100 (2.4%)
0.01 M EDDS	95.0 (0.8%)	73.6 (1.8%)	51.0 (1.2%)

Deionised water extracted higher amounts of Pb and Zn from tailings fertilised with OSM compared to the unfertilised tailings and the TPP-fertilised tailings (Table 6.2). The aqueous extractability of Pb in the unfertilised (control) tailings and the OSM-fertilised tailings exceeded the ANZECC Groundwater Investigation Limits (0.1 μg Pb g^{-1}) indicating that tailings dam leachates could be environmentally hazardous (ANZECC, 1999). Furthermore, the concentration of Pb in deionised water extractions of the TPP-fertilised tailings was equivalent to the ANZECC Groundwater Investigation Limits (0.1 μg Pb g^{-1}). This indicates that the addition of OSM to the mine tailings significantly raised the solubility of Pb (1.9 μg g^{-1}) compared to the unfertilised mine tailings (0.3 μg Pb g^{-1}) and the TPP fertilised mine tailings (0.1 μg Pb g^{-1}). Zinc solubility was also significantly raised by the addition of OSM fertiliser (33.1 μg Zn g^{-1}) to the mine tailings compared to the unfertilised mine tailings (1.7 μg Zn g^{-1}).

The 0.01 M EDTA and 0.005 M DTPA extractions are commonly used to estimate the concentrations of plant-available metals and metalloids in contaminated soil (Norvell, 1991; Ma and Lindsay, 1993; Li and Shuman, 1996). The EDTA and DTPA extractions performed in this study removed slightly less Pb and Zn from fertilised tailings compared to unfertilised tailings (Table 6.2). The extraction of Ag using 0.01 M EDTA, 0.005 M DTPA and 0.01 M EDDS were below detection limit for both fertilised and unfertilised tailings (Appendix I). The 0.01 M EDDS extraction removed a higher concentration of Pb from the unfertilised mine tailings (902 μg Pb g^{-1})

compared to 0.01 M EDTA (892 μg Pb g^{-1}) and 0.005 M DTPA (208 μg g^{-1}) (Table 6.2). In contrast, 0.01 M EDDS dissolved lower concentrations of Zn from the OMS-fertilised tailings (73.6 μg Zn g^{-1}) and the TPP-fertilised tailings (51.0 μg Zn g^{-1}) compared to 0.01 M EDTA and 0.005 M DTPA. Consistently higher concentrations of Pb were extracted from the fertilised mine tailings by EDTA and EDDS compared to Zn (Table 6.2). This indicates that the plant available concentrations of Pb in both substrate types were significantly higher than the plant available concentrations of Zn.

6.3.2. Plant growth

The growth of *Chloris gayana* on a soil contaminated with 12.5 wt% mine tailings was unaffected by repeated weekly applications of both concentrations of EDTA and EDDS (0.5 and 2.0 g kg^{-1}), as well as 2 g kg^{-1} DTPA and 0.5 g kg^{-1} TSP amendments. Visible phytotoxicity (yellowing of leaves and leaf-tip necrosis) to the 2 g kg^{-1} TSP treatment became apparent after four weekly amendments to the contaminated soil, whereas 0.5 and 2.0 g kg^{-1} SCN and THIO amendments resulted in plant death over the first two weeks of treatment. Chemical amendments of SCN (2.0 g kg^{-1}) to fertilised tailings resulted in rapid plant death and were associated with elevated concentrations of Ag in *Chloris gayana* compared to 2.0 g kg^{-1} amendments of EDTA and EDDS (Section 6.3.3). The 2.0 g kg^{-1} EDTA and EDDS amendments were non-toxic to *Chloris gayana* and resulted in slightly higher Pb uptake and significantly higher Zn uptake by the plant (Section 6.3.3).

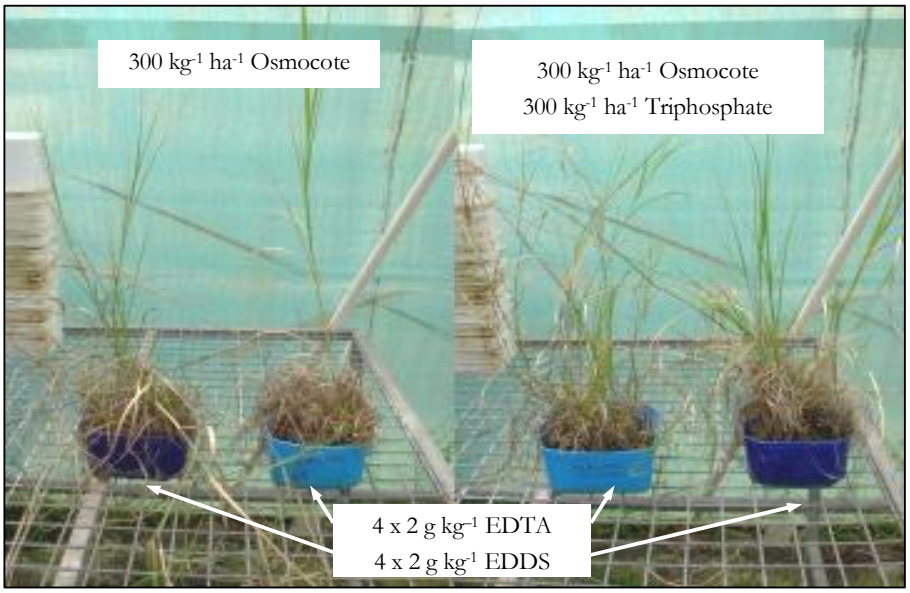

Figure 6-1. The growth of *Chloris gayana* after 42 weeks of cultivation on fertilised mine tailings, having received four applications of the chelates; EDTA and EDDS.

Plants cultivated on fertilised mine tailings that were not amended with the chemical reagents (OSM-Cont. and TPP-Cont.) remained healthy for approximately 9 months of the experiment. After nine months, the health of *Chloris gayana* declined slightly and plants developed minor leaf tip necrosis and chlorotic foliage. However, at the completion of the experiment (week 42), both fertiliser treatments still maintained a groundcover over the mine tailings (Figure 6.1). In addition, plants grown on the OSM-fertilised tailings did not flower or develop seed heads compared to plants grown on mine tailings receiving OSM and TPP fertilisers.

6.3.3. Plant chemistry for contaminated soil

Chelate amendments of 2.0 g kg^{-1} EDTA and DTPA promoted maximum As accumulations (6.3 ± 3.5 and 5.5 ± 0.5 μg As g^{-1} respectively) in *Chloris gayana* (Figure 6.2) (Appendix G). Lead uptake by *Chloris gayana* peaked after four weekly chemical amendments with 2.0 g EDTA kg^{-1} (146 ± 61.3 μg Pb g^{-1}) and decreased thereafter (98.1 ± 30.8 μg Pb g^{-1}) (Figure 6.3). In contrast, soil amendments of 2.0 g kg^{-1} DTPA promoted maximum Pb uptake in *Chloris gayana* (68.1 ± 14.4 μg Pb g^{-1}) after two and four weekly treatments respectively. In contrast, Cd uptake using 2 g kg^{-1} EDDS (4.7 ± 3.0 μg Cd g^{-1}) were maximal after three weekly chemical treatments (Figure 6.4). Chemically-assisted uptake of Sb by *Chloris gayana* from soil contaminated with 12.5 wt% mine tailings (Figure 6.5) was greatest after one treatment of 2 g kg^{-1} DTPA (0.3 ± 0.06 μg Sb g^{-1}) and then decreased with further weekly chemical amendments. Soil amendments of 0.5 g kg^{-1} TSP and 2.0 g kg^{-1} EDDS also promoted a modest level of Sb uptake (≈0.2 μg Sb g^{-1}) in *Chloris gayana* after three weekly chemical treatments. *Chloris gayana* accumulated a maximum concentration of Zn from the contaminated soil (Figure 6.7) after four weekly chemical treatments of 2.0 g kg^{-1} EDDS (417 ± 347 μg Zn g^{-1}) and then decreased slightly after a further application of the chelate (391 ± 374 μg Zn g^{-1}). In contrast, 2 g kg^{-1} DTPA promoted maximum accumulation of Zn in *Chloris gayana* after one chemical treatment (300 ± 118 μg Zn g^{-1}). Zinc uptake by *Chloris gayana* then progressively decreased with further weekly amendments of 2.0 g kg^{-1} DTPA.

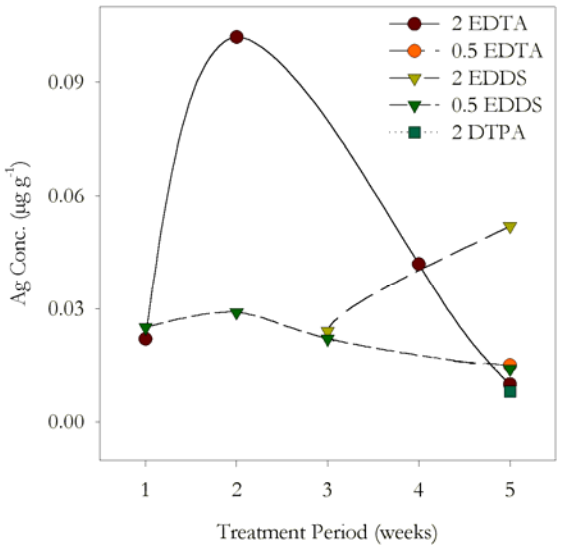

Figure 6-2. The Ag concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with EDTA, EDDS and DTPA (e.g. 2.0 EDTA = 2.0 g EDTA per kg contaminated soil; 0.5 EDTA = 0.5 g EDTA per kg contaminated soil; etc).

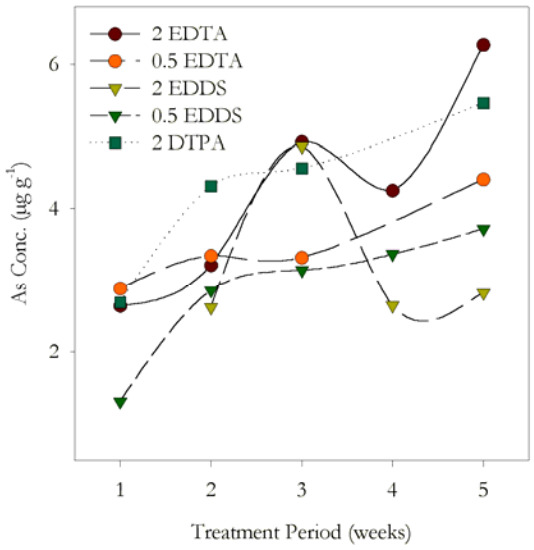

Figure 6-3. The As concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with EDTA, EDDS and DTPA (e.g. 2.0 EDTA = 2.0 g EDTA per kg contaminated soil; 0.5 EDTA = 0.5 g EDTA per kg contaminated soil; etc).

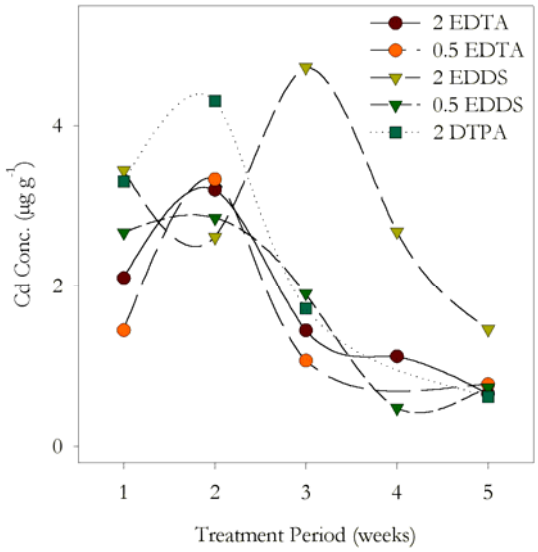

Figure 6-4. The Cd concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with EDTA, EDDS and DTPA (e.g. 2.0 EDTA = 2.0 g EDTA per kg contaminated soil; 0.5 EDTA = 0.5 g EDTA per kg contaminated soil; etc).

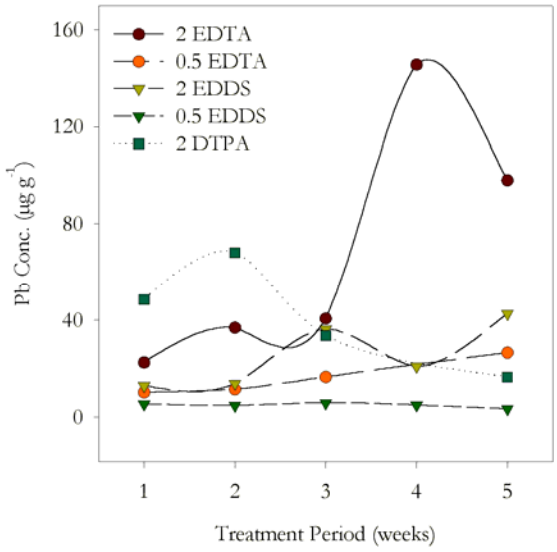

Figure 6-5. The Pb concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with EDTA, EDDS and DTPA (e.g. 2.0 EDTA = 2.0 g EDTA per kg contaminated soil; 0.5 EDTA = 0.5 g EDTA per kg contaminated soil; etc).

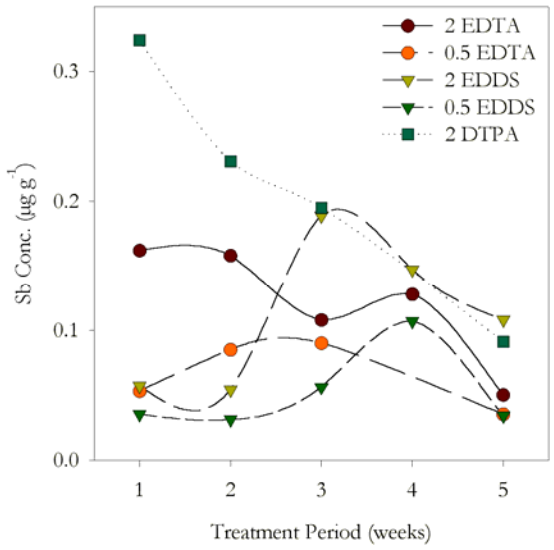

Figure 6-6. The Sb concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with EDTA, EDDS and DTPA (e.g. 2.0 EDTA = 2.0 g EDTA per kg contaminated soil; 0.5 EDTA = 0.5 g EDTA per kg contaminated soil; etc).

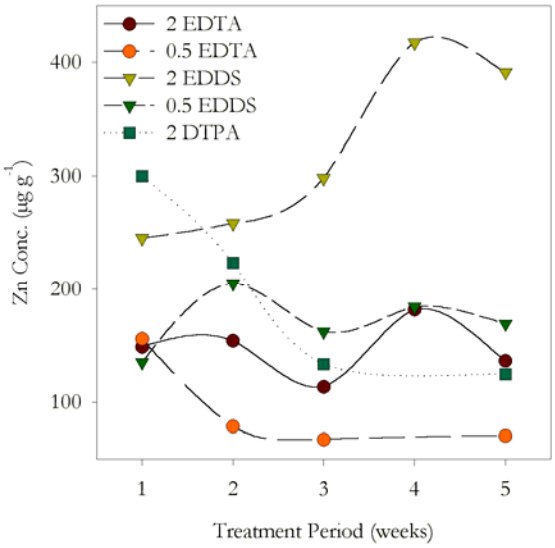

Figure 6-7. The Zn concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with EDTA, EDDS and DTPA (e.g. 2.0 EDTA = 2.0 g EDTA per kg contaminated soil; 0.5 EDTA = 0.5 g EDTA per kg contaminated soil; etc).

In contrast, soil amendments of 2.0 g kg^{-1} DTPA and TSP promoted maximum Pb uptake in *Chloris gayana* (68.1 ± 14.4 and 90.7 ± 62.0 µg Pb g^{-1}, respectively) after two and four weekly treatments respectively. Silver and Cd uptake (Figure 6.4 and 6.5 respectively) by *Chloris gayana* was highest after four weekly amendments of 2 g TSP kg^{-1} (1.8 ± 0.7 µg Pb g^{-1} and 5.8 ± 2.6 µg Pb g^{-1} respectively), compared to the other chemical treatments.

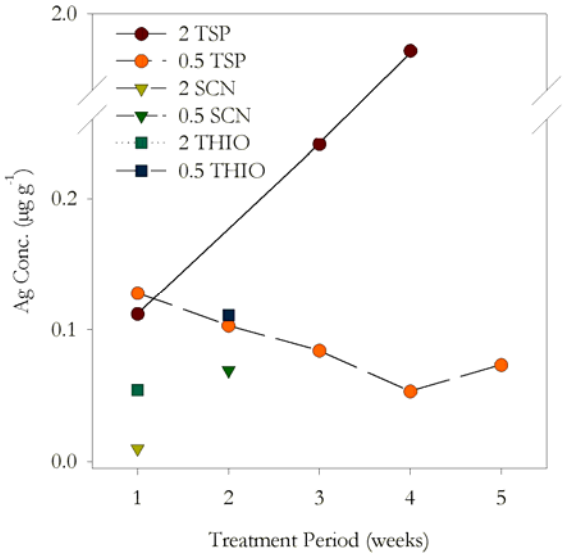

Figure 6-8. The Ag concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with ammonium thiosulphate (TSP), ammonium thiocyanate (SCN) and thiourea (THIO) (e.g. 2.0 TSP = 2.0 g TSP per kg contaminated soil; 0.5 TSP = 0.5 g TSP per kg of soil; etc).

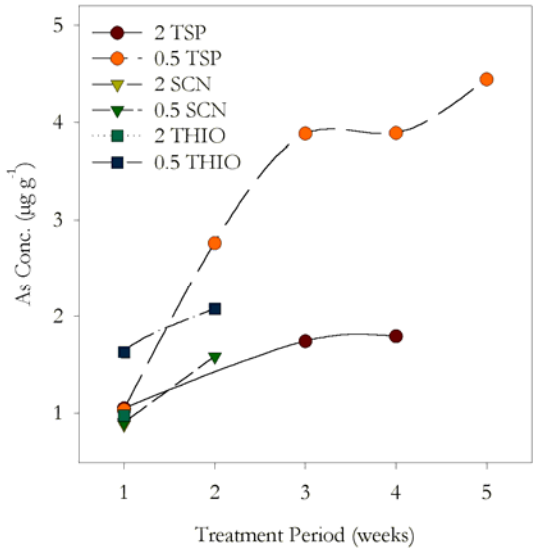

Figure 6-9. The As concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with ammonium thiosulphate (TSP), ammonium thiocyanate (SCN) and thiourea (THIO) (e.g. 2.0 TSP = 2.0 g TSP per kg contaminated soil; 0.5 TSP = 0.5 g TSP per kg of soil; etc).

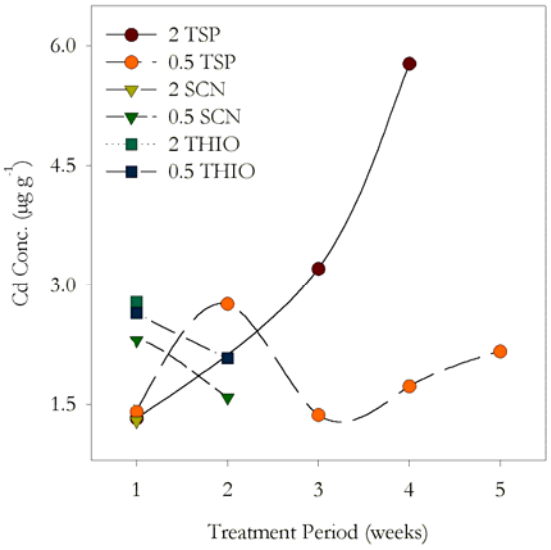

Figure 6-10. The Cd concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with ammonium thiosulphate (TSP), ammonium thiocyanate (SCN) and thiourea (THIO) (e.g. 2.0 TSP = 2.0 g TSP per kg contaminated soil; 0.5 TSP = 0.5 g TSP per kg of soil; etc).

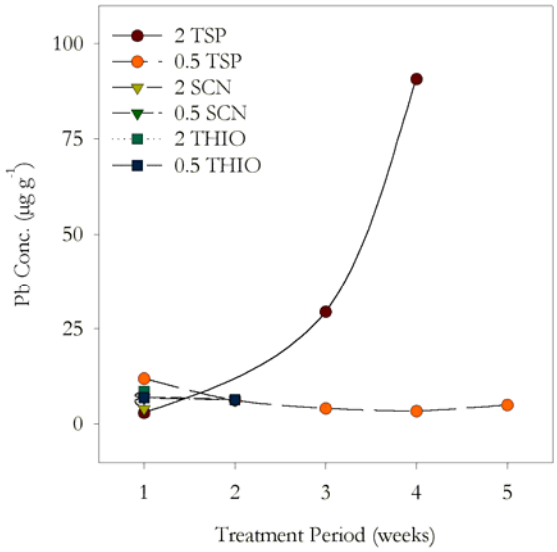

Figure 6-11. The Pb concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with ammonium thiosulphate (TSP), ammonium thiocyanate (SCN) and thiourea (THIO) (e.g. 2.0 TSP = 2.0 g TSP per kg contaminated soil; 0.5 TSP = 0.5 g TSP per kg of soil; etc).

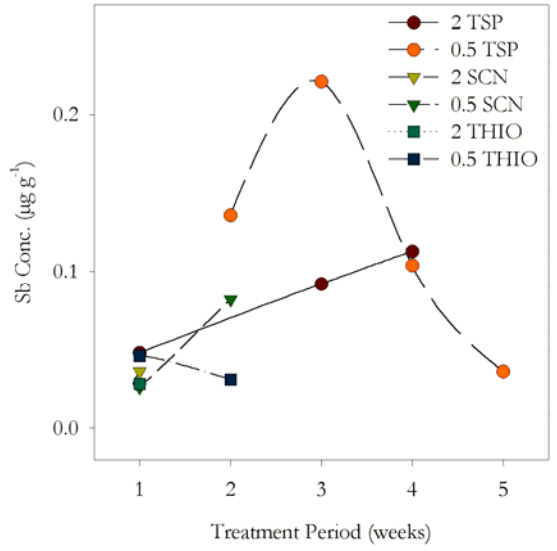

Figure 6-12. The Sb concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with ammonium thiosulphate (TSP), ammonium thiocyanate (SCN) and thiourea (THIO) (e.g. 2.0 TSP = 2.0 g TSP per kg contaminated soil; 0.5 TSP = 0.5 g TSP per kg of soil; etc).

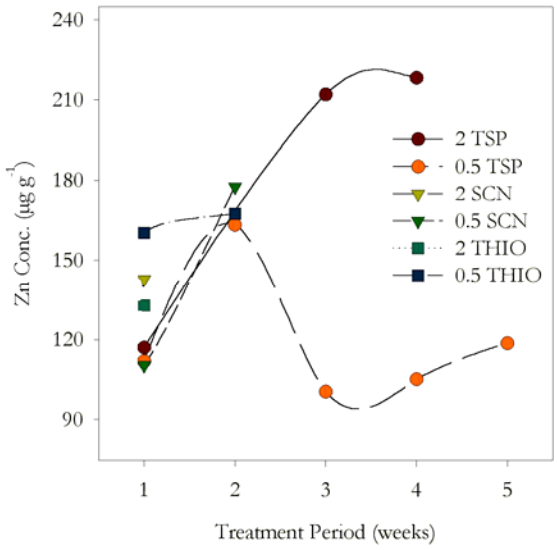

Figure 6-13. The Zn concentrations (µg g^{-1}) in *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings having received weekly amendment with ammonium thiosulphate (TSP), ammonium thiocyanate (SCN) and thiourea (THIO) (e.g. 2.0 TSP = 2.0 g TSP per kg of soil; etc).

6.3.4. Plant chemistry for fertilised tailings

The Pb concentration in the leaves of *Chloris gayana* grown on the TPP-fertilised tailings (Figure 6.8) that were not amended with the chemical reagents (TPP-Cont.) was significantly higher (69 µg Pb g^{-1}) than the Pb concentration in leaves collected from the unamended OSM-fertilised tailings (OSM-Cont.) (17 µg Pb g^{-1}) and all of the chemical treatments (Appendix G). By comparison, the concentration of Zn in *Chloris gayana* grown on the fertilised mine tailings in the absence of the chemical reagents (OSM-Cont. and TPP-Cont.) were much lower (≈180 µg Zn g^{-1}) than the concentrations of Zn accumulated by the plant following application of the chemical amendments (Figure 6.9). In contrast, Ag uptake from control pots

containing OSM-fertilised tailings (0.21 µg Ag g^{-1}) was approximately equivalent to Ag uptake from the TPP-fertilised tailings (0.16 µg Ag g^{-1}). Control samples of *Chloris gayana* contained higher concentrations of Ag compared to samples collected from fertilised mine tailings that were amended with 2.0 g kg^{-1} of the chelates (EDTA and EDDS).

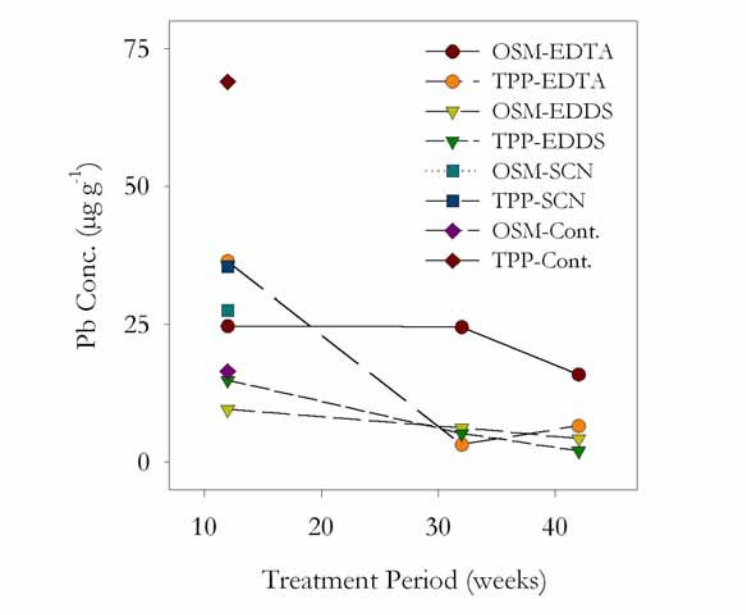

Figure 6-14. The Pb concentration (µg g^{-1}) of *Chloris gayana* grown on fertilised mine tailings over 42 weeks and treated with chemical amendments (e.g. OSM-EDTA = mine tailings amended with 300 kg Osmocote fertiliser ha^{-1} in addition to receiving periodic amendments of 2.0 g EDTA kg^{-1} of fertilised mine tailings; TPP-EDTA = mine tailings amended with 300 kg Osmocote fertiliser ha^{-1} + 300 kg Triphosphate fertiliser ha^{-1} in addition to receiving periodic amendments of 2.0 g EDTA kg^{-1} fertilised mine tailings).

Periodic application (10-week intervals) of the chelate treatments (2.0 g kg^{-1} EDTA and EDDS) did not result in a deleterious plant-growth response by *Chloris gayana* over the course of the experiment (Figure 6.8). In contrast, the

application of 2.0 g kg^{-1} SCN to mine tailings amended with both fertiliser treatments resulted in rapid plant death. Compared to the concentration of Pb in control samples, only the first amendment of 2.0 g kg^{-1} EDTA and SCN to OSM-fertilised tailings resulted in elevated concentrations of Pb in the leaves of *Chloris gayana*. The concentrations of Pb in the leaves of *Chloris gayana* then decreased with subsequent amendments of EDTA and SCN to OSM-fertilised tailings. Periodic applications of the remaining chemical amendments to the fertilised mine tailings (TPP-EDTA, OSM-EDDS, TPP-EDDS, TPP-EDDS) did not result in plant death nor did they elevate the concentration of Pb in *Chloris gayana* compared to control samples. Chemical amendments (2.0 g kg^{-1} SCN, EDTA and EDDS) to TPP-fertilised tailings promoted higher Pb concentrations in the leaves of *Chloris gayana* (34.4, 36.4 and 14.9 μg Pb g^{-1} respectively) compared to chemically-assisted Pb uptake from the OSM-fertilised tailings (27.6, 24.7 and 9.6 μg Pb g^{-1} respectively).

The concentrations of Zn in the leaves of *Chloris gayana* decreased over time for all chemical amendments applied to both types of fertilised mine tailings (Figure 6.9). The exception was EDDS amendments to mine tailings fertilised with only Osmocote, which raised the concentration of Zn in *Chloris gayana* after three 10-week treatments from 773.4 μg Zn g^{-1} to 831.4 μg Zn g^{-1}. Zinc concentrations in *Chloris gayana* were greatest following one application of 2.0 g kg^{-1} EDTA to the TPP-fertilised tailings (1907.5 μg g^{-1}). One application of 2.0 g kg^{-1} EDDS to the same substrate also elevated the Zn concentration in *Chloris gayana* (1079.1 μg g^{-1}) compared to unamended control plants (140.9 μg

g^{-1}). In contrast, EDTA amendments to OSM-fertilised tailings progressively reduced the concentrations of Zn in the leaves of *Chloris gayana* from 696.9 μg g^{-1} to 399.2 μg g^{-1} over the treatment period. The Zn concentration in *Chloris gayana* after four applications of 2.0 g kg^{-1} EDTA (to both the OSM- and TPP-fertilised tailings) in addition to the 2.0 g kg^{-1} EDDS treatment (to TPP-fertilised tailings only) was similar to the concentrations of Zn in control samples.

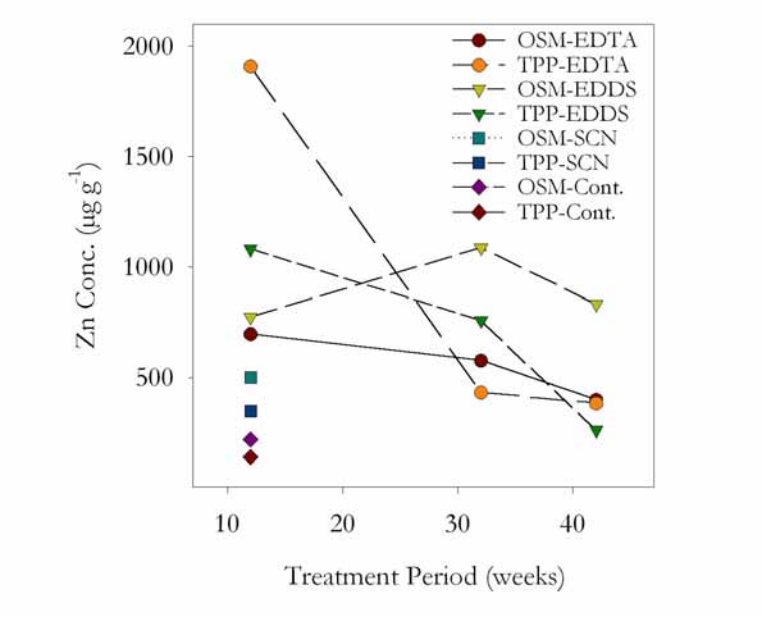

Figure 6-15. The Zn concentration (μg g^{-1}) in *Chloris gayana* grown on fertilised mine tailings over 42 weeks and treated with chemical amendments (e.g. OSM-EDTA = mine tailings amended with 300 kg Osmocote fertiliser ha^{-1} in addition to receiving periodic amendments of 2.0 g EDTA kg^{-1} fertilised mine tailings; TPP-EDTA = mine tailings amended with 300 kg Osmocote fertiliser ha^{-1} + 300 kg of Triphosphate fertiliser ha^{-1} in addition to receiving periodic amendments of 2.0 g EDTA kg^{-1} fertilised mine tailings).

The CAP of Ag from fertilised mine tailings using the chelate amendments (2.0 g kg^{-1} EDTA and EDDS) was low (0.01-0.1 μg Ag g^{-1}) compared to Ag concentrations in control samples (approximately 0.2 μg Ag g^{-1}). The application of 2.0 g kg^{-1} SCN to OSM-fertilised tailings did not appreciably elevate the concentration of Ag in the leaves of *Chloris gayana* compared to the control. In contrast, the application of 2.0 g kg^{-1} SCN to mine tailings fertilised with Osmocote plus Triphosphate (TPP) promoted high accumulations of Ag (2.4 μg g^{-1}) in the leaves of *Chloris gayana* (Figure 6.10).

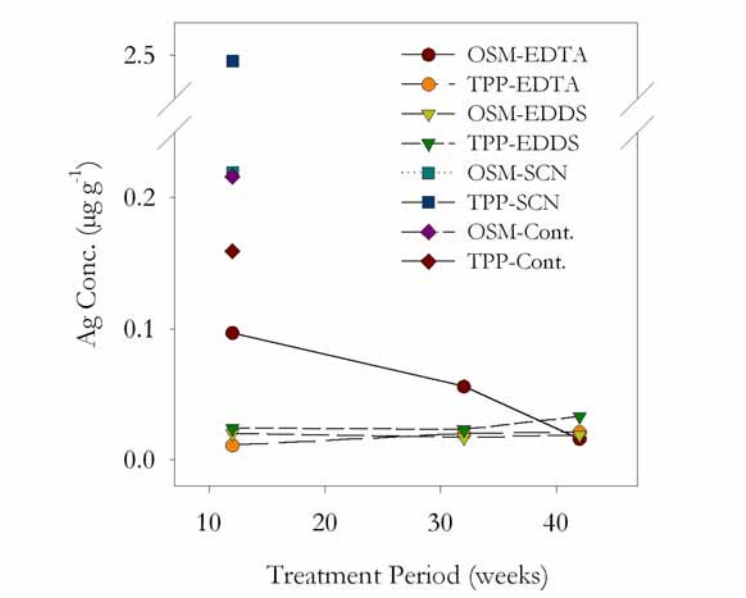

Figure 6-16. The Ag concentration (μg g^{-1}) in *Chloris gayana* grown on fertilised mine tailings over 42 weeks and treated with chemical amendments (e.g. OSM-EDTA = mine tailings amended with 300 kg Osmocote fertiliser ha^{-1} in addition to receiving periodic amendments of 2.0 g EDTA kg^{-1} fertilised mine tailings; TPP-EDTA = mine tailings amended with 300 kg of Osmocote fertiliser ha^{-1} + 300 kg Triphosphate fertiliser ha^{-1} in addition to receiving periodic amendments of 2.0 g EDTA kg^{-1} fertilised mine tailings).

6.4. Discussion

The rehabilitation of soils and mine waste materials containing elevated concentrations of heavy metals and metalloids can be undertaken to achieve several different goals. Contaminated soils and mine wastes can simply be bound together using the root systems of cover vegetation thus reducing the physical migration of contaminants to surrounding environments (Cunningham and Berti, 2000). Once established on contaminated soil or mine waste, the cover vegetation may then be returned to an agricultural land use capability. Alternatively, chemical amendments can then be applied to the soil to promote the uptake of heavy metals and metalloids by the cover vegetation (i.e. phytoremediation). The metal-rich biomass can then be harvested and removed from site thereby reducing the soil's contaminant load. In addition, chemical amendments may also promote the uptake of valuable heavy metals by the cover vegetation (i.e. phytomining), which may also be harvested to recover the metals for sale (Brooks *et al.*, 1998). Successful field demonstrations of chemically-assisted Pb phytoremediation, in addition to the phytoremediation of other heavy metals and metalloids, are increasing in the literature (Cunningham and Ow, 1996; Blaylock *et al.*, 1997; Raskin and Ensley, 2000); however, phytomining remains unproven for any metal.

Determining the biological effects of chemical amendments used to facilitate heavy metal and metalloid phytoextraction is critically important to the long-term persistence of vegetation cover on a contaminated soil. For example,

having revegetated a contaminated soil, it would be unwise to then use a chemical amendment that would significantly impact upon plant health and vegetation cover persistence. Ideally, one would seek to employ a reagent that promoted a high level of metal uptake while maintaining a healthy vegetation cover thus removing the need to perform the costly revegetation process after each phytoextractive treatment, or series of treatments. Furthermore, the long-term use of fertiliser amendments in agriculture has often been plagued by plant and soil accumulations of trace contaminants contained in the fertilisers themselves (e.g. Cd) (Loganathan *et al.*, 1997). However, for the purpose of CAP, both the introduction of trace quantities of contaminants contained in the fertilisers and the accumulation of these contaminants and other metals and metalloids in plants resulting from their use is not a limitation.

6.4.1. Chemical tolerance and plant growth

Chloris gayana was found to grow well on the contaminated soil while undergoing repeated applications of 0.5 and 2.0 g kg^{-1} EDTA and EDDS, as well as 2 g DTPA kg^{-1} and 0.5 g TSP kg^{-1}. In addition, the chelates (EDTA, EDDS and DTPA) achieved greater uptake of all metals and metalloids, except Ag, in *Chloris gayana* compared to soil amendments of THIO and SCN (Section 6.3.3). Silver phytoextraction was pronounced from the soil contaminated with 12.5 wt% mine tailings using 2.0 g kg^{-1} TSP amendments and increased with ongoing applications of the reagent (Figure 6.5). However, after four weekly treatments of 2.0 g kg^{-1} TSP the health of *Chloris gayana*

began to deteriorate. This indicates that the health and cover persistence of *Chloris gayana* grown on soil contaminated with 12.5 wt% mine tailings would not be affected by repeated applications of EDTA, DTPA and EDDS.

Both fertiliser applications (OSM and TPP) resulted in vigorous plant growth on metalliferous mine tailings containing 1.2 wt% Pb and 0.42 wt% Zn (Figure 6.1). The biomass production of *Chloris gayana* grown on both fertiliser treatments to the mine tailings was estimated to be 5 t ha^{-1}; a level very similar to the open pasture biomass estimate from the Cannington mine (Malone, 2004). The presence of P in the fertiliser treatment significantly elevated the concentration of Pb in control samples of *Chloris gayana* indicating a relationship between P in the substrate and Pb uptake by this species. The possible immobilisation of Pb resulting from the application of TSP fertiliser (Section 6.4.2) suggests that Pb may have accumulated in the mine tailings as insoluble phosphate minerals. It is therefore likely that the elevated Pb concentration of *Chloris gayana* grown on TSP fertilised mine tailings may have resulted from persistence of Pb in the plant growth medium. The absence of P from the fertiliser treatment (i.e. OSM) delayed flower and seed development in *Chloris gayana* suggesting a mechanism for managing the species in ecologically sensitive areas. In addition, *Chloris gayana* was able to tolerate the effects of ongoing applications of high-strength (2.0 g kg^{-1}) EDTA and EDDS amendments while growing on the fertilised mine tailings. In contrast, the application of 2.0 g SCN kg^{-1} resulted in rapid plant death. This indicates that (a) fertiliser amendments can promote a healthy

vegetation cover over undiluted mine tailings, (b) chelate amendments to the fertilised mine tailings did not significantly affect plant health or vegetation persistence, and (c) addition of P to the mine tailings raises the level of naturally accumulated Pb by *Chloris gayana*.

6.4.2. Fertiliser-induced heavy metal solubility

Increased aqueous solubility of Pb and Zn in the OSM-fertilised mine tailings (Table 6.2) could have resulted from the production of acidity via nitrification of the applied ammonium nitrate load (48 kg ha^{-1}) and the oxidation of sulphide minerals contained in the mine tailings (Williams, 1990; Armstrong *et al.*, 1997), although no difference in substrate acidity was measured. Nitrification is the two-step conversion (Equation 1 and 2) of ammonium to nitrate by bacteria (*Nitrosomonas* and *Nitrobacter*) in soil which produces protons (acidity):

$$2NH_4^- + O_4 \leftrightarrow 2NO_2^- + 2H_2O + H^+ \quad [1]$$

Ammonium Oxygen Nitrite Water Protons

$$2NO_2^- + O_2 \leftrightarrow 2NO_3^+ \quad [2]$$

Nitrite Oxygen Nitrate

The nitrification process is inhibited at low pH conditions which results in the direct uptake of NH_4^+ by plants. In addition, NH_4^+ may successfully compete with exchange sites under these soil conditions resulting in the leaching of basic cations from the profile (Armstrong *et al.*, 1997).

Furthermore, if nitrification were to proceed rapidly in sufficiently moist soil, the leaching of basic cation would also occur via the formation of soluble nitrate complexes. Increased Pb and Zn solubility could also be attributed to the formation of other soluble metallic compounds in the mine tailings resulting from the addition of K (30 kg ha^{-1}) and S (7.5 kg ha^{-1}) contained in the OSM fertiliser.

The concomitant addition of P (13.2 kg ha^{-1}) contained in the OSM-fertiliser would also suggest that poorly soluble phosphate minerals (such as orthophosphate) may have formed in the mine tailings resulting in a decrease in heavy metal solubility (Basta *et al.*, 2001; Stanforth and Qiu, 2001). The immobilisation of heavy metals by the formation of phosphate minerals (Barrow, 1987; Ruby *et al.*, 1994; Ma *et al.*, 1997) may also explain the low extractability of Pb and Zn in the TPP-fertilised mine tailings (75.3 kg P ha^{-1}) compared to the unfertilised mine tailings. The data would therefore suggest that some degree of heavy metal immobilisation is likely to have occurred in mine tailings amended with both fertiliser treatments. Furthermore, small quantities of these fertilisers appear capable of immobilising high concentrations of Pb and Zn contained in the mine tailings.

6.4.3. Phytoremediation of contaminated soil

The highest concentration of Ag (1.7 μg g^{-1}), As (6.3 μg g^{-1}), Cd (5.8 μg g^{-1}), and Pb (145.6 μg g^{-1}) accumulated by *Chloris gayana* from the contaminated

soil occurred after soil treatment with 2.0 g kg^{-1} EDTA and TSP (Figures 6-2, 6-3, 6-4 and 6-5). EDDS amendments (2.0 g kg^{-1}) promoted maximum uptake of Zn (417.2 μg g^{-1}) from the contaminated soil (Figure 6.7), in addition to high levels of Cd phytoextraction (4.7 μg g^{-1}) (Figure 6.4). Antimony uptake from the contaminated soil by *Chloris gayana* was greatest (0.3 μg g^{-1}) using soil amendments of 2.0 g kg^{-1} DTPA (Figure 6.4). This indicates that the phytoremediation of As and Pb from a soil contaminated with 12.5 wt% mine tailings using *Chloris gayana* would be best achieved using ongoing treatments of EDTA, which did not affect the health of the plant. The health of *Chloris gayana* was also unaffected by ongoing applications of DTPA that promoted a high level of As, in addition to maximum Sb, phytoextraction. By comparison, maximum Zn phytoremediation, in addition to high As, Cd and Sb phytoremediation, was achieved using ongoing amendments of EDDS, which also did not affect plant health. In contrast, Ag and Cd phytoremediation was optimal using TSP amendments; however, the health of *Chloris gayana* was significantly reduced after one month of weekly chemical treatments. The data suggests that the use of several chemical reagents may be necessary to promote the simultaneous phytoextraction of Ag, As, Cd, Pb, Sb and Zn from the contaminated soil.

The concentrations of all metals and metalloids (except Ag) accumulated by the monocot *Chloris gayana* using chemical amendments were significantly lower than reported levels for other, predominantly dicotyledonous, agricultural plant species (e.g. Huang *et al.*, 1997a). These authors report

EDTA-extractable Pb concentrations of 1600 mg L^{-1} from contaminated soils containing 2500 μg Pb g^{-1}. Using a 2 g kg^{-1} EDTA amendment to the contaminated soil, the Pb concentration in *Zea mays* L. cv. Fiesta was raised from 30 μg g^{-1} to over 11000 μg g^{-1}. By comparison, the contaminated soil used in this investigation contained a total Pb concentration of 1520 μg g^{-1} and an EDTA-extractable Pb concentration of 1502 μg g^{-1}. *Chloris gayana* accumulated 146 μg Pb g^{-1} in its leaves when cultivated on this contaminated soil after treatment with 2.0 g kg^{-1} EDTA. The data indicate that *Chloris gayana* is highly efficient at excluding metals and metalloids from uptake even when high concentrations of heavy metals were extracted from the substrates using 0.1 M EDTA and 0.005 M EDTA.

Raskin and Ensley (2000) concluded that plant species which accumulate >1 wt% Pb (in dry plant tissues) and produce >20 t ha^{-1} yr^{-1} of biomass were economically viable for the phytoremediation of Pb contaminated soils. This implies that Pb phytoremediation is only viable when the plant removes ≥200 kg Pb ha^{-1} yr^{-1}. Although there are no published biomass estimates for *Chloris gayana* grown on anthroposol soils or soils contaminated by base metals, natural pasture fuel loads for the Cannington region suggest a biomass production of 5 t ha^{-1} (Malone, pers. com. 2004). At this level of biomass production, a crop of *Chloris gayana* treated with the above reagents (EDTA, DTPA, EDDS and TSP) is estimated to phytoremediate 8.8 g Ag, 31 g As, 29 g Cd, 0.73 kg Pb, 1.6 g Sb and 2.1 kg Zn ha^{-1} $crop^{-1}$. Clearly, this indicates that the chemically-assisted phytoremediation of metals and metalloids from soil

contaminated with mine tailings using *Chloris gayana* is not viable according to the performance indicators (i.e. ≥200 kg Pb ha^{-1} yr^{-1} from ≥20 t biomass ha^{-1} yr^{-1}) proposed by Raskin and Ensley (2000). Nonetheless, the chemically-assisted uptake of metals and metalloids by *Chloris gayana* does suggest that an annual input of approximately 1100 μg Pb m^2 could be phytoremediated from the soil using the chelate amendments (2.0 g kg^{-1} EDTA and EDDS).

6.4.4. Phytoextraction of mine tailings

The application of chelate amendments (EDTA, EDDS and DTPA) to fertilised mine tailings did not result in elevated concentration of Ag in *Chloris gayana* (Figure 6.5). However, one 'toxic' application of SCN to the TPP-fertilised tailings resulted in a significant increase in the Ag concentration in *Chloris gayana* (2.4 μg g^{-1}) compared to Ag uptake from the OSM-fertilised tailings (0.2 μg g^{-1}) and by the unamended control plants (Figure 6.9). The chemically-assisted phytoextraction of Ag by *Chloris gayana* may indicate the influence of metal speciation on the complexing potential of the chemical reagents. Under natural conditions, Ag occurs predominantly as monovalent cations whereas heavy metals such as Cd, Pb and Zn occur predominantly as divalent cations (Cotton and Wilkinson, 1980; McBride, 1994). However, the application of fertiliser amendments is likely to have formed a range of secondary minerals of varying solubility in the mine tailings used in this study (Section 6.4.2). This indicates that predominantly divalent heavy metals such as Cd, Pb and Zn would be strongly associated with relatively insoluble minerals (such as orthophosphate) in the TPP-fertilised mine tailings.

Whereas predominantly monovalent heavy metals such as Ag would be strongly associated with relatively soluble minerals (such as nitrates) in the OSM-fertilised mine tailings. Complexes of Ag and SCN are noted to occur under natural condition (Alloway, 1995) and SCN has been used to selectively phytoextract Au (another predominantly monovalent cation in solution) from mine wastes containing elevated concentrations of a range of heavy metals (Anderson *et al.*, 1999b; Keeling, 2002). This suggests that the chemically-assisted phytoextraction of Ag from fertilised mine tailings by *Chloris gayana* is strongly influence by fertiliser composition. In addition, the data further demonstrates the effectiveness of SCN amendments for the dissolution of precious metals from acidic mine wastes.

In contrast, chelates are well known for promoting the phytoaccumulation of a range of divalent heavy metals from polymetallic contaminated soil (e.g. Blaylock *et al.*, 1997; Wu *et al.*, 1999). *Chloris gayana* tolerated ongoing chelate amendments (EDTA and EDDS) and accumulated greater Pb and Zn concentrations from the TPP-fertilised tailings compared to the OSM-fertilised tailings (cf. Figures 6.7 and 6.8). Lead uptake by control plants grown on TPP-fertilised tailings was high (69 $\mu g\ g^{-1}$) compared to Pb uptake by control plants for the OSM-fertilised tailings (17 $\mu g\ g^{-1}$) and all of the chemical treatments (Figure 6.7). This indicates that P availability may influence natural Pb uptake in *Chloris gayana*, perhaps by the formation of poorly soluble phosphate minerals in the substrate. The converse may also apply to a reduced level of chemically-assisted metal uptake by *Chloris gayana*

from the OSM-fertilised tailings where N availability may have caused highly soluble nitrate minerals to form or may have acidified the soil via nitrification resulting in increased metal leaching (Armstrong *et al.*, 1997; Sumner, 2000). However, there was no significant difference between pH and EC of leachates from both fertiliser treatments (Table 6.2). This suggests that the application of the chemical amendments has resulted in leaching of metals and metalloids from the fertilised mine tailings before the plants were able to accumulate them from the draining pore fluids.

The potential of a plant species to recover heavy metals and metalloids from a contaminated soil (i.e. phytoremediation potential, g of element ha^{-1} $crop^{-1}$) can be estimated by multiplying the plant concentration (μg g^{-1}) of an accumulated element by the plants dry matter production rate (t ha^{-1}) (McGrath *et al.*, 1993). The biomass production of *Chloris gayana* grown on fertilised mine tailings was estimated to be 5 t ha^{-1} $crop^{-1}$, indicating that the species could phytoextract approximately 12 g Ag (2.0 g SCN kg^{-1} tailings), 182 g Pb (2.0 g EDTA kg^{-1} tailings) and 9.5 kg Zn (2.0 g EDTA kg^{-1} tailings) per hectare per crop from the TPP-fertilised tailings. By comparison, approximately 1.1 g Ag (2.0 g SCN kg^{-1} tailings), 124 g Pb (2.0 g EDTA kg^{-1} tailings) and 5.4 kg Zn (2.0 g EDDS kg^{-1} tailings) per hectare per crop could be phytoextracted by *Chloris gayana* grown on the OSM-fertilised tailings. Lead uptake by *Chloris gayana* was conspicuously low considering (a) the applied chelating agents are well documented for promoting high concentrations of Pb in plants (Greman *et al.*, 2003; Kos and Lestan, 2003)

and (b) the relatively high concentrations of chelate-extractable Pb contained in the mine tailings (Table 6.2). Lead uptake by *Chloris gayana* from the contaminated soil using 2.0 g EDTA kg^{-1} (146 μg Pb g^{-1}) was far greater than Pb uptake from the fertilised tailings (10-36 μg Pb g^{-1}). It is possible that the high concentrations of plant-available Zn in the fertilised tailings (≈51-98 μg g^{-1}) interfered with Pb uptake, although this was not reflected by a high concentration of Zn in the leaves of *Chloris gayana*.

6.5. Conclusions

The study has shown *Chloris gayana* to be an extremely hardy species capable of surviving in soil contaminated with 12.5 wt% mine tailings, in addition to undiluted mine tailings (1.2 wt% Pb and 0.42 wt% Zn) that have received N and N+P fertiliser applications. Both fertiliser regimes (300 kg ha^{-1} Osmocote and 300 kg ha^{-1} Osmocote plus 300 kg ha^{-1} Triphosphate) promoted vigorous plant growth on undiluted mine tailings indicating that nutrient deficiency is the limiting factor to effective direct revegetation of this waste material with *Chloris gayana*. In addition, the absence of P in the fertilised mine tailings was found to limit plant reproduction indicating a mechanism for controlling *Chloris gayana* in ecologically sensitive areas.

Chelate amendments (EDTA, EDDS and DTPA) applied to the contaminated soil did not significantly affect the growth of *Chloris gayana*, whereas amendments with thio-compounds resulted in toxicity symptoms

(TSP) or rapid plant death (SCN and THIO). Similarly, *Chloris gayana* grown on fertilised mine tailings was capable of surviving the effects of ongoing applications of EDTA and EDDS, whereas one application of SCN was sufficient to kill the plant.

By today's published standards (i.e. soil phytoremediation of ≥200 kg Pb ha^{-1} yr^{-1} from ≥20 t biomass ha^{-1} yr^{-1}), no chemical treatment was found to be effective for the phytoremediation of Pb, or the other elements tested, from the contaminated soil using *Chloris gayana*. However, higher levels of metal and metalloid uptake by *Chloris gayana* may be possible using higher strength chelate amendments, although this will inevitably lead to further environmental problems. EDTA-assisted Pb uptake was estimated as sufficient to phytoremediate an annual contamination load of approximately 1100 μg Pb m^{-1} from a soil. Chemical amendments promoted higher levels of metal uptake from tailings amended with Osmocote and Triphosphate fertilisers compared to mine tailings amended with Osmocote fertiliser only. The CAP of metals by *Chloris gayana* grown on fertilised tailings (biomass production of 5 t ha^{-1}) was estimated to be 12 g Ag (2.0 g SCN kg^{-1} to TPP-fertilised tailings), 1.2 kg Pb (2.0 g EDTA kg^{-1} to OSM-fertilised tailings) and 9.5 kg Zn (2.0 g EDTA kg^{-1} to TPP-fertilised tailings) per hectare per crop. This indicates that *Chloris gayana* is unsuitable for the CAP of Ag, Pb and Zn from fertilised mine tailings. However, the growth of *Chloris gayana* was unaffected by repeated application of EDTA and EDDS, which dissolved high concentrations of Pb and Zn from the tailings. This suggests that the

chelates could be used to leach the fertilised tailings of Pb and Zn, while *Chloris gayana* is simply used to phytostabilise the surface of the tailings dams thereby reducing the short-term and long-term environmental risk of the mine waste.

Chapter 7. The Leaching of Ag, Pb and Zn from Cannington Mine Tailings using Novel Chemical Reagents

7.1. Introduction

The Cannington Ag-Pb-Zn mine annually produces approximately 0.5 million tonnes of Pb and Zn mineral concentrates from approximately 2.1 million tonnes of sulphide ore (Walters, 1998). Approximately two thirds of the mine waste produced annually is disposed of as backfill in underground voids (BHPBilliton, 2002). The remaining tailings solids (approximately 0.5 million tonnes per annum) are thickened and pumped as slurry into a conventional tailings dam repository: the Process Residue Facility (PRF) (refer Figure 2.2). This indicates that approximately 3.5-4.0 million tonnes of mine tailings have been deposited in the PRF since mining began eight years ago. The average concentration of commodity metals contained in the mine tailings is 60 μg Ag g^{-1}, 1.2 wt% Pb, and 0.42 wt% Zn (Appendix A). This indicates that approximately 8 M oz Ag, 44,000 tonnes Pb, and 12,700 tonnes Zn, with a combined value of US$113 million[1], are presently contained in the Process Residue Facility. The PRF has been designed to eventually occupy approximately 125 ha of land surface immediately adjacent to the mines ore-processing infrastructure (BHPBilliton, 2002). Three tailings dam cells are scheduled for construction over the life of the mine (currently 22 years); each

[1] Assuming: US$7.20 oz^{-1} Ag, US$980 t^{-1} Pb, US$1020 t^{-1} Zn, 7 years of 2 M TPA production to realise 0.5 M tonnes of concentrates, 65% of tailings solids to backfill, 35% of tailings solids to the PRF.

composed of a 'keyed in' bund wall (15 m) lined with compacted clay and containing dewatering infrastructure. Process waters won from the deposited tailings slurry are directed back to the ore processing operation to minimise the mines net water usage. Eventually the PRF will be rehabilitated by means of a 'store-and-release' cover system vegetated with native pasture plant species.

Hydrometallurgical processing of base-metal ores generally involves the addition of chemical reagents to modify the hydraulic properties of ore minerals such that they may be separated from gangue minerals by flotation. The rate of mineral recovery from base metal ores largely depends on the existing ore mineralogy, mineral chemistry and elemental interferences as well as the type and strength of reagent applied (Zipperian *et al.*, 1988; Jeffery, 2001). As a result, existing hydrometallurgical treatment techniques produce tailings that contain variable concentrations of residual metals. The residual mass of metals contained in waste materials derived from hydrometallurgically treated base-metal ores can be considered as resources if metal recovery from tailings is economically and environmentally feasible using appropriate technologies, such as chemical leaching.

The hydrometallurgical processing of Au-Ag, Ni-Co and U ores relies on the use of chemical reagents that dissolve ore minerals and release target elements to process waters (e.g. Bartlett, 1992; Akretche *et al.*, 1995). The

leaching of Ag from geologic materials has been reported using a variety of reagents, such as chlorides, cyanide, thiosulphate and thiourea (e.g. Li and Wadsworth, 1993; Akretche *et al.*, 1995; Liu and Yen, 1995; Maudos *et al.*, 1996; Jeffery, 2001). The rate of Ag dissolution is usually reported as being controlled by two factors: (1) O_2 diffusion at the mineral surface and (2) sensitivity to reagent concentrations (Jeffery *et al.*, 2001). High reagent concentrations often reduce Ag dissolution by excessive sulphide mineral disassociation (Liu and Yen, 1995), presumably causing elemental interference (i.e. by Cu, Fe, and Zn) and increased reagent consumption. Cotton and Wilkinson (1980) report that Ag complexation by ammoniacal cyanide or thiosulphate solutions, in addition to aminopolycarboxylate chelating agents (EDTA, DTPA), is likely under standard conditions.

The effects of aminopolycarboxylate chelating agents on the dissolution of heavy metals in soil materials has been reported by numerous authors from a variety of perspectives (e.g. Means *et al.*, 1980; Norvell, 1984, 1991; Li and Shuman, 1996; Yordanov and Roundhill, 1998; Greet and Smart, 2002; Nowack, 2002). Chelating agents (e.g. EDTA or DTPA) have been demonstrated as effective tools for the dissolution of plant nutrients and metals, particularly Pb and Zn, in soils. In addition, chelating agents have been used to wash heavy metal contamination from soil (Kos and Lestan, 2003). The natural occurrence of thiosulphate in the weathering zone has been implicated in the transport and deposition of heavy and precious metals (Mann, 1984; Gray *et al.*, 1992; Kucha *et al.*, 1995). Thiosulphate ($S_2O_3^{2-}$)

solutions have also been investigated extensively for the leaching of Au and Ag from refractory and low-grade ores (cf. Zipperian *et al.*, 1988; Maudos *et al.*, 1996; Breuer and Jeffery, 2000; Jeffery, 2001; Molleman and Dreisinger, 2002). Thiosulphate, and thiocyanate (SCN^-), complexes are simple inorganic ligands and their use may significantly reduce the risks associated with conventional leaching techniques. Both compounds are considerably less toxic compared to other chemical reagents that are currently employed to leach heavy metals (e.g., NaCN) (Moran, 1998). In addition, thiocyanate and thiosulphate have much shorter biological half-lives compared to NaCN and would therefore not persist in leached materials to the same degree (Goncalves *et al.*, 1998). This suggests that hydrometallurgical leaching using thiocyanate and thiosulphate solutions may result in significantly less toxic residual materials compared to NaCN, for example.

While EDTA has been documented to dissolve Ag, Pb and Zn from soils and TSP has been investigated for its ability to dissolve precious metals from ores, the application of EDTA, TSP and SCN in the hydrometallurgical leaching of metals from base metal mine tailings remains largely unexplored. The chemical reagents used to promote heavy metal dissolution in soil contaminated with mine tailings and undiluted mine tailings (Chapter 4 and 6) and their uptake by plants indicated that the metals may have been leaching from the plant growth medium before the plants were able to accumulate them. In addition, the persistence of EDTA in soil has been attributed to the leaching of heavy metals resulting in groundwater contamination (Means *et*

al., 1980; Kinnersley, 1993). Due to these processes and the potential experimental losses of dissolved heavy metals (Chapter 4 and 6) this study was conducted to evaluate the potential leaching of heavy and precious metals from Cannington mine tailings using 0.1 M and 1 M solutions of various chemical reagents (EDTA, TSP, SCN, THIO and NaCN). The aim of the experiment was to determine whether low ionic strength solutions of these chemical reagents could be used to dissolve the residual commodity metals (Ag, Pb and Zn) contained in the mine's waste. Moreover, the effects of the chemical reagents on the dissolution of metals contained in the mine tailings required evaluation because the CAP experiments (Chapter 4 and 6) alluded to the potential loss of metals. Leaching was performed over a three-month period to take advantage of natural processes such as O_2 replacement in interstitial pore fluids and voids in the tailings, and the continued oxidation of previously leached ore minerals to recover the metals.

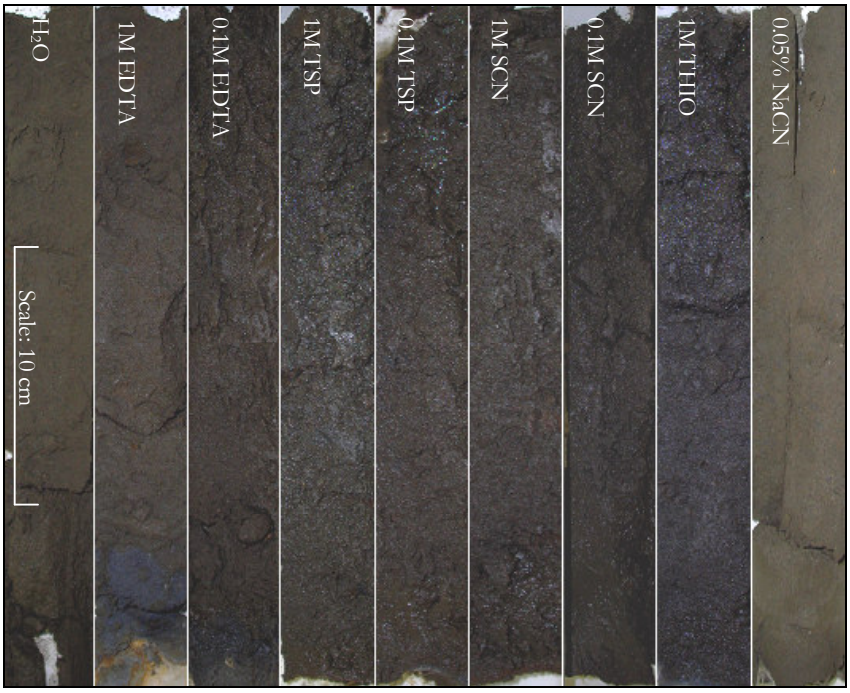

Figure 7-1. Vertical cross sections of the leach columns containing Cannington mine tailings after 12 weekly treatments with 0.1 M and 1 M EDTA, TSP and SCN, and 1 M THIO. 1 M EDTA was applied once only and treatment with 0.05% NaCN occurred over 8 weeks.

7.2. Materials and methods

This column leach experiment (Chapter 2, Section 2.13) was conducted using 2 kg charges of air-dried Cannington mine tailings (Chapter 2, Section 2.11.2). Samples were loaded into 300 mm columns of transparent polycarbonate tubing of 65 mm diameter and mounted vertically. Leaching solutions (200 mL) of EDTA, TSP, SCN, THIO and NaCN were applied weekly at concentrations of 0.1 M and 1 M reagent per kg of mine tailings (Chapter 2, Table 2.4). A disodium salt of EDTA (Na_2EDTA) was chosen for this leaching experiment because the reagent is readily available in bulk quantities

from the manufacturer (Sigma). Chemical reagents were prepared fresh each week and allowed to completely drain from the column before being analysed (Chapter 2, Section 2.14) for Ag, Pb and Zn (Appendix J).

7.3. Results

The 200 mL weekly amendment of the leaching solutions completely drained from the columns in approximately 120-130 hours. All columns were completely drained of leachate prior to subsequent chemical treatments. This would suggest that a degree of O_2 replacement in pore fluids and voids in the tailings would have occurred via the ingress of atmospheric gases between successive weekly chemical treatments.

7.3.1. Leachate discolouration

Discolouration became progressively more apparent in leachate solutions of EDTA and SCN as the experiment progressed. The 0.1 M and 1 M EDTA leachates took on a deep red-brown opaque colour indicating leachates contained a high concentration of Fe. The 1 M SCN leachates became pale transparent blue in colour suggesting the presence of Cu, Cr, Co, or potentially V, in solution (Saunders, 2005). In contrast, the 0.1 M SCN leachates became a pale transparent pink colour indicating that Co, Mn or V may have been present in solution (Saunders, 2005). The 0.1 M EDTA leachate sample for week five was damaged during shipping to the laboratory and could not be included in the dataset.

Table 7.1. The proportion of Ag, Pb and Zn (% of total metal concentration) leached from the Cannington mine tailings by the various chemical reagents (EDTA, TSP, SCN, THIO, and NaCN). Total metal concentrations (μg g^{-1}) of the mine tailings are included.

Solution	Leached Metal (% of total element concentration)		
	Ag	Pb	Zn
dH_2O	<0.01%	0.01%	0.7%
1 M EDTA*	<0.01%	14.1%	6.0%
0.1 M EDTA	<0.01%	26.7%	13.0%
1 M TSP	3.9%	6.2%	12.5%
0.1 M TSP	2.7%	3.3%	3.1%
1 M SCN	83.7%	0.01%	8.8%
0.1 M SCN	0.5%	<0.01%	0.2%
1 M THIO	8.2%	<0.01%	0.2%
0.05% NaCN**	1.0%	0.01%	0.4%
Total Metal Conc. (μg g^{-1})	60	11950	4150

* = one chemical treatment, ** = eight periodic chemical treatments.

7.3.2. Silver extraction

Leachate analyses (Appendix J) indicated that EDTA extracted very low concentrations of Ag (<0.01% of the total element concentration - TEC) at both reagent concentrations (0.1 M and 1 M) over the 12-week leach period (Table 7.1). Following an initial two-week period where very little metal was detected in leachate solutions, Ag dissolution using 1 M THIO was relatively constant (≈4.5 μg Ag g^{-1}) over the remaining ten-week treatment period (Figure 7.2 and 7.3 respectively). Although low by comparison, Ag dissolution from the mine tailings increased at a constant rate using the 0.05% NaCN solution (<2 μg Ag g^{-1}) over an eight-week treatment period (Figure 7.4 and Figure 7.5, respectively). Both concentrations of TSP (0.1 M and 1 M) dissolved low levels of Ag from the mine tailings at relatively constant concentrations (≈2 and 4 μg Ag g^{-1} respectively). After an initial

peak (≈1 μg Ag g^{-1}), Ag extraction from the mine tailings using the 0.1 M SCN was similarly low and constant (≈0.2 μg g^{-1}). The 1 M SCN solutions dissolved a significant amount of Ag (≈83.7% TEC) from the mine tailings with dissolution peaking at >320 μg Ag g^{-1} after two weeks of treatment (Figure 7.2). Approximately 90% of the Ag extracted using 1 M SCN was leached from the mine tailings in the initial four weeks of treatment (Figure 7.3).

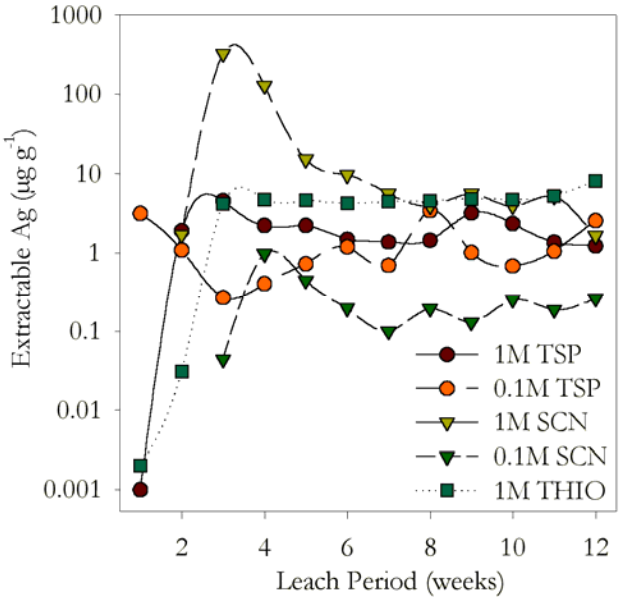

Figure 7-2. Silver (μg g^{-1}) dissolution from Cannington mine tailings using ammonium thiosulphate (TSP), ammonium thiocyanate (SCN) and thiourea (THIO) (e.g. 1 M TSP = 1 M TSP kg^{-1} mine tailings, 0.1 M TSP = 0.1 M TSP kg^{-1} mine tailings, etc.).

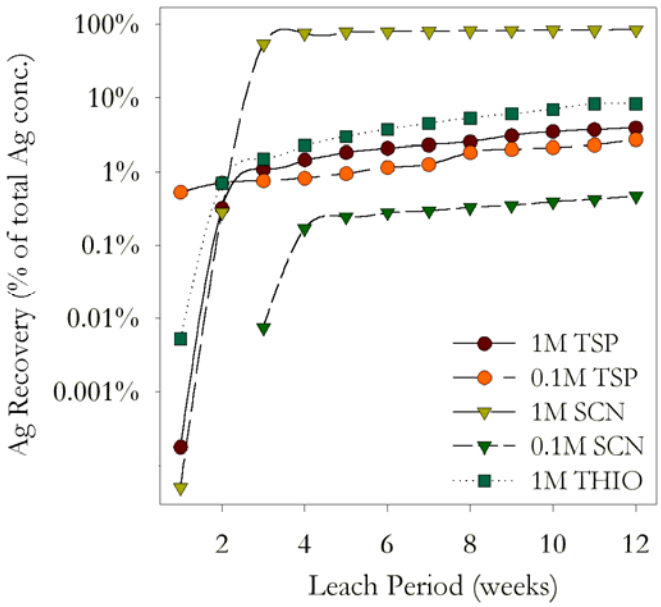

Figure 7-3. Cumulative Ag recovery from Cannington mine tailings leached with ammonium thiosulphate (TSP), ammonium thiocyanate (SCN) and thiourea (THIO) (e.g. 1 M TSP = 1 M TSP kg^{-1} mine tailings, 0.1 M TSP = 0.1 M TSP kg^{-1} mine tailings, etc.).

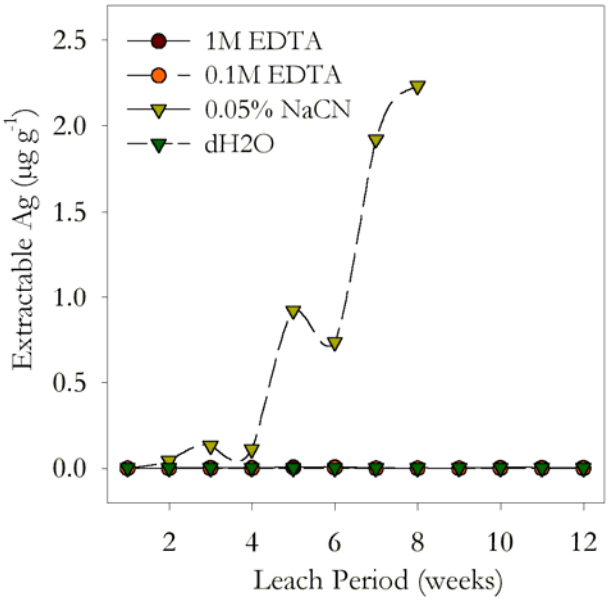

Figure 7-4. Silver (µg g^{-1}) dissolution from Cannington mine tailings using EDTA and sodium cyanide solutions (e.g. 1 M EDTA = 1 M EDTA kg^{-1} mine tailings, 0.1 M EDTA = 0.1 M EDTA kg^{-1} mine tailings, etc.).

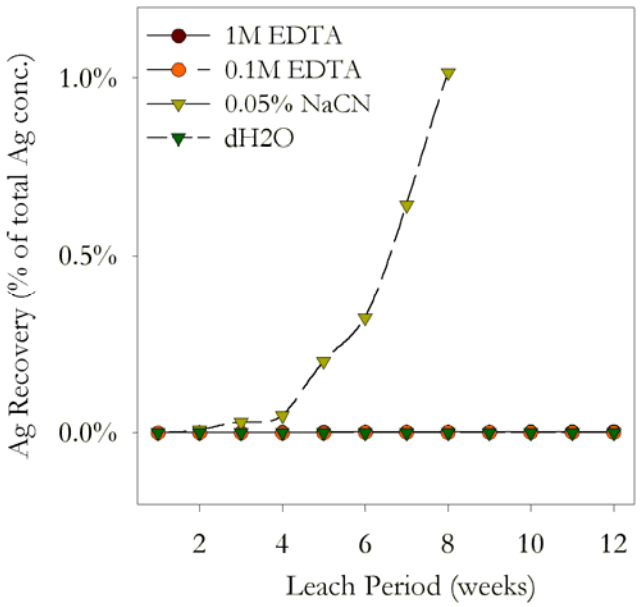

Figure 7-5. Cumulative Ag recovery from Cannington mine tailings leached with EDTA and sodium cyanide solutions (e.g. 1 M EDTA = 1 M EDTA kg^{-1} mine tailings, 0.1 M EDTA = 0.1 M EDTA kg^{-1} mine tailings, etc.).

7.3.3. Lead extraction

Leachate analyses indicated that SCN, THIO and NaCN dissolved low levels of Pb (Figure 7.6) from the mine tailings (<1 μg g^{-1}, 2.1 μg g^{-1} and 3.3 μg g^{-1} respectively). Lead extraction using TSP was significant (Table 7.1) at both concentrations (0.1M, 3.3% TEC; 1M, 6.2% TEC). The application of a single amendment of 1 M EDTA followed by weekly amendments of deionised water produced very high Pb dissolution (1.5 mg g^{-1}) after the first three weeks of treatment (Figure 7.7). This treatment effectively leached 14.1% of the Pb contained in the mine tailings over the 12-week period with 98% of the recovery occurring in the first six weeks of treatment (Figure 7.7).

The 0.1 M EDTA treatments also produced significant Pb dissolution (0.8 mg g^{-1}) removing 26.7% of the Pb contained in the mine tailings over an eight-week treatment period (Table 7.1).

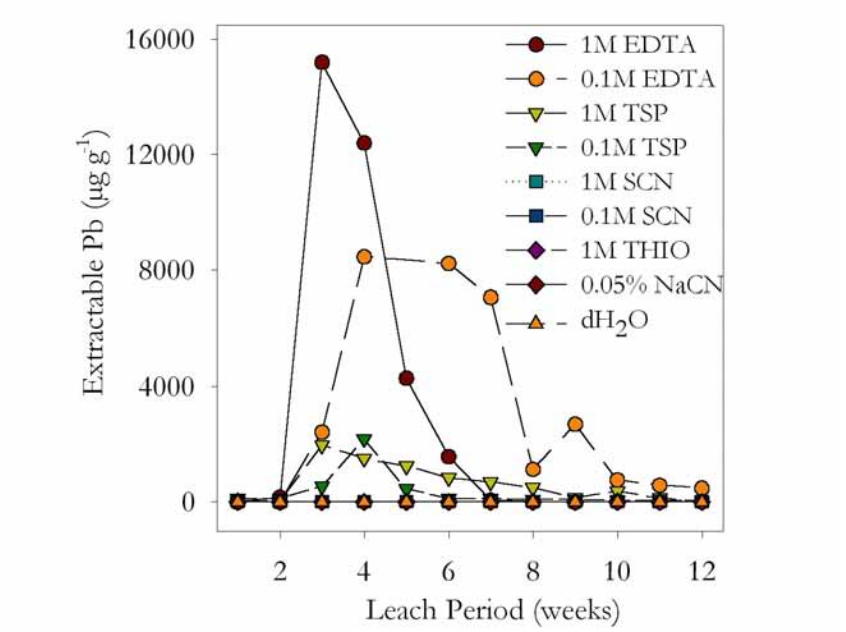

Figure 7-6. Lead (µg g^{-1}) dissolution from Cannington mine tailings using the various chemical treatments (e.g. 1 M EDTA = 1 M EDTA kg^{-1} mine tailings, 0.1 M EDTA = 0.1 M EDTA kg^{-1} mine tailings, etc.).

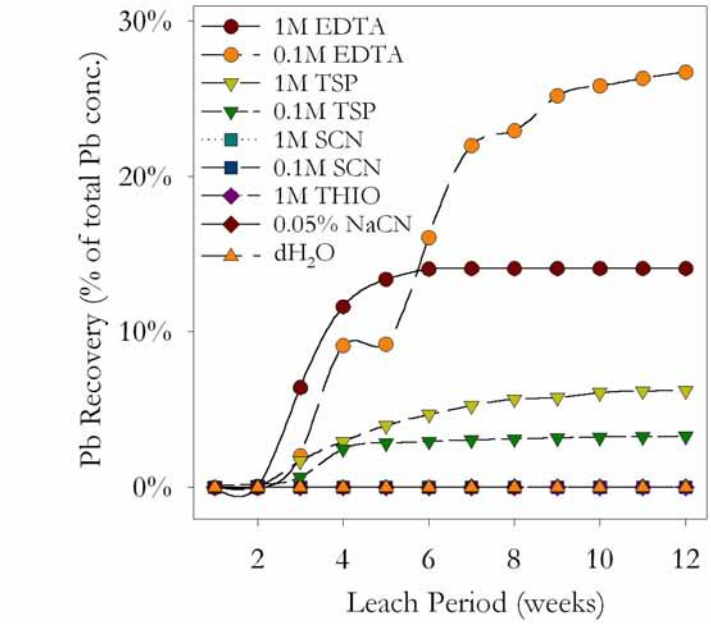

Figure 7-7. Cumulative Pb recovery from Cannington mine tailings leached with various chemical reagents (e.g. 1 M EDTA = 1 M EDTA kg^{-1} mine tailings, 0.1 M EDTA = 0.1 M EDTA kg^{-1} mine tailings, etc.).

7.3.4. Zinc extraction

Zinc extraction from the mine tailings using water (dH_2O), 1 M THIO, 0.05% NaCN, 0.1 M SCN, and 0.1 M TSP was very low (≤10 μg Zn g^{-1}) over the twelve-week treatment period (Figure 7.8). Zinc dissolution from the mine tailings using 0.1 M EDTA and 1 M TSP was pronounced (13.0% TEC and 12.5% TEC respectively). Approximately 85% of Zn leached from the mine tailings using 0.1 M EDTA and 1 M TSP occurred in the first five-weeks of treatment (Figure 7.9). In contrast, 1 M EDTA leached 6.0% of the mine tailings total Zn concentration compared to 0.1 M EDTA and TSP but over an eight-week treatment period. Zinc extraction by 0.1 M and 1 M EDTA and 1 M TSP then decreased to a relatively constant rate (≈250 μg Zn

g^{-1}) for the remaining weekly treatments. Solutions of 0.1 M TSP and 1 M SCN extracted small quantities of Zn (3.1% TEC and 8.8% TEC respectively) from the mine tailings with Zn recovery by 0.1 M TSP peaking at 710 μg Zn g^{-1} after four weeks of leaching. Zinc extraction using 1 M SCN also peaked after four weeks of leaching (513 μg Zn g^{-1}), however, the Zn concentration of subsequent leachates of 1 M SCN decreased at a much lower rate compared to 0.1 M TSP, which has accounted for the greater recovery of Zn by 1 M SCN.

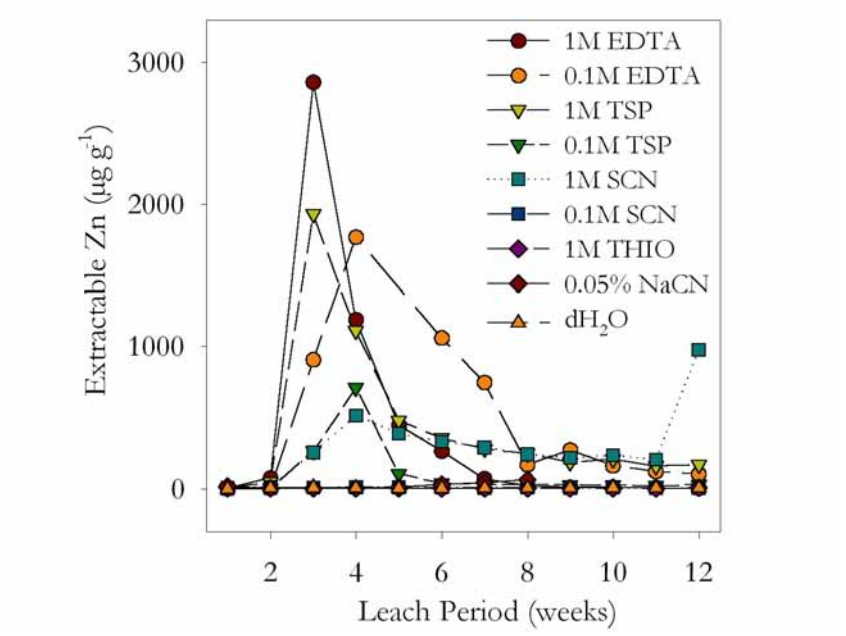

Figure 7-8. Zinc (μg g^{-1}) dissolution from Cannington mine tailings using the various chemical treatments (e.g. 1 M EDTA = 1 M EDTA kg^{-1} mine tailings, 0.1 M EDTA = 0.1 M EDTA kg^{-1} mine tailings, etc.).

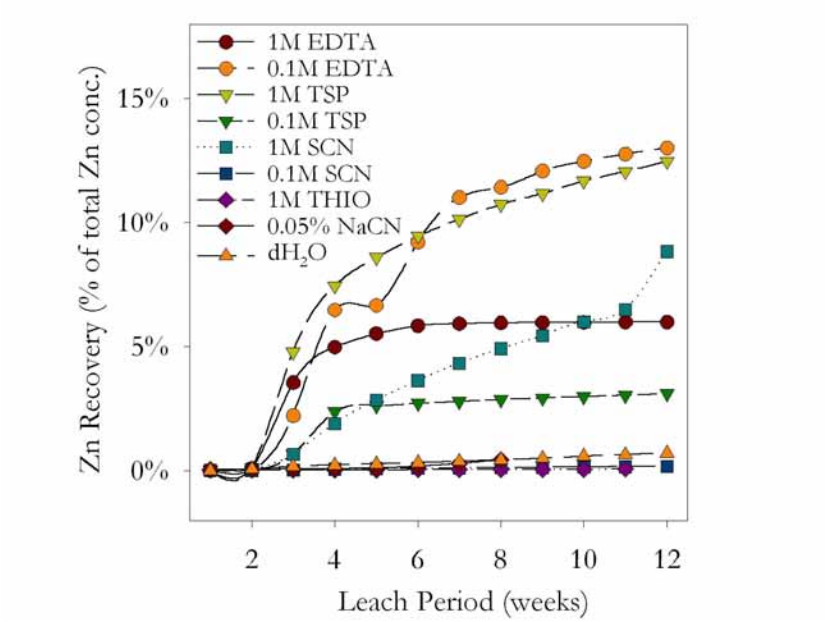

Figure 7-9. Cumulative Zn recovery from Cannington mine tailings leached with various chemical reagents (e.g. 1 M EDTA = 1 M EDTA kg^{-1} mine tailings, 0.1 M EDTA = 0.1 M EDTA kg^{-1} mine tailings, etc.).

7.4. Discussion

The dissolution rates of Ag, Pb, and Zn from the Cannington mine tailings indicate that approximately 90% of metal dissolution occurred within the first two months of treatment. Silver recovery was notable only for the 1 M SCN leach solution (≈84% TEC). The estimated mass of Ag (50.2 g t^{-1}) leached from the mine tailings (Table 7.2) using 1 M SCN and the rapid rate of Ag dissolution (2-8 weeks) suggest that a higher concentration of applied SCN over this period may result in more complete Ag extraction.

Table 7.2. Mass of metal removed from Cannington mine tailings after three months of weekly leaching, including total metal concentrations of the tailings

Lixiviant	Metal Extractability		
	Ag (g t^{-1})	Pb (kg t^{-1})	Zn (kg t^{-1})
dH_2O	<0.01	<0.01	0.06
1 M EDTA*	<0.01	3.37	0.50
0.1 M EDTA	<0.01	6.39	1.08
1 M TSP	4.68	1.49	1.04
0.1 M TSP	3.25	0.79	0.26
1 M SCN	50.2	<0.01	0.73
0.1 M SCN	0.56	<0.01	0.01
1 M THIO	9.90	<0.01	<0.01
0.05% NaCN**	2.05	<0.01	0.28
Total Metal Conc.	65	12	3.6

* = one chemical treatment, ** = eight periodic chemical treatments.

The leaching of Ag, and other heavy metals, from polymetallic ore with solutions of THIO has been reported to increase with increasing reagent concentrations and decreasing pH (Akretche *et al.*, 1995). Akretche *et al.* (1995) also found that the presence and abundance of various sulphide minerals in the polymetallic ore (e.g. pyrite, pyrrhotite, arsenopyrite) strongly influences leach efficiency using THIO solutions. These same authors reported that the leaching of Ag using THIO solutions was improved with the use of other oxidative reagents (e.g. H_2SO_4 and Fe^{3+}) and the introduction of saline water. In the present study, Ag recovery from the mine tailings (pH 5.4) using a simple 1 M THIO solution was low (≈4.5 μg Ag g^{-1}) and occurred at a relatively constant rate over the three-month treatment period. The constant low rate of Ag extraction from the mine tailings using 1 M THIO may be the result of restricted mineral dissolution that is limiting metal complexation and extraction by thiourea. In addition, the oxidation of other sulphide minerals such as pyrrhotite (Appendix A) or the pH and redox potential of the 1 M THIO solution may have reduced leach efficiency (Akretche *et al.*, 1995). The data indicates that improved Ag extraction from

the mine tailings using 1 M THIO would require the use of additional reagents (such as H_2SO_4) to lower the pH, and Fe^{3+} and Cl^- to modify the redox potential, of the solution. Furthermore, in a practical sense the availability of saline groundwaters at the Cannington mine may offer a relatively inexpensive means of producing large volumes of saline solutions of thiourea for the leaching of mine tailings.

The dissolution of Ag and Au from polymetallic ores using cyanide solutions is best achieved under alkaline pH conditions (pH 10.3) (Li and Wadsworth, 1993). The pH of the cyanide leaching solution is adjusted to this level by the addition of sodium hydroxide to avoid the evolution of hydrocyanic acid vapour at lower pH levels, thus reducing reagent consumption. In addition, the leach solutions should contain dissolved oxygen in the range 8-32 $\mu g\ g^{-1}$ to achieve high leach recoveries of the Au and Ag (Liu and Yen, 1995). These authors found that the abundance of other heavy metals (e.g. Cu, Fe and Zn) in the ore significantly increased the consumption of both cyanide and oxygen in the leach solution thus reducing the efficiency of the method. Silver recovery from the Cannington mine tailings using 0.05 wt% solutions of NaCN (1.0% TEC) is likely to have resulted from low pH conditions in the column (pH 5.4) causing high levels of NaCN consumption. In addition, the rate of Ag dissolution from the mine tailings using NaCN may have been influenced by the bulk mineral assemblage of the mine tailings. The extraction of Ag from sulphidic ores using cyanide solutions has been shown to decrease in the presence of sulphide minerals such as chalcopyrite,

pyrrhotite and stibnite (Liu and Yen, 1995). However, the presence of galena was shown to improve the extraction of Ag from sulphidic ores (Liu and Yen, 1995). This suggests that the composition and oxidation of the sulphide mineral assemblage in the mine tailings may have interfered with the leach efficiency of NaCN. In addition, interference from elements other than those investigated (such as Cu and Sb) may have also effected the leaching efficiency of NaCN solutions.

Experimental leaching of Pb using EDTA solutions from materials containing galena and its oxidation products was found almost exclusively to dissolve secondary minerals (carbonates, hydroxides, sulphates and oxides) without attacking galena itself (Greet and Smart, 2002). These authors also found that a 0.1 M EDTA solution will rapidly dissolve the oxidation products of galena. In an earlier investigation, Wang and Forssberg (1990) suggested that EDTA may actually attack galena if the reagent is present in excess in the leach solution and the material being leached is low in total S. The bulk mineralogy of the Cannington mine tailings, determined by x-ray diffraction (Appendix A), indicated that galena (2 vol%) was the only detectable ore mineral contained in the mine waste. Gilfedder (2004) reported that a range of secondary minerals were present in exposed surficial mine tailings (gypsum, anglesite, plumbojarosite, natrojarosite, halite, illite and native sulphur). Therefore, it could be expected that the maximum quantity of Pb available for recovery by leaching from the Cannington mine tailings using solutions of EDTA would be significantly less than the total

concentration of Pb contained in the tailings, and that metal recovery would be limited by mineral oxidation. Lead recovery from the mine tailings was most efficient using solutions of EDTA (0.1 M, 26.7% TEC; 1 M, 14.1% TEC) (Figure 7.4). The removal of Zn from the mine tailings was also significant using a leach solution of EDTA (0.1M, 13.0% TEC; 1 M, 6.0% TEC). This indicates that a low ionic strength solution of EDTA (0.1 M) is significantly more efficient at leaching Pb from the mine tailings compared to a high strength EDTA solution. The low strength solution would also be safer to use in the natural environment and considerably less costly to use compared to the high strength solution..

The commercial application of hydrometallurgical methods to reprocess Cannington mine tailings would be based upon purely economic parameters. Essentially, the revenue from metal production would have to exceed the cost of the leaching process by a certain percentage to generate profit. The total value of residual Ag, Pb, and Zn contained in the mine tailings was estimated to be low (US$31[†] per tonne) indicating that low-cost methods would be required to hydrometallurgically reprocess the Cannington mine tailings. An estimate of the costs of the leaching procedures (Table 7.3) indicates that the leaching reagents could not extracted sufficient quantities of valuable metals from the mine tailings (by any reasonable proportion) to offset the cost of their use.

[†] The mass of extracted metals is taken from Table 7.2, to which metal prices (US$7.20 oz^{-1} Ag, US$980 t^{-1} Pb and US$1020 t^{-1} Zn) were applied.

Table 7.3. The estimated quantity (kg t^{-1}) and cost (US$ t^{-1}; reagent cost of US$5 t^{-1}) of the leaching reagents used to extract Ag, Pb, and Zn from the mine tailings, included are estimates of metal revenue‡ (US$ t^{-1}) and process value (US$ t^{-1}).

Reagent	Application (kg t^{-1})	Reagent Cost (US$ t^{-1})	Metal Revenue (US$ t^{-1})	Process Value (US$ t^{-1})
dH_2O	-	-	0.06	0.06
1 M EDTA*	74.5	372.24	3.81	-368.43
0.1 M EDTA	59.6	297.80	7.36	-290.44
1 M TSP	237	1185.68	3.64	-1182.04
0.1 M TSP	23.7	118.56	1.82	-116.74
1 M SCN	122	608.96	12.79	-596.17
0.1 M SCN	12.2	60.88	0.14	-60.74
1 M THIO	121	608.96	2.38	-606.58
0.05% NaCN**	0.04	0.20	0.78	0.58

* = one chemical treatment, ** = eight periodic chemical treatments.

7.5. Conclusions

This study has demonstrated that EDTA, TSP and SCN are capable of leaching appreciable concentrations of Ag, Pb, and Zn from Cannington mine tailings. Weekly applications of a low ionic strength solution of EDTA (0.1 M kg^{-1}) removed significant quantities of Pb (26.7% TEC) and Zn (13.0% TEC) from the mine tailings over an eight-week treatment period. In contrast, a single amendment of EDTA (1 M kg^{-1}) followed by weekly amendments of deionised water removed 14.1% of the Pb and 6.0% of the Zn contained in the mine tailings over a six-week period. The selective leaching of Zn from the mine tailings could be achieved using weekly applications of a 1 M TSP solution over a five-week treatment period, whereas a high proportion of the Ag contained in the mine tailings (83.7%) could be selectively leached using solutions of 1 M SCN applied over an

‡ US$7.20 oz^{-1} Ag, US$980 t^{-1} Pb and US$1020 t^{-1} Zn

eight-week period.

At present, it appears that a means of unlocking the potential resources contained in the Cannington mine's waste may not be possible using simple solutions of low ionic strength reagents (EDTA, TSP and SCN). However, the chemical leaching of Cannington mine tailings with these reagents could be improved by investigating the effects adding various secondary reagents to modify the materials pH and Eh conditions. However, care must be taken with the introduction of other reagents into the leaching solution because their cost may also outweigh the geochemical advantage of their use. At present, the leaching of heavy metals from the Cannington mine tailings could be investigated as a means of reducing the mine wastes short-term environmental risk. Chemical leaching would significantly reduce the surface concentrations of heavy metals contained in the tailings thereby reducing environmental risks associated with a large area of exposed mine tailings. Furthermore, chemical leaching could conceivably reduce the cost of rehabilitating the Process Residue Facility by potentially reducing the engineering standards required to rehabilitate a less hazardous mine waste.

Chapter 8. The Role of Phytotechnology in the Rehabilitation of the BHPBilliton Cannington Ag-Pb-Zn Mine: A General Conclusion

8.1. The EMOS for the Cannington Ag-Pb-Zn Mine

This research project sought to determine the suitability of a selection of pasture plant species for the revegetation and phytoremediation of soil contaminated with mine tailings at the BHPBilliton Cannington Ag-Pb-Zn Mine, Queensland. The EMOS for the Cannington mine details what are presently considered as acceptable land rehabilitation principles and practices for the operation (BHPBilliton, 2002). The Environment Department of the Cannington mine (the collaborative partner) required that this project be developed in accordance with these principles and practices. Two aspects of the Cannington mine's current EMOS strongly influenced the directions this research project could be taken and as a result have affected the outcomes of the study.

The Environment Department of the Cannington mine has adopted a policy of using only 'tenement hosted' plant species for all land rehabilitation projects (BHPBilliton, 2002). This policy restricted the selection of plants for the present study to a collection of native pasture species that had not previously been investigated for phytoextractive purposes. In addition, the

principles and practices of phytoextraction had to be integrated into the plant selection and chemical treatment procedures when very little was known regarding the response of these plants to heavy metals in soil. The exclusion of 'exotic' plant species (such as *Brassica juncea*, Huang and Cunningham, 1996), that have been demonstrated as highly effective for the chemically-assisted phytoremediation of a range of heavy metals in contaminated soil (e.g. Blaylock *et al.*, 1997; Ebbs *et al.*, 1997), has prevented the development of alternative land rehabilitation strategies for the Cannington mine. In reality, the rigid nature of the Cannington mine's current EMOS will preclude numerous demonstrable phytoextraction technologies from being adopted.

The Cannington mine's EMOS indicates that acceptable concentrations of heavy metals and metalloids contained in rehabilitated soil at the mine site must be no higher than background concentrations in surrounding pasture soil. It is obvious that this environmental objective is unattainable with any existing *in situ* soil remediation process, including phytoextraction. This land rehabilitation objective will inevitably lead to significant losses of valuable topsoil through the use of conventional rehabilitation techniques, such as excavation and disposal. It would therefore be prudent for the Cannington mine to reconsider what are achievable, rather than desirable, levels of residual heavy metals and metalloids in remediated soil, such as those outlined by the Australian and New Zealand Environment and Conservation Council (ANZECC, 1999).

The results of this study have been compared to published laboratory and field tested examples of CAP. The recommended threshold for the technology's viability has been reported as being a crop producing 20 tonnes of biomass per hectare that contains >1 wt% Pb, accumulated by natural or chemical means (Raskin and Ensley, 2000). There are very few herbaceous plant species capable of generating this quantity of biomass production and none were present on the Cannington mine tenement. Furthermore, a concentration of 1 wt% Pb in plant tissues has only been achieved in a small number of well-known agricultural crop species, none of which were grasses.

8.2. The revegetation of soil contaminated with mine tailings

The revegetation of soil contaminated with ≤35 wt% mine tailings (equivalent to 718 μg As g^{-1}, 4202 μg Pb g^{-1} and 1499 μg Zn g^{-1}) can be undertaken at the Cannington mine site using *Crotalaria novae-hollandiae*, *Cymbopogon ambiguus*, *Cymbopogon bombycinus*, *Cyperus victoriensis*, *Gomphrena canescens* and *Triodia molesta*. This study concludes that the native pasture plant species available for land rehabilitation at the Cannington mine will satisfy the environmental principles and practices outlined in the mine's EMOS. Furthermore, plant selection may be performed by comparing the Pb concentration of a contaminated soil requiring rehabilitation with the reported levels for plant tolerance to contaminated soil determined by this study. It must be stated, however, that plant growth will be significantly reduced when cultivated on soil contaminated with ≥25 wt% mine tailings (equivalent to 515 μg As g^{-1}, 3010 μg Pb g^{-1} and 1091 μg Zn g^{-1}), indicating

the need to reduce the toxicity of these soils to achieve effective revegetation.

In general, the pasture plant species investigated were found to exclude heavy metals and metalloids from uptake, even when soil extractions indicated that high levels of plant-available metals were present in the substrate. This study concludes that the natural uptake of metals and metalloids by the native pasture plant species when cultivated on soil contaminated with mine tailings is low. However, the revegetation of soil contaminated with mine tailings destined for a pastoral land use capability using high proportions of *Triodia molesta*, *Gomphrena canescens*, *Cyperus victoriensis*, *Crotalaria novae-hollandiae* in the cover system is not recommended because of the potential for these plants to accumulate high concentrations of some heavy metals and metalloids in above-ground plant tissues.

8.2.1. Improved revegetation of contaminated soil using limestone amendments

Limestone amendments (10 wt% and 20 wt%) applied to soil contaminated with mine tailings effectively inhibit the natural accumulation of Ag, As, Cd, Pb and Zn in *Cymbopogon ambiguus*, *Cymbopogon bombycinus* and *Crotalaria novae-hollandiae* while maintaining a high level of biomass production. This study concludes that the pasture quality of these species cultivated on modestly contaminated soils (≤25 wt% mine tailings) would be improved with the use of small amendments of limestone (e.g. ≤10 wt.%). Effective revegetation of

soil contaminated with higher proportions of mine tailings (>25 wt%) using these species will require using larger quantities of limestone (≤20 wt%).

8.2.2. Revegetation of contaminated soil and mine tailings using **Chloris gayana**

Although contradicting the principles and practices of the Cannington mine's EMOS, the exotic pasture grass *Chloris gayana* was included in this study because none of the native pasture plant species indicated the potential to grow in highly metalliferous materials, such as undiluted mine tailings (≤0.2 wt% As, ≤1.2 wt% Pb and ≤0.4 wt% Zn). Furthermore, the Environment Department of the Cannington mine expressed a desire to investigate methods for directly revegetating the mine's tailings, and to potentially phytoremediate the residual concentrations of Ag, Pb and Zn contained therein.

This study concludes that *Chloris gayana* is extremely well suited to the revegetation of soil contaminated with a range of mine tailings proportions (equivalent to ≤715 μg As g^{-1}, ≤4202 μg Pb g^{-1}, ≤1499 μg Zn g^{-1}). Furthermore, the biomass production of *Chloris gayana* cultivated on these contaminated soils was dramatically higher than the biomass production of plants grown on uncontaminated soil. *Chloris gayana* naturally accumulated lower concentrations of As, Cd and Pb from these contaminated soils indicating a low potential for fodder toxicity in pastoral applications.

Moreover, the uptake of elements by *Chloris gayana* from contaminated soil was lower than reported levels for Vetiver grass (Truong, 2000) suggesting a greater potential to revegetate metalliferous soil compared to other pasture species currently used for this purpose. This study also concludes that Cannington mine tailings can be directly revegetated with *Chloris gayana* following the application of 300 kg^{-1} ha^{-1} of P and/or N fertiliser and suggests that highly contaminated soil (>35 wt% mine tailings) may also be effectively revegetated by this species using fertiliser amendments.

8.3. The phytoremediation of mine tailings and soil contaminated by mine tailings

This study determined that chelate amendments (EDTA, DTPA, EDDS) applied to soil contaminated with mine tailings resulted in significant uptake of base metals and metalloids by *Chloris gayana*, *Crotalaria novae-hollandiae*, *Cyperus victoriensis* and *Cymbopogon bombycinus*. In contrast, soil amendments of thiosulphate resulted in elevated concentrations of Ag in *Chloris gayana*, *Cyperus victoriensis* and *Crotalaria novae-hollandiae*. However, in comparison to the existing literature this study must conclude that the chemically-assisted phytoremediation of heavy metals and metalloids from soil contaminated with mine tailings is not viable using the pasture plant species investigated.

Of the plant species and chemical amendments tested, *Cyperus victoriensis* indicated the highest potential to tolerate on-going soil amendments of

EDTA and EDDS in addition to phytoremediating large quantities of Pb (approximately 6.0-6.5 kg^{-1} ha^{-1} $crop^{-1}$) and Zn (approximately 7.0-7.2 kg^{-1} ha^{-1} $crop^{-1}$) from the contaminated soil using these reagents. These quantities of metal equate to a surface contamination load of approximately 1.5 g Pb m^{-2} yr^{-1} and 1.4 g Zn m^{-2} yr^{-1}; a level similar to the current rate of metallic dust deposition at the Cannington mine (Wilson, March 2002). Therefore, this study can also conclude that pastures of *Cyperus victoriensis* may be useful for the phytoremediation of low levels of heavy metal and metalloid contamination to pasture soil at the Cannington mine.

Chemically-assisted phytoextraction of Ag, Pb and Zn from fertilised mine tailings using *Chloris gayana* met with limited success. Although soil extractions indicated that large concentrations of plant-available metals were present in the fertilised mine tailings, *Chloris gayana* was estimated to phytoremediate a low quantity of Pb (equivalent to approximately 1.2 kg ha^{-1} $crop^{-1}$) and a modest quantity of Zn (equivalent to approximately 9.5 kg ha^{-1} $crop^{-1}$) from the fertilised mine tailings using relatively non-toxic EDTA amendments. This study concludes that while *Chloris gayana* is highly effective for the direct revegetation of fertilised mine tailings and is highly tolerant to the effects of the chemical amendments used to dissolve large quantities of the residual metals contained in the tailings, this species is not suitable for the CAP of metals from fertilised mine tailings.

8.3.1. A potential new approach to tailings dam management

The leaching of heavy metals from Cannington mine tailings using the chemical treatments employed for their phytoextraction determined that low ionic strength solutions of EDTA, TSP and SCN dissolved large quantities of the tailings contained Ag, Pb and Zn. This study concludes that selective leaching of commodity metals from Cannington mine tailings is possible using solutions of EDTA (28.1% Pb and 12.6% Zn), TSP (12.1% Zn) and SCN (83.7% Ag). The study suggests that the mine tailings presently contained in the Process Residue Facility may be leached of approximately 201 tonnes Ag, 12,300 tonnes Pb and 1600 tonnes of Zn over approximately 8-10 weeks of treatment using these chemical reagents. The *in situ* chemical leaching of the mine tailings would significantly reduce its metal load and may result in a waste material requiring a less costly rehabilitation solution. Furthermore, heavy metal and metalloid depletion in the surface horizon of the mine waste resulting from chemical leaching may facilitate the introduction of the less heavy-metal tolerant native pasture species. At present, however, this study alludes to the possibility of both directly revegetating the tailings with *Chloris gayana* while also leaching significant quantities of Ag, Pb and Zn from the tailings using low ionic strength solutions of novel chemical reagents.

8.4. Further research

The identification and phytoextractive evaluation of dicotyledonous native

pasture species is needed at the Cannington mine site because all successful demonstrations of chemically-assisted heavy-metal phytoextraction employ this class of plant.

Re-evaluation of the occurrence of heavy metal and metalloid tolerance and accumulation in the plants of northern and central Australia, most likely situated on or near surface gossans, is required and efforts must be made to fully describe these species in the literature.

Should either of the above recommendations prove fruitless, other chemical reagents, such as analogues to natural phytochelatins or metallothioneins in grass species, could be investigated to promote high levels of heavy metal and metalloid uptake by these plants from contaminated soil.

Knowledge regarding the physiological mechanisms behind heavy metal uptake and exclusion by native pasture plant species is required and should include the role of mycorrhyzae in this process.

Finally, the leaching of metals from mine tailings using EDTA, TSP and SCN requires evaluation at a larger scale to determine the effects of stratification in the tailings profile. In addition, a microscopic study should be undertaken to evaluate mineral surface reactions promoted by these chemical reagents.

Bibliography

Akretche, D.-E., Slimane, S.K., and Kerdjoudj, H., 1995, Selective leaching of a polymetallic complex ore by sulphuric acid and thiourea mixed with sea water: Hydrometallurgy, v. 38, p. 189-204.

Albasel, N., and Cottenie, A., 1985, Heavy metal uptake from contaminated soils as affected by peat, lime, and chelates: Soil Science Society of America Journal, v. 49, p. 386-390.

Alloway, B.J., 1995, Heavy Metals in Soils: London, Blackie Academic and Professional, 368 p.

Almås, Å., Singh, B.R., and Salbu, B., 1999, Mobility of cadmium^{-109} and zinc^{-65} in soil influenced by equilibrium time, temperature, and organic matter: Journal of Environmental Quality, v. 28, p. 1742-1750.

ANBG, 2004, Australian National Botanical Gardens Integrated Botanical Information Systems (IBIS), www.anhg.gov.au, Volume 2004.

Anderson, C.W.N., 2000, Practical Aspects of Phytoextraction [PhD thesis]: Palmerston North, Massey University.

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

Anderson, C.W.N., Brooks, R.R., Simcock, R., and Robinson, B.H., 1999b, The phytoremediation and phytomining of heavy metals, *in* G.Weber, ed., PACRIM '99 Congress: Bali, Indonesia, Australasian Institute of Mining and Metallurgy, p. 127-135.

ANRA, 2004, Australian National Resource Atlas, Volume 2004, Australian Government, Environment Australia, www.audit.ea.gov.au/anra/atlas_home.cfm.

Antonovics, J., Bradshaw, A.D., and Turner, R.G., 1971, Heavy metal tolerance in plants: Advances in Ecological Research, v. 7, p. 1-85.

ANZECC, 1999, Australian and New Zealand Guidelines for the Assessment and Management of Contaminated Sites: Canberra, Australian and New Zealand Environmental Conservation Council, and National Health and Medical Research Council, p. 16.

Aplin, T.E.H., 1976, Cyanogenic Plants of Western Australia: Western Australian Department Of Agriculture Bulletin, v. 3967, p. 2-15.

AQIS, 2005, Icon Plant Database, Volume 2005, Australian Quarantine Inspection Service.

Armstrong, R.D., McKosker, K., Millar, G., and Probert, M.E., 1997, Fluxes in nitrogen derived from plant residues and fertiliser on a cracking clay soil in a semi-arid environment: Australian Journal of Agricultural Research, v. 49, p. 437-450.

Ayres, B., Silveira, C., Ellice, C., Christensen, D., and O'Kane, M., 2003, Development of a cover system design for potentially acid-forming tailings at Peak Gold Mines, Proceeding of the 6th International Conference on Acid Rock Drainage: Cairns, Queensland, p. 957-963.

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

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

Baker, A.J.M., Reeves, R.D., and Hajar, A.S.M., 1994, Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae): New Phytologist, v. 127, p. 61-68.

Banuelos, G., 1993, Boron and selenium removal in boron-laden soils by four sprinkler irrigated plant species: Journal of Environmental Quality, v. 22, p. 786-792.

Barrow, N.J., 1987, The effects of phosphate on zinc sorption by a soil: Journal of the Soil Science Society of America, v. 38, p. 453-459.

Bartlett, R.W., 1992, Solution Mining: Amsterdam, Gordon and Breach Science Publishers, 443 p.

Basta, N.T., and Gradwohl, R., 2000, Estimation of Cd, Pb, and Zn Bioavailability in Smelter-Contaminated Soils by a Sequential Extraction Procedure: Journal of Soil Contamination, v. 9, p. 149-164.

Basta, N.T., Gradwohl, R., Snethen, K.L., and Schroder, J.L., 2001, Chemical immobilization of lead, zinc, and cadmium in smelter-contaminated soils using biosolids and rock phosphate: Journal of Environmental Quality, v. 30, p. 1222-1230.

Batianoff, G.N., Specht, R.L., and Reeves, R.D., 1990, *Stackhousia tryonii* Bailey: a nickel-accumulating serpentine-endemic species of Central Queensland.: Australian Journal of Botany, v. 38, p. 121-130.

Begonia, M.F.T., Begonia, G.B., Butler, A., Burrell, M., Ighoavodha, O., and Crudup, B., 2002, Chelate-assisted phytoextraction of lead from a contaminated soil using wheat (*Triticum aestivum* L.): Bulletin of Environmental Contamination and Toxicology, v. 68, p. 705-711.

Bell, M.A., 1986, Soil immobilization and Plant Toxicity of Arsenic, Copper and Nickel: Brisbane, University of Queensland.

Bernal, M.P., Walker, D.J., Clemente, R., and Roig, A., 2002, The effect of organic amendments and lime on the bioavailability of heavy metals in contaminated soils, 17th World Conference on Soils and Sediments: Thailand, p. 25-31.

Bhatia, N.P., Bhatia, P., and Ashwath, N., 2002, Asexual propagation of *Stackhousia tryonii*: a step towards restoration of a rare metallophyte: Australian Journal of Botany, v. 50, p. 577-582.

BHPBilliton, 2002, Environmental Management Overview Strategy (EMOS). Cannington Mine, Queensland, Australia, BHP Billiton Minerals Pty Ltd, p. 58.

Bidwell, S.D., Woodrow, I.E., Batianoff, G.N., and Sommer-Knudsen, J., 2002, Hyperaccumulation of manganese in the rainforest tree *Austromyrtus bidwellii* (Myrtaceae) from Queensland, Australia: Functional Plant Biology, v. 29, p. 899-905.

BioTech, 2004, Life Science Dictionary, Volume 2004, University of Texas, Institute of Cellular and Molecular Biology, http://biotech.icmb.utexas.edu/.

Blaylock, M.J., 1999, Field demonstration of phytoremediation of lead contaminated soils, *in* Banuelos, G., and Terry, N., eds., Phytoremediation of Trace Elements: Ann Arbor, MI, Ann Arbor Press, p. 304.

Blaylock, M.J., 2000, Field demonstrations of phytoremediation of lead-contaminated soils: Florida, CRC Press, 1-12 p.

Blaylock, M.J., and Huang, C.P., 2000, Phytoextraction of Metals, *in* Raskin, I., and Ensley, B.D., eds., Phytoremediation of Toxic Metals: Canada, Wiley International.

Blaylock, M.J., Salt, D.E., Dushenkov, V., Zakharova, 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, v. 31, p. 860-865.

Blissett, A.H., 1966, Copper-tolerant plants from the Upakaringa Copper Mine, Williamstown: Quarterly Geological Notes, v. 18, p. 1-4.

Bodon, S.B., 1998, Paragenetic relationships and their implications for ore genesis at the Cannington Ag-Pb-Zn deposit, Mount Isa inlier, Queensland, Australia: Economic Geology, v. 93, p. 1463-1488.

Bonaparte, R., and Giroud, J.P., 1996, Waste containment systems for pollution control: Part II - Hydraulic design and performance, *in* Aral, M.M., ed., Advances in groundwater pollution control and remediation: Dordrecht, The Netherlands, Kluwer Academic Publishers, p. 437-477.

Breuer, P.L., and Jeffery, M.I., 2000, Thiosulfate leaching kinetics of gold in the presence of copper and ammonia: Mineral Engineering, v. 13, p. 1071-1081.

Brooks, R.R., 1980, Accumulation of nickel by terrestrial plants, *in* Nriagu, J.O., ed., Nickel in the Environment, John Wiley & Sons, Inc., p. 407-427.

Brooks, R.R., 1987, Serpentine and its Vegetation: A Multidisciplinary Approach: Portland, Oregon, Dioscorides Press, 324 p.

Brooks, R.R., 1998, Plants that Hyperaccumulate Heavy Metals: their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining: Wallingford, CAB International, 278 p.

Brooks, R.R., Chambers, M.F., Nicks, L.J., and Robinson, B.H., 1998, Phytomining: Trends in Plant Science, v. 3, p. 359-362.

Brooks, R.R., and Radford, C.C., 1978, Nickel accumulation by European species of the genus *Alyssum*: Proceedings of the Royal Society London, v. 200, p. 217-224.

Brooks, R.R., Reeves, R.D., Morrison, R.S., and Malaisse, F., 1980, Hyperaccumulation of copper and cobalt - A review: Bulletin of the Royal Society of Botany, Belgium, v. 113, p. 166-172.

Brown, D., 2004, Poisonous Plants Information Database, Volume 2004, Department of Animal Science, Cornell University, http://www.ansci.cornell.edu/plants/index.html.

Brown, K.S., 1995, The green clean: BioScience, v. 45, p. 579-582.

Brown, S.L., Chaney, R.L., Angle, J.S., and Baker, A.J.M., 1994, Phytoremediation potential of *Thlaspi caerulescens* and Bladder Campion for zinc- and cadmium-contaminated soil: Journal of Environmental Quality, v. 23, p. 1151-1157.

Bruce, S.L., Noller, B.N., Grigg, A.H., Mullen, B.F., Mulligan, D.R., Richie, P.J., Currey, N., and Ng, J.C., 2003, A field study conducted at Kidson Gold Mine, to evaluate the impact of arsenic and zinc from mine tailings to grazing cattle: Toxicology Letters, v. 137, p. 23-34.

Burbridge, N.T., 1953, The genus *Triodia* R.Br. (gramineae): Australian Journal of Botany, v. 1, p. 121-166.

Burbridge, N.T., 1960, Further notes on *Triodia* R.Br. (Graminae) with description of five new species and one variety: Australian Journal of Botany (580 P4) Cairns, v. 8, p. 381-395.

Carroll, C., Merton, L., and Burger, P., 2000, Impact of vegetation cover and slope on runoff, and water quality for field plots on a range of soil and spoil materials on central Queensland coal mines: Australian Journal of Soil Research, v. 38, p. 313-323.

Catalan, L.J.J., Buset, K.C., and Yin, G., 2002, Reactivity of oxidized sulfidic mine tailings during lime treatment: Environmental Science and Technology, v. 36, p. 2766-2771.

Catt, P.C., 2000, Biogeochemistry, geochemistry, geobotany and spectral reflectance integrated as a tool for mineral exploration in a tropical savannah environment [PhD thesis]: Townsville, James Cook University.

Chan, K.Y., Bowman, A.M., and Friend, J.J., 1997, Restoration of soil fertility of dredged vertisols using a pasture including a native grass (*Astrebla lappacea*): Tropical Grasslands, v. 31, p. 145-155.

Chaney, R.L., 1983, Plant uptake of inorganic waste constituents, *in* Parr, J.F., Marsh, P.B., and Kla, J.M., eds., Land Treatment of Hazardous Wastes: Park Ridge, New Jersey, Noyes Data Corp, p. 50-76.

Chaney, R.L., 2000, personal communication to R.R. Brooks regarding the cost of using EDTA in "chelator-induced' phytoextraction.

Chaney, R.L., Brown, S.L., Li, Y., Angle, J.S., Stuczynski, T.I., Daniels, W.L., Henry, C.L., Siebielec, G., Malik, M., Ryan, J.A., and Compton, H., 2000, Progress in Risk Assessment for Soil Metals, and In-Situ Remediation and Phytoextraction of Metals from Hazardous Contaminated Soils, Phytoremediation: State of Science Conference: Boston, MA, US-EPA, p. 1-14.

Chaney, R.L., Brown, S.L., Stuczynski, T.I., Daniels, W.L., Henry, C.L., Li, Y.-M., Siebielec, G., Malik, M., Angle, J.S., Ryan, J.A., and Compton, H., 1999, In-situ remediation and phytoextraction of metals from hazardous contaminated soils, Innovative Clean-Up Approaches: Investments in Technology Development, Results & Outlook for the Future: Bloomingdale, Illinois, United States Environmental Protection Agency, p. 1-29.

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

Clem, R.L., and Hall, T.J., 1994, Persistence and productivity of tropical pasture legumes on three cracking clay soils (Vertisols) in north-eastern Queensland: Australian Journal of Experimental Agriculture, v. 34, p. 161-171.

Cole, M.M., 1973, Geobotanical and biogeochemical investigations in the sclerophyllous woodland and shrub associations of the Eastern Goldfields area of Western Australia with particular reference to the role of *Hybanthus floribundus* (Lindl.) F. Muell. as a nickel indicator and accumulator plant: Applied Ecology, v. 10, p. 269-320.

Cole, M.M., Provan, D.M.L., and Tooms, J.S., 1968, Geobotany, biogeochemistry and geochemistry in mineral exploration in the Bulman-Waimuna Springs area, Northern Territory, Australia: Transactions of the Institute of Mining and Metallurgy, v. 11, p. 81-104.

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, v. 28, p. 1709-1719.

Correll, R.L., and Taylor, R.G., 1974, Occurrence of *Polycarpaea spirostylis* at Daisy Bell copper mine, Emuford, North Queensland, Australia: Transactions of the Institute of Mining and Metallurgy, v. 83, p. 28-34.

Costelloe, M., 2003, Environmental review of the Mary Kathleen uranium mine [MSc thesis]: Cairns, Queensland, James Cook University.

Cotton, F.A., and Wilkinson, G., 1980, Advanced Inorganic Chemistry, John Wiley & Sons, Inc., 245 p.

Cunningham, S.D., and Berti, W.R., 2000, Phytoextraction and phytostabilization: technical, economic, and regulatory considerations of the soil-lead issue, *in* Terry, N., and Banuelos, G., eds., Phytoremediation of Contaminated Soil and Water: Florida, CRC Press, p. 359-376.
Cunningham, S.D., Berti, W.R., and Huang, J.W., 1995, Phytoremediation of contaminated soils: Trends in Biotechnology, v. 13, p. 393-397.
Cunningham, S.D., and Ow, D.W., 1996, Promises and prospects of phytoremediation: Plant Physiology, v. 110, p. 715-719.
Cunningham, S.D., Shann, J.R., Crowley, D.E., and Anderson, T.A., 1997, Phytoremediation of Contaminated Water and Soil, *in* Kruger, E.L., Anderson, T.A., and Coats, J.A., eds., Phytoremediation of Soil and Water Contamination: Washington, American Chemical Society, p. 1-24.
Davies, J.G., Hossner, L.R., and Persuad, N., 1993, Elemental toxicity effects on the germination and growth of pearl millet seedlings: Journal of Plant Nutrition, v. 16, p. 1957-1968.
Deram, A., Petit, D., Robinson, B.H., Brooks, R.R., Gregg, P.E.H., and Halluwyn, C.V., 2000, Natural and induced heavy-metal accumulation by *Arrhenatherum elatis*: Implications for phytoremediation: Communications in Soil Science and Plant Analysis, v. 31, p. 413-421.
Duke, A., 1983, Handbook of Energy Crops, Volume 2004, www.hort.purdue.edu/newcrop/duke energy/Chloris gayana.html.
Dushenkov, V., Kumar, N.P.B.A., Motto, H., and Raskin, I., 1995, Rhizofiltration: The use of plants to remove heavy metals from aqueous streams: Environmental Science and Technology, v. 29, p. 1239-1245.
Ebbs, S.D., and Kochian, L.V., 1998, Phytoextraction of zinc by Oat (*Avena sativa*), Barley (*Hordeum vulgare*), and Indian Mustard (*Brassica juncea*): Environmental Science and Technology, v. 32, p. 802-806.
Ebbs, S.D., Lasat, M.M., Brady, D.J., Cornish, J., Gordon, R., and Kochian, L.V., 1997, Phytoextraction of cadmium and zinc from a contaminated soil: Journal of Environmental Quality, v. 26, p. 1424-1430.
Ensley, B.D., 2000, Rationale for the use of phytoremediation, *in* Raskin, I., and Ensley, B.D., eds., Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment: New York, John Wiley & Sons, Inc, p. 304.
Ernst, W.H.O., 1996, Bioavailability of heavy metals and decontamination of soils by plants: Applied Geochemistry, v. 11, p. 163-167.
Farago, M.E., Clark, A.J., and Pitt, M.J., 1977, Plants which accumulate metals. Part I. The metal content of three Australian plants growing over mineralised sites: Inorganica Chimica Acta, v. 24, p. 53-56.
Farago, M.E., and Mahmoud, I.E.D.A.W., 1983, Plants which accumulate metals (Part VI): further studies of an Australian nickel accumulating plant: Minerals and the Environment, v. 5, p. 113-121.
Feng, D., and Van Deventer, J.S.J., 2002, Leaching behaviour of sulphides in ammoniacal thiosulphate systems: Hydrometallurgy, v. 63, p. 189-200.
FloraBase, 2004, FloraBase, Volume 2004, Department of Conservation and Land Management, Western Australian State Government, www.florabase.calm.wa.gov.au.
French, S., 2001, Impact of heavy metals for mine site rehabilitation [BSc (Hon) thesis]: Brisbane, University of Queensland.
GA, 2004, The Australian Atlas of Mineral Resources, Mines and Processing Centres, Volume 2004, Geoscience Australia, www.australianmineatlas.gov.au/TOC.jsp.
Gatehouse, S., Russell, D.W., and Van Moort, J.C., 1977, Sequential soil analysis in exploration geochemistry: Journal of Geochemical Exploration, v. 8, p. 483-494.
Gatliff, E.G., 1994, Vegetative remediation process offers advantages over traditional pump-and-treat technologies: Remediation Summer, v. 4, p. 343-352.
Gent, D.B., Larson, S.L., Fabian, G., Granade, S., Bricka, R.M., and Alshawabkeh, A.N., 2004, Bench- and field-scale evaluation of chromium and cadmium extraction by electrokinetics: Journal of Hazardous Materials, v. 110, p. 53-62.
Gilfedder, B., 2004, Tailings geochemistry, mineralogical processes and the effectiveness of soil covers at the Cannington Ag-Pb-Zn mine, northwest Queensland [BSc (Hon) thesis]: Cairns, James Cook University.
Glass, B.D., 2000, Economic Potential of Phytoremediation, *in* Raskin, I., and Ensley, B.D., eds., Phytoremediation of Toxic Metals: Canada, Wiley International, p. 320.
Glendinning, J.S., 2000, Australian Soil Fertility Manual: Melbourne, CSIRO Publishing, 154 p.

Goncalves, M.M.M., Pinto, A.F., and Granato, M., 1998, Biodegradation of free cyanide, thiocyanate and metal complexed cyanides in solutions with different compositions: Environmental Technology, v. 19, p. 133-142.
Graham, G., and Lambert, G., 1996, Sown Pasture Notes - Central Queensland, *in* Graham, G., and Lambert, G., eds., Information Series QI96118: Emerald, Department of Primary Industries, p. 1-2.
Gray, D.J., Butt, C.R.M., and Lawrence, L.M., 1992, Regolith Geochemistry in Tropical and Subtropical Terrains, *in* Butt, C.R.M., and Zeegers, H., eds., Geochemistry of gold in laterite terraines. Hand Book of Exploration Geochemistry, Volume 4: London, Chapman Hall, p. 461-482.
Greet, C., and Smart, R.S.C., 2002, Diagnostic leaching of galena and its oxidation products with EDTA: Mineral Engineering, v. 15, p. 515-522.
Greman, H., Vodnik, D., Velikonja-Bolta, S., and Lestan, D., 2003, Ethylenediaminedissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction: Journal of Environmental Quality, v. 32, p. 500-506.
Grice, T., 2001, Weed ecology and dynamics, *in* McCullough, M., and Musso, B., eds., The Grazing Lands of the Upper Burdikan River Catchment, Volume 1, Integrated Science and Management: Townsville, Tropical Savanna CRC, Queensland Department of Primary Industries, CSIRO - Land and Water Division, p. A5/1-A5/61.
Grigg, A., Shelton, M., and Mullen, B., 2000, The nature and management of rehabilitated pastures on open-cut coalmines in central Queensland, Tropical Grasslands Conference 2000, p. 70-85.
Hall, T.J., 1982, Species associations in a grassland on a heavy cracking clay soil in north-west Queensland: their structure, soil associations and effects of flooding: Australian Journal of Ecology, v. 7, p. 249-259.
Hamon, R., Wundke, J., McLaughlin, M.J., and Naidu, R., 1997, Availability of zinc and cadmium for different plant species: Australian Journal of Soil Research, v. 35, p. 1267-1277.
Hamon, R.E., McLaughlin, M.J., and Cozens, G., 2002, Mechanisms of attenuation of metal availability in *in situ* remediation treatments: Environmental Science and Technology, v. 36, p. 3991-3996.
Haq, A.U., Bates, T.E., and Soon, Y.K., 1980, Comparison of extractants for plant-available zinc, cadmium, nickel and copper in contaminated soils: Soil Science Society of America Journal, v. 44, p. 772-777.
Harwood, M.R., Hacker, J.B., and Mott, J.J., 1999, Field evaluation of seven grasses for use in the revegetation of lands disturbed by coal mining in central Queensland: Australian Journal of Experimental Agriculture, v. 39, p. 307-316.
Hayes, W.J., Chaudhry, T.M., Buckney, R.T., and Khan, A.G., 2003, Phytoaccumulation of trace metals at the Sunny Corner Mine, New South Wales, with suggestions for a possible remediation strategy: Australian Journal of Ecotoxicology, v. 9, p. 69-82.
Huang, J.W., Chen, J., Berti, W.R., and Cunningham, S.D., 1997a, Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction: Environmental Science and Technology, v. 31, p. 800-805.
Huang, J.W., Chen, J., and Cunningham, S.D., 1997b, Phytoextraction of lead from contaminated soils, *in* Kruger, E.L., Anderson, T.A., and Coats, J.A., eds., Phytoremediation of Soil and Water Contamination: Washington, American Chemical Society, p. 283-298.
Huang, J.W., and Cunningham, S.D., 1996, Lead phytoextraction: species variation in lead uptake and translocation: New Phytologist, v. 134, p. 75-84.
Hunt, C.B., 1972, Geology of soils; their evolution, classification, and use: San Francisco, W.H. Freeman, 344 p.
Isbell, R.F., 1996, The Australian Soil Classification: Melbourne, Australia, CSIRO Publishing, 143 p.
Islam, M., and Adams, M.A., 1999, Mineral content and nutrient value of native grasses and the response to added phosphorous in a Pilbara rangeland: Tropical Grasslands, v. 33, p. 193-200.
James, D., 1995, The threat of exotic grasses to the biodiversity of semiarid ecosystems: Arid Lands Newsletter, www.ag.arizona.edu/OALS/ALN/aln37/james.html, v. 2004, p. 37.
James, S.H., 1981, Cytoevolutionary patterns, genetic systems and the phytogeography of Australia, *in* Keast, A., ed., In Ecological Biogreography of Australia, Volume 1: The Hague, Dr W junk, p. 761-805.

Jasper, D.A., Robson, A.D., and Abbott, L.K., 1988, Revegetation in an iron-ore mine - nutrient requirements for plant growth and the potential role of vascular-arbuscilar (VA) mycorrhizal fungi: Australian Journal of Soil Research, v. 26, p. 497-508.
Jeffery, M.I., 2001, Kinetic aspects of gold and silver leaching in ammonia-thiosulfate solutions: Hydrometallurgy, v. 60, p. 7-16.
Jeffery, M.I., Breuer, P.L., and Choo, W.L., 2001, A kinetic study that compares the leaching of gold in the cyanide, thiosulfate, and chloride systems: Metallurgical and Materials Transactions B, v. 32B, p. 979-986.
Kabata-Pendias, A., and Pendias, H., 1984, Trace Elements in Soils and Plants: Florida, CRC Press.
Kayser, A., Wenger, K., Keller, A., Attinger, W., Felix, H.R., 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, v. 34, p. 1778-1783.
Keeling, S.M., 2000, The economic significance of the phytoextraction of nickel, cobalt and gold from metalliferous soils [MSc thesis]: Palmerston North, New Zealand, Massey University.
Keeling, S.M., 2001, Heavy metal phytoextraction: Turning waste into ore, Green Processing 2002: Cairns, Australia, Australasian Institute of Mining and Metallurgy, p. 137-146.
Keeling, S.M., 2002, Heavy metal phytoextraction: Turning waste into ore, Green Processing 2002: 4/2002: Cairns, Australia, Australasian Institute of Mining and Metallurgy, p. 137-146.
Keeling, S.M., Stewart, R.B., Anderson, C.W.N., and Robinson, B.H., 2003, Nickel and Cobalt Phytoextraction by the Hyperaccumulator *Berkheya coddii:* Implications for Polymetallic Phytomining and Phytoremediation: International Journal of Phytoremediation, v. 5, p. 12-26.
Kelly, R.J., and Guerin, T.F., 1994, Metal accumulating plants and implications for the mining industry: Australian Journal of Mining, v. November, p. 38-40.
Kinnersley, A.M., 1993, The role of phytochelatins in plant growth and productivity: Plant Growth Regulation, v. 12, p. 207-218.
Kochian, L.V., 2000, Phytoremediation: using plants to clean up soil: Agricultural Research, v. June, p. 1-9.
Kos, B., and Lestan, D., 2003, Induced phytoextraction/soil washing of lead using biodegradable chelate and permeable barriers: Environmental Science and Technology, v. 37, p. 624-629.
Kucha, H., Prohaska, W., and Stumpfl, E.F., 1995, Deposition and transport of gold by thiosulphates, Veitsch, Austria, Mineralogical Magazine, Volume 59, p. 253-258.
Lasat, M.M., 2002, Phytoextraction of toxic metals: a review of biological mechanisms: Journal of Environmental Quality, v. 31, p. 109-120.
Lazarides, M., 2004, A Revision of *Triodia* including *Plectrachne* (Poaceae, Eragrostideae, Triodiinae): Australian Systematic Botany, v. 10, p. 381-489.
Leblanc, M., Petit, D., Deram, A., Robinson, B.H., and Brooks, R.R., 1999, The phytomining and environmental significance of hyperaccumulation of thallium by *Iberis intermedia* from Southern France: Economic Geology, v. 94, p. 109-114.
Lee, A.T., 1978, Some species of Crotalaria in Australia: Telopea, v. 1, p. 319-356.
Li, J., and Wadsworth, M.E., 1993, Electrochemical study of silver dissolution in cyanide solutions: Journal of the Electrochemical Society, v. 140, p. 1921-1927.
Li, Y., Chaney, R.L., Siebielec, G., and Kerschner, B.A., 2000, Response of four turfgrass cultivars to limestone and biosolids compost amendment of a zinc and cadmium contaminated soil at Palmerton, PA: Environmental Quality, v. 29, p. 1440-1447.
Li, Z., and Shuman, L.M., 1996, Extractability of zinc, cadmium, and nickel in soils amended with EDTA: Soil Science, v. 161, p. 226-241.
Licht, L.A., and Schnoor, J.L., 1993, Tree buffers protect shallow groundwater at contaminated sites., EPA Ground Water Currents, Office of Solid Waste and Emergency Response, p. EPA/542/N-93/011.
Liles, J., 2004, Themeda triandra, Volume 2004, Australian National Botanical Gardens, www.anhg.gov.au.
Lindsay, W.L., and Norvell, W.A., 1978, Development of a DTPA soil test for zinc, iron, manganese and copper: Soil Science Society of America Journal, v. 42, p. 421-428.
Liu, G.Q., and Yen, W.T., 1995, Effects of sulphide minerals and dissolved oxygen on the gold and silver dissolution in cyanide solutions: Mineral Engineering, v. 8, p. 111-123.
Lodge, G.M., Murphy, S.R., and Harden, S., 2003, Effects of grazing and management on herbage mass, persistence, animal production and soil water content of native pastures. 2. A mixed

native pasture, Manilla, North West Slopes, New South Wales: Australian Journal of Experimental Agriculture, v. 43, p. 891-905.
Loganathan, P., Hedley, M.J., Gregg, P.E.H., and Currie, L.D., 1997, Effects of phosphate fertiliser type on the accumulation and plant availability of cadmium in grassland soils: Nutrient Cycling and Agroecosystems, v. 47, p. 169-178.
Ma, L.Q., Choate, A.L., and Rao, G.N., 1997, Effects of incubation and phosphate rock on lead extractability and speciation in contaminated soils: Journal of Environmental Quality, v. 26, p. 801-807.
Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., and Kennelley, E.D., 2001, A fern that hyperaccumulates arsenic: Nature, v. 409, p. 579.
Ma, L.Q., and Lindsay, W.L., 1993, Measurements of free zinc2+ activity in uncontaminated and contaminated soils using chelation: Soil Science Society of America Journal, v. 57, p. 963-967.
Ma, L.Q., Traina, S.A., and Logan, T.J., 1993, *In situ* lead immobilisation by apatite: Environmental Science and Technology, v. 27, p. 1-8.
MacKenzie, J., Mayer, R., and Bisset, W.J., 1982, Productivity of five subtropical grasses on a black earth of the eastern Darling Downs of Queensland: Tropical Grasslands, v. 16, p. 170-180.
Malone, S., 2004, Senior Environmentalist at the BHPBilliton Cannington Ag-Pb-Zn Mine, personal communication on estimated pasture fuel loads.
Malone, S., pers. com. 2004, Senior Environmentalist at the BHPBilliton Cannington Ag-Pb-Zn Mine, personal communication on estimated pasture fuel loads.
Mann, A.W., 1984, Mobility of gold and silver in lateritic weathering profiles: Some observations from Western Australia: Economic Geology, v. 79, p. 35-49.
Marschner, H., 1995, The Mineral Nutrition of Higher Plants: London, Academic Press, 887 p.
Maudos, I., Chimenos, J.M., Segarra, M., and Espiell, M., 1996, Kinetic study of silver chloride dissolution in complexing media: Hydrometallurgy, v. 40, p. 153-167.
McBride, M.B., 1994, Environmental Chemistry of Soils: New York, Oxford University Press, Inc.
McGrath, S.P., 1998, Phytoextraction for Soil Remediation, *in* Brooks, R.R., ed., Plants that Hyperaccumulate Heavy Metals: New York, CAB International, p. 261-285.
McGrath, S.P., Chang, A.C., Page, A.L., and Witter, E., 1994, Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States: Environmental Review, v. 2, p. 108-118.
McGrath, S.P., Shen, Z.G., and Zhao, F.J., 1997, Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* growth in contaminated soils: Plant and Soil, v. 188, p. 153-159.
McGrath, S.P., Sidoli, C.M.D., Baker, A.J.M., and Reeves, R.D., 1993, The potential for the use of metal-accumulating plants for the *in situ* decontamination of metal-polluted soils, *in* Eijackers, H.J.P., and Hamers, T., eds., Integrated Soil and Sediment Research: A Basis for Proper Protection: Netherlands, Kluwer Academic Publishers, p. 673-676.
McGrath, S.P., Zhao, F.J., and Dunham, S.J., 1999, Constraints to the growth and metal uptake by hyperaccumulator plants, *in* Wenzel, W.W., Adriano, D.C., Alloway, B.J., Doner, H.E., Keller, C., Lepp, N.W., Mench, M., Naidu, R., and Pierzynski, G.M., eds., Proceedings of the 5th International Conference On The Biogeochemistry of Trace Elements: Vienna, p. 10-11.
Means, J.L., Kucak, T., and Crerar, D.A., 1980, Relative degradation rates of NTA, EDTA and DTPA and environmental implications: Environmental Pollution, v. (Series B), p. 45-60.
Meecham, J.R., and Bell, L.C., 1977, Revegetation of Alumina Refinery Wastes: 2. Glasshouse Experiments: Australian Journal of Experimental Agriculture and Animal Husbandry, v. 17, p. 689-696.
Mentis, M.T., 2001, Diagnosis of the rehabilitation of opencast coal mines on the Highveld of South Africa: South African Journal of Science, v. 95, p. 210-215.
Menzies, N.W., and Mulligan, D.R., 2000, Vegetation dieback on clay-capped pyritic mine waste: Journal of Environmental Quality, v. 29, p. 437-442.
Miller, W.P., Martens, D.C., and Zelazny, L.W., 1987, Short-term transformations of copper in copper-amended soils: Journal of Environmental Quality, v. 16, p. 176-180.
Milson, J., 2000, Pasture plants of north-west Queensland: Brisbane, Department of Primary Industries, 160 p.
Molleman, E., and Dreisinger, D., 2002, The treatment of copper-gold ores by ammonium thiosulphate leaching: Hydrometallurgy, v. 66, p. 1-21.

Moran, R., 1998, Cyanide uncertainties - Observations on the chemistry, toxicity, and analysis of cyanide and mining-related waters: Washington, DC, Mineral Policy Centre, p. 1-13.
Morrey, D.R., Balkwill, K., and Balkwill, M.-J., 1989, Studies on serpentine flora: Preliminary analyses of soils and vegetation associated with serpentine rock formation in the south-eastern Transvaal: South African Journal of Botany, v. 55, p. 171-177.
Morrison, R.S., 1980, Aspects of the accumulation of cobalt, copper and nickel by plants [PhD thesis]: Palmerston North, New Zealand, Massey University.
Mortvedt, J.J., Cox, F.R., Shuman, L.M., and R.M., W., 1991, Micronutrients in Agriculture: Madison, Soil Science Society of America, 673 p.
Mott, J.J., Ludlow, M.M., Richards, J.H., and Parsons, A.D., 2004, Effects of moisture supply in the dry season and subsequent defoliation on persistence of the savanna grasses *Themeda triandra*, *Heteropogon contortus* and *Panicum maximum*.: Australian Journal of Agricultural Research, v. 43, p. 241-260.
Naidu, B.P., and Harwood, M.R., 1997, Opportunities for landscape stabilisation and revegetating disturbed lands in stressful environments with exotic or native forages: Tropical Grasslands, v. 31, p. 364-369.
Narayanan, M., Davies, L.C., and Erickson, L.E., 1995, Fate of volatile chlorinated organic compounds in a laboratory chamber with alfalfa plants: Environmental Science and Technology, v. 29, p. 2437-2444.
Nellessen, J.E., and Fletcher, J.S., 1993, Assessment of published literature on the uptake/accumulation, translocation, adhesion and biotransformation of organic chemicals by vascular plants: Environmental Toxicology and Chemistry, v. 12, p. 1669-1680.
Nicholas, D.J.D., and Egan, A.R., 1975, Trace elements in soil-plant-animal systems: Glen Osmond, South Australia, Academic Press, 417 p.
Nicks, L.J., and Chambers, M.F., 1995, Farming of metals: Mining Environmental Management, v. September, p. 15-18.
Nicks, L.J., and Chambers, M.F., 1998, A pioneering study of the potential of phytomining for nickel, *in* Brooks, R.R., ed., Plants that Hyperaccumulate Heavy Metals: Wallingford, CAB International, p. 313-326.
Niedzwiedz-Siegien, I., 1998, Cyanogenic glucosides in *Linum usitatissimum*: Phytochemistry, v. 49, p. 59-63.
Norvell, W.A., 1984, Comparison of chelating agents as extractants for metals in diverse soil materials: Journal of the Soil Science Society of America, v. 48, p. 1285-1292.
Norvell, W.A., 1991, Reactions of metal chelates in soils and nutrient solutions, *in* Mortvedt, J.J., ed., Micronutrients in Agriculture: Madison, Soil Science Society of America, p. 187-227.
Nowack, B., 2002, Environmental chemistry of aminopolycarboxylate chelating agents: Environmental Science and Technology, v. 36, p. 4009-4016.
Nriagu, J.O., 1974, Lead orthophosphates: IV. Formation and stability in the environment: Geochemica et Cosmochemica Acta, v. 38, p. 887-898.
O'Kane, M., and Waters, P., 2003, Dry cover trials at Mt Whaleback - A summary of overburden storage area cover system performance, Proceedings of the 6th International Conference on Acid Rock Drainage: Cairns, Queensland, p. 147-153.
Oliver, J., 2004, Jamie's Dinners: London, Penguin Books Ltd, 320 p.
Orr, D., 1975, A review of Astrebla (Mitchell Grass) pastures in Australia: Tropical Grasslands, v. 9, p. 32-41.
Outridge, P.M., and Noller, B.N., 1991, Accumulation of toxic trace elements by freshwater vascular plants: Reviews of Environmental Contamination and Toxicology, v. 121, p. 1-63.
Pampura, T.B., Pinskiy, D.L., Ostoumov, V.G., Gershevich, V.D., and Bashkin, V.N., 1993, Experimental study of the buffer capacity of a Chernozem contaminated with copper and zinc: Eurasian Soil Science, v. 25, p. 27-38.
Papp, C.S.E., Filipek, L.H., and Smith, K.S., 1991, Selectivity and effectiveness of extractants used to release metals associated with organic matter: Applied Geochemistry, v. 6, p. 349-353.
Phelps, D., 2004, Mitchell grass - Australia's own grass, Volume 2004, Department of Primary Industries and Fisheries, Queensland. www.dpi.qld.gov.au/sheep/5014.html.
Pitts, B., and Albrecht, D., 2000, Buffel grass (*Cenchrus ciliaris*) control in central Australia, Danthonia: The newsletter of the Australian Network for Plant Conservation Inc, Volume 9, p. 7-8.
Plenderleith, R.W., 1990, Tolerance of twelve tropical grasses to high soil concentrations of copper: Tropical Grasslands, v. 24, p. 103-110.

Plimer, I.R., and Williams, P.A., 1987, New mechanisms for the mobilization of the platinum-group elements in the supergene zone: Proceedings of the Symposium "Geo-Platinum", p. 15-21.
Queensland_Government, 2002, Rhodes grass *Chloris gayana*: Environmental Weeds Information Series, v. EW25.
Raskin, I., and Ensley, B.D., 2000, Phytoremediation of Toxic Metals: Canada, Wiley International.
Raskin, I., Kumar, N.P.B.A., Dushenkov, S., and Salt, D.E., 1994, Bioconcentration of heavy metals by plants: Current Opinions in Biotechnology, v. 5, p. 285-290.
Raven, P.H., Evert, R.F., and Eichhorn, S.E., 1998, Biology of Plants: New York, Worth Publishers, 875 p.
Reeves, R.D., and Baker, A.J.M., 2000, Metal-accumulating Plants, *in* Raskin, I., and Ensley, B.D., eds., Phytoremediation of Toxic Metals: Canada, Wiley International, p. 190-227.
Reeves, R.D., Baker, A.J.M., and Brooks, R.R., 1995, Abnormal accumulation of trace metals by plants: Mining Environmental Management, v. September, p. 4-8.
Reeves, R.D., and Brooks, R.R., 1983, European species of Thlaspi L. (Crucifarae) as indicators of nickel and zinc: Journal of Geochemical Exploration, v. 18, p. 275-283.
Reichman, S., 2000, The response of plants to metal toxicity: A review focusing on copper, manganese and zinc: Melbourne, Australian Minerals and Energy Environmental Foundation, 54 p.
Rendell, P.S., Batley, G.E., and Cameron, A.J., 1980, Absorption as a control of metal concentrations in sediment extracts: Environmental Science and Technology, v. 14, p. 314-318.
Rethman, N., 2000, Approaches to biodiversity on rehabilitated minelands in South Africa, Tropical Grasslands Conference 2000, p. 77-79.
Robinson, B.H., 1997, The phytoextraction of heavy metals from metalliferous soils [PhD thesis]: Palmerston North, New Zealand, Massey University.
Robinson, B.H., Brooks, R.R., Howes, A.W., Kirkman, J.H., and Gregg, P.E.H., 1997, The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining: Journal of Geochemical Exploration, v. 60, p. 115-126.
Robinson, B.H., Green, S.R., Mills, T.M., Clothier, B.E., van der Velde, M., Laplane, R., Fung, L.E., Deurer, M., Hurst, S., Thayalakumaran, T., and van den Dijssel, C., 2003, Phytoremediation: using plants as biopumps to improve degraded environments: Australian Journal of Soil Research, v. 41, p. 599-611.
Rock, S., 1997, Phytoremediation, *in* Freeman, H., ed., Standard Handbook of Hazardous Waste Treatment and Disposal, Volume Practical Approaches to Implementation: New York, McGraw Hill, p. 8-1-8-9.
Rossi, G., Figliolia, A., Socciarelli, S., and Pennelli, B., 2002, Capability of *Brassica napus* to accumulate cadmium, zinc and copper from soil: Acta Biotechnologia, v. 22, p. 133-140.
Ruby, M.V., Davies, A., and Nicholson, A., 1994, *In situ* formation of lead phosphates in soils as a method to immobilize lead: Environmental Science and Technology, v. 28, p. 646-654.
Russell, M.J., and Roberts, B.R., 1986, Revegetation of coal mine spoil using pasture on the Darling Downs of Queensland, Australia: Reclamation and Revegetation Research, v. 5, p. 509-519.
Salt, D.E., and Kramer, U., 2000, Mechanisms of Metal Hyperaccumulation in Plants, *in* Raskin, I., and Ensley, B.D., eds., Phytoremediation of Toxic Metals: Canada, Wiley International, p. 231-245.
Sarret, G., Vangronsveld, J., Musso, M., D'Haen, J., Menthennex, J.-J., and Hazemann, J.-L., 2001, Accumulation forms of Zn and Pb in *Phaseolus vulgaris* in the presence and absence of EDTA: Environmental Science and Technology, v. 2001, p. 2854-2859.
Saunders, N., 2005, Creative Chemistry, www.creative-chemistry.org.uk/ alevel/ module5/ documents/ N-ch5-07.pdf, Volume 2005.
Schnoor, J.L., Licht, L.A., McClutcheon, S.C., Wolfe, N.L., and Carreira, L.H., 1995, Phytoremediation of organic and nutrient contaminants: Environmental Science and Technology, v. 29, p. 318A-323A.
Severne, B.C., and Brooks, R.R., 1972, A nickel-hyperaccumulating plant from Western Australia: Planta, v. 103, p. 91-94.
Sheppard, S.C., and Evenden, W.G., 1985, Mobility and uptake by plants of elements placed near a shallow water table interface: Journal of Environmental Quality, v. 14, p. 554-560.
Shimp, J.F., Tracey, J.C., Davies, L.C., Lee, E., Huang, W., Erickson, L.J., and Schnoor, J.L., 1993, Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials: Critical Reviews in Environmental Science and Technology, v. 23, p. 41-77.

Singh, B.R., and Narwal, R.P., 1984, Plant availability of heavy metals in a sludge treated soil: II. Metal extractability compared with plant metal uptake: Journal of Environmental Quality, v. 13, p. 344-349.
SSSA, 2004, Environmental Glossary, Volume 2004, Soil Science Society of America, http://soilslab.cfr.washington.edu/S-7/envglossary.html.
Stanforth, R., and Qiu, J., 2001, Effects of phosphate treatment on the solubility of lead in contaminated soil: Environmental Geology, v. 41, p. 1-10.
Sumner, M.E., 2000, Handbook of Soil Science: Boca Raton, Florida, CRC Press, A-1-I-33 p.
't Mannetje, L., and Kersten, S.M.M., 1992, Chloris gayana Kunth, *in* Mannetje, L., and Jones, R.M., eds., Plant Resources of South-East Asia No 4. Forages: Wageningen, Pudoc-DLO, p. 90-92.
Taylor, G., Spain, A., Nefiodovas, A., Timms, G., Kuznetsov, V., and Bennett, J.W., 2003, Determination of the reasons for deterioration of the Rum Jungle waste rock cover: Kenmore, QLD 4069, Australian Centre for Mining Environmental Research, p. 108.
Tejowulun, R.S., and Hendershot, W.H., 1998, Removal of trace metals from contaminated soils using EDTA incorporated resin trapping techniques: Environmental Pollution, v. 103, p. 135-142.
Tessier, A., Campbell, P.G.C., and Bisson, M., 1979, Sequential extraction procedure for the speciation of particulate trace metals: Analytical Chemistry, v. 51, p. 844-851.
Thornton, I., 1999, Bioavailability of trace elements in the foodchain, *in* Wenzel, W.W., Adriano, D.C., Alloway, B.J., Doner, H.E., Keller, C., Lepp, N.W., Mench, M., Naidu, R., and Pierzynski, G.M., eds., Proceedings of Extended Abstracts: 5th International Conference on the Biogeochemistry of Trace Elements: Vienna, Austria, p. XI-XIV.
Tipping, E., Hetherington, N.B., Hilton, J., Thompson, D., Bowles, E., and Hamilton-Taylor, J., 1985, Artefacts in the use of selective chemical extraction to determine the distribution of metals between oxides of manganese and iron: Analytical Chemistry, v. 57, p. 1944-1946.
Tix, D., 2000, *Cenchrus ciliaris* invasion and control in Southwestern U.S. grasslands and shrub lands, Volume 2004, Restoration and Reclamation Review, www.hort.agri.umn.edu/h5015/00papers/tix.htm.
Truong, P., 1998, Vetiver Grass Technology: A Tool Against Environmental Degradation, Tavira Vetiver Workshop: Portugal, European and Mediterranean Vetiver Network, p. 1-16.
Truong, P., 2000, Vetiver Grass for Mine Site Rehabilitation and Reclamation, Remade Lands International Conference: Fremantle, WA, p. 85-86.
Truong, P., April 1999, Vetiver grass technology for land stabilisation, erosion and sediment control in the Asia Pacific region, First Asia-Pacific Conference on Ground and Water Bio-engineering for Erosion Control and Slope Stabilisation: Manila, Philippines, p. 72-84.
Truong, P., and Baker, D., 1998, Vetiver Grass System for Environmental Protection, Volume 2004: Bangkok, Thailand, Pacific Rim Vetiver Network, Office of the Royal Development Projects Board, www.vetiver.com/AUS_toxaures.htm.
USEPA, 1997, Cleaning Up the Nation's Waste Sites: Markets and Technology Trends: Washington, DC, Office of Solid Waste and Emergency Response, p. EPA/542/R-96/005.
USEPA, 2000a, Introduction to phytoremediation: Cincinnati, Office of Research and Development, p. EPA 600/R-99/107.
USEPA, 2000b, Electrokinetic and phytoremediation in situ treatment of metal-contaminated soil: State-of-the-practice: Washington, DC, Office of Solid Waste and Emergency Response Technology Innovation Office, p. EPA/542/R-00/XXX (draft for final review).
USEPA, 2004, Terms of Environment, Volume 2004, United States Environmental Protection Agency, http://www.epa.gov/OCEPAterms/cterms.html.
Vandevivere, P.C., Hammes, F., Verstraete, W., Feijtel, T., and Schowanek, D., 2001a, Metal decontamination of soil, sediment, and sewage sludge by means of transition metal chelant [S,S]-EDDS: Journal of Environmental Engineering, v. September, p. 802-811.
Vandevivere, P.C., Saveyn, H., Verstraete, W., Feijtel, T.C.J., and Schowanek, D.R., 2001b, Biodegradation of metal-[S,S]-EDDS complexes: Environmental Science and Technology, v. 35, p. 1765-1770.
Walters, S., 1998, Geology and mineralization of the Cannington Ag-Pb-Zn deposit: An example of broken hill-type mineralization in the eastern succession. Mount Isa Inlier, Australia: Economic Geology, v. 93, p. 1307-1329.
Wang, X., and Forssberg, E., 1990, EDTA induced floatation of sulphide minerals: Journal of Colloid and Interfacial Science, v. 140, p. 217-226.

Waters, C.M., 2004, Guiding the delimitation of local provenance for Australian native grasses, Volume 2004, Online Community Publishing, www.regional.org.au/au/stipa/papers/stipa2001-09.html.
Watson, L., and Dallwitz, M.J., 1992, Grass Genera of the World: Wallingford, UK, CAB International, 1038 p.
White, R., 2004, LegumeWeb, Volume 2004, International Legume Database and Information Service, www.ildis.org/LegumeWeb.
Whitten, M.G., and Richie, G.S.P., 1991, Calcium chloride extractable cadmium as an estimate of cadmium uptake by subterranean clover: Australian Journal of Soil Research, v. 29, p. 215-221.
Williams, P.A., 1990, Oxide zone geochemistry: Chichester, England, Ellis Horwood, 286 p.
Wilson, R., 2002, Manager of the Environment Department at the Cannington Ag-Pb-Zn Mine, personal communication.
Wilson, R., March 2002, Manager of the Environment Department at the Cannington Ag-Pb-Zn Mine, personal communication on dust deposition.
Wolverton, B.C., 1975, Water hyacinths and alligator weeds for removal of lead and mercury from polluted waters, Volume TM-X-72723, NASA Technical Memorandum.
Woolnough, A.P., and Foley, W.J., 2002, Rapid evaluation of pasture quality for a critically endangered mammal, the northern hairy-nosed wombat (*Lasiorhinus krefftii*): Wildlife Research, v. 29, p. 91-100.
Wu, J., Hsu, F.C., and Cunningham, S.D., 1999, Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints: Environmental Science and Technology, v. 33, p. 1898-1904.
Ye, Z.H., Wong, J.W.C., Wong, M.H., Baker, A.J.M., Shu, W.S., and Lan, C.Y., 2000, Revegetation of Zn/Pb mine tailings, Guangdong Province, China: Restoration Ecology, v. 8, p. 87-92.
Yordanov, A.T., and Roundhill, D.M., 1998, Solution extraction of transition and post-transition heavy and precious metals by chelate and macrocyclic ligands: Coordination Chemistry Review, v. 170, p. 93-124.
Zhang, Z.Q., Wong, M.H., Nie, X.P., and Lan, C.Y., 1998, Effects of Zinc (zinc sulphate) on rhzobia-earleaf acacia (*Acacia auriculaeformis*) symbiotic association: Bioresource Technology, v. 64, p. 97-104.
Zipperian, D., Raghavan, S., and Wilson, J.P., 1988, Gold and silver extraction by ammoniacal thiosulphate leaching from a rhyolite ore: Hydrometallurgy, v. 19, p. 361-375.

Appendices

APPENDIX A. GEOCHEMICAL AND MINERALOGICAL DATA OF SUBSTRATES.

Table A.1. X-ray diffraction analysis of the different substrates used in this study. Detection limit (>2.0 vol%).

Anthroposol Soil		Tailings		Limestone	
Mineral	Vol%	Mineral	Vol%	Mineral	Vol%
Quartz	60	Quartz	47	Calcite	89
Albite	13	Magnetite	17	Quartz	6
Microcline	8	Fluorite	12	Expanding clays	5
Kaolin	6	Kaolin	4		
Expanding clays	6	Anorthite	4		
Muscovite	3	Cordierite	4		
Illite	3	Wollastonite	4		
		Pyrrhotite	3		
		Rutile	3		
		Huntite	2		
		Galena	2		

Table A.2. Total metal concentrations (μg g^{-1}) of the different substrates used in this study.

Replicate	Anthroposol Soil (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn
1	<0.005	7	<0.005	28	<0.005	70
2	<0.005	6	<0.005	23	<0.005	65
3	<0.005	7	<0.005	35	<0.005	75
4	<0.005	8	<0.005	33	<0.005	77
Mean Conc.	<0.005	7	<0.005	30	<0.005	72
Standard Deviation	<0.005	0.7	<0.005	4.7	<0.005	4.7
	Mine Tailings (μg g^{-1})					
1	59	2030	28	11700	333	4050
2	65	2087	33	12800	340	4650
3	67	2100	34	13200	348	4500
4	49	1940	25	10100	310	3400
Mean Conc.	60	2039	30	11950	332	4150
Standard Deviation	7.0	63.1	3.7	1201	14.2	486
	Limestone (μg g^{-1})					
1	<0.005	31	6	29	Not tested	420
2	<0.005	33	7	32	Not tested	364
3	<0.005	34	8	33	Not tested	376
4	<0.005	33	7	32	Not tested	267
Mean Conc.	<0.005	33	7	32	Not tested	357
Standard Deviation	<0.005	1.1	0.7	1.5	Not tested	55.9

Table A.3. Chemical properties (total element concentrations and pH) of the substrate components used in this study.

Parameter	Soil	Tailings	Limestone
P_{total} (µg g^{-1})	270	770	770
Na_{total} (wt%)	0.83	0.36	0.006
K_{total} (wt%)	1.32	0.63	0.17
Ca_{total} (wt%)	0.41	7.22	29.59
Mg_{total} (wt%)	0.42	1.09	0.26
pH	8.4	5.4	8.6
Cu_{total} (µg g^{-1})	32	135	55
Fe_{total} (wt%)	3.07	17.1	1.66
Mn_{total} (wt%)	610	2.2	1230
S_{total} (wt%)	232	2.09	91

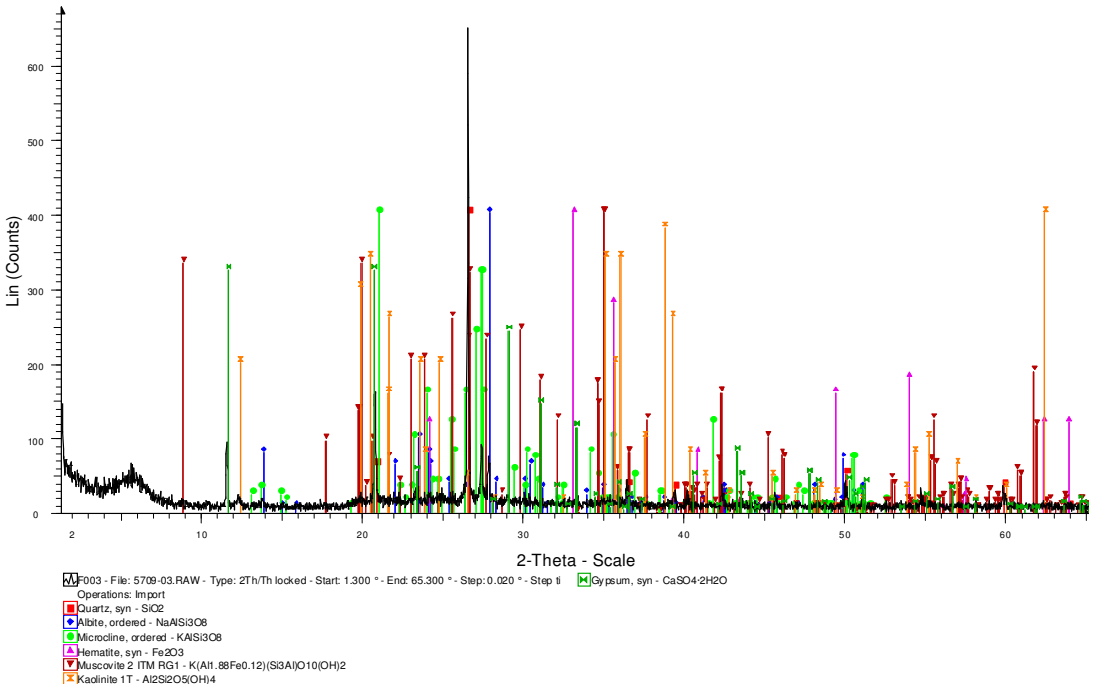

Figure A.1. X-ray diffractogram of the soil component (replicate 1 of Table A.2) used in this study.

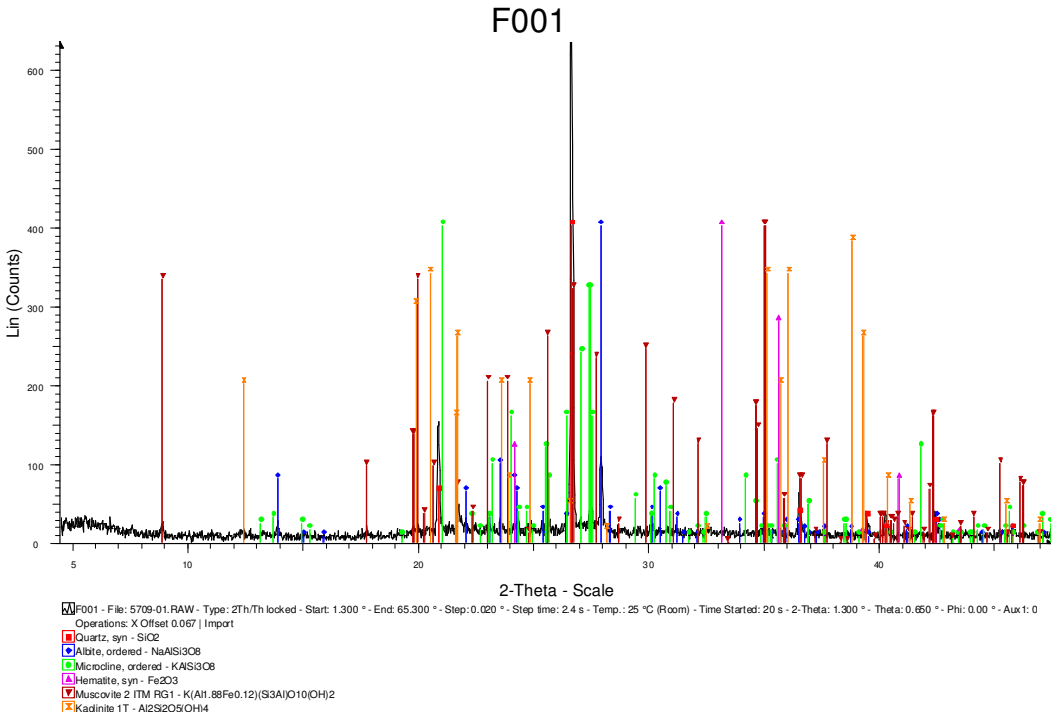

Figure A.2. X-ray diffractogram of the soil component (replicate 1 of Table A.2) used in this study.

APPENDIX B. PLANT CHEMISTRY AND BIOMASS PRODUCTION OF SELECTED NATIVE PASTURE PLANT SPECIES GROWN ON THE ST MIXTURES.

The concentration of metals and arsenic present in the dry weight (DW) of each plant sample (P) was determined by multiplying the element concentration in the liquid sample (E) by the sample dilution factor (10) and expressing this concentration as a proportion of the plant biomass (DW) present in the sample. The following equation was used to calculate such DW element concentrations: [P = (E x 10)/DW] (Chapter 2, Section 2-11.1). Plants cultivated in substrates that died prior to harvesting have been annotated in the tables with 'ns' (no sample).

Table B.1 Biomass production (Total DW; g plant^{-1}) and plant chemistry (μg g^{-1}) of *Triodia molesta* grown on soil contaminated with mine tailings.

Substrate	DW (g)	Total DW (g)	Sample Conc. (μg g^{-1})					Plant Conc. (μg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns											
	0.24	0.24	0.002	0.001	0.011	0.039	1.130	0.10	0.04	0.46	1.59	46.5
	0.26	0.26	0.002	<0.001	0.006	0.014	1.090	0.07	<0.01	0.23	0.51	41.3
	0.08	0.08	0.001	<0.001	0.004	0.010	0.311	0.11	<0.01	0.49	1.33	41.5
5 wt%	0.09	0.07	0.001	0.153	0.007	0.040	2.830	0.17	17.8	0.87	4.62	329
Tailings	0.03	0.03	0.001	<0.001	0.011	0.029	0.564	0.23	<0.01	3.33	8.70	171
	0.06	0.06	<0.001	0.002	0.013	0.175	0.786	<0.01	0.32	2.16	30.1	136
	0.1	0.1	<0.001	0.136	0.033	0.041	1.420	<0.01	14.0	3.38	4.18	146
15 wt%	0.03	0.03	0.002	0.090	0.008	1.030	1.790	0.77	30.0	2.53	343	597
Tailings	0.01	0.01	0.001	0.002	0.008	0.138	0.402	0.82	1.98	7.29	125	365
	0.04	0.04	0.003	0.109	0.005	0.194	1.120	0.89	30.3	1.35	53.9	311
	ns											

ns = no sample

Table B.1.1 Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) for *Triodia molesta* grown on soil contaminated with mine tailings.

Substrate	Biomass		Plant Chemistry (μg g^{-1})					Standard Deviation (μg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.19	0.10	0.09	0.04	0.39	1.14	43.1	0.02	<0.01	0.14	0.56	2.96
5 wt% Tailings	0.07	0.03	0.20	10.7	2.44	11.9	195	0.05	9.19	1.19	12.3	90.3
15 wt% Tailings	0.03	0.01	0.83	20.8	3.73	174	424	0.06	16.3	3.14	150	151

Table B.2. Biomass production (Total DW; g plant^{-1}) and plant chemistry (µg g^{-1}) of *Cymbopogon ambiguus* grown on soil contaminated with mine tailings.

Substrate	DW (g)	Total DW (g)	Sample Conc. (µg g^{-1})					Plant Conc. (µg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.02	0.02	0.001	0.003	<0.005	0.074	0.143	0.67	1.82	<0.01	43.5	84.1
	0.08	0.08	0.002	0.012	0.001	0.111	0.699	0.19	1.43	0.17	13.5	85.2
	0.78	0.78	0.002	<0.005	0.023	0.242	1.790	0.02	<0.01	0.30	3.17	23.5
	0.70	0.70	0.009	<0.005	0.026	0.203	3.610	0.12	<0.01	0.37	2.90	51.5
5 wt%	0.15	0.15	0.002	<0.005	0.002	0.027	0.489	0.12	<0.01	0.13	1.82	33.0
Tailings	0.22	0.22	0.002	<0.005	0.017	0.407	1.420	0.07	<0.01	0.74	18.3	63.8
	0.10	0.10	0.002	0.021	0.006	0.367	0.489	0.21	2.10	0.54	36.0	47.9
	0.19	0.19	0.001	<0.005	0.014	0.227	1.340	0.07	<0.01	0.71	11.8	69.4
15 wt%	0.18	0.18	0.003	0.064	0.015	1.440	4.370	0.16	3.61	0.81	81.0	241
Tailings	0.05	0.05	0.001	0.083	0.005	0.095	1.390	0.21	18.5	1.21	21.1	309
	0.21	0.21	0.002	0.029	0.021	1.050	3.770	0.08	1.36	0.98	49.3	177
	0.04	0.04	0.001	0.004	0.006	0.483	0.692	0.18	1.07	1.54	120	173

Table B.2.1. Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) for *Cymbopogon ambiguus* grown on soil contaminated with mine tailings.

Substrate	Biomass		Plant Chemistry (µg g^{-1})					Standard Deviation (µg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.40	0.40	0.25	1.63	0.21	15.8	61.1	0.29	0.28	0.16	19.1	29.6
5 wt% Tailings	0.17	0.05	0.12	2.10	0.53	17.0	54.0	0.07	<0.01	0.28	14.4	16.4
15 wt% Tailings	0.12	0.09	0.16	6.13	1.13	68.0	226	0.06	8.31	0.31	42.8	64.5

Table B.3. Biomass production (Total DW, g plant^{-1}) and plant chemistry (µg g^{-1}) for *Cymbopogon bombycinus* grown on soil contaminated with mine tailings.

Substrate	DW (g)	Total DW (g)	Sample Conc. (µg g^{-1})					Plant Conc. (µg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns											
	0.09	0.09	0.001	0.001	<0.005	0.009	0.564	0.08	0.11	<0.01	1.05	64.1
	0.68	0.68	0.002	<0.005	0.003	0.024	2.23	0.03	<0.01	0.04	0.35	32.7
	1.48	1.48	0.002	<0.005	0.016	0.098	4.75	0.01	<0.01	0.11	0.67	32.2
5 wt%	0.16	0.16	0.001	<0.005	0.004	0.176	2.06	0.06	<0.01	0.26	11.1	130.
Tailings	0.15	0.15	0.001	<0.005	0.004	0.060	2.78	0.06	<0.01	0.30	3.97	183
	0.12	0.12	0.001	<0.005	0.005	0.102	3.26	0.07	<0.01	0.39	8.36	267
	0.27	0.27	0.001	0.159	0.006	0.044	3.14	0.05	5.85	0.23	1.61	115
15 wt%	0.37	0.37	0.002	<0.005	0.079	0.759	13.8	0.07	<0.01	2.15	20.7	376
Tailings	0.71	0.71	0.007	0.080	0.123	0.915	25.2	0.10	1.13	1.73	12.8	353
	0.17	0.17	0.005	0.259	0.051	0.821	9.28	0.27	14.9	2.93	47.2	533
	0.17	0.17	0.001	0.026	0.018	0.305	3.99	0.06	1.55	1.09	18.5	242
25 wt%	0.19	0.19	0.001	0.019	0.020	0.452	7.12	0.07	1.01	1.06	23.7	373
Tailings	0.03	0.03	0.003	<0.005	0.004	0.127	2.80	1.08	<0.01	1.68	50.8	1120
	0.04	0.04	0.001	0.047	0.004	0.347	1.31	0.23	12.8	1.00	93.8	354
	0.37	0.37	0.002	0.152	0.019	0.309	4.14	0.04	4.09	0.51	8.31	111
35 wt%	0.33	0.33	0.007	0.815	0.129	2.780	17.4	0.21	24.5	3.87	83.5	523
Tailings	0.01	0.01	0.001	0.004	0.003	0.054	1.06	1.41	3.84	3.28	54.0	1060
	0.09	0.09	0.002	0.280	0.019	1.250	3.24	0.28	31.5	2.08	140	364
	ns											
50 wt%	0.13	0.13	0.009	0.389	0.024	5.28	5.03	0.71	30.873	1.90	419	399
Tailings	0.13	0.13	0.004	0.492	0.022	0.360	12.2	0.31	37.273	1.64	27.3	924
	0.08	0.08	0.004	0.189	0.021	2.24	8.61	0.53	22.8	2.51	269	1037
	ns											

ns = no sample

Table B.3.1 Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) for *Cymbopogon bombycinus* grown on soil contaminated with mine tailings.

Substrate	Biomass		Plant Chemistry (µg g^{-1})					Standard Deviation (µg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.75	0.70	0.04	0.11	0.08	0.69	43.0	0.04	<0.01	0.05	0.40	18.3
5 wt% Tailings	0.18	0.07	0.06	5.85	0.29	6.27	173	0.01	<0.01	0.07	4.30	68.6
15 wt% Tailings	0.35	0.26	0.12	5.85	1.97	24.8	376	0.10	7.83	0.78	15.3	120
25 wt% Tailings	0.16	0.16	0.36	5.96	1.06	44.1	489	0.49	6.10	0.48	37.5	437
35 wt% Tailings	0.14	0.17	0.63	19.9	3.08	92.6	649	0.67	14.4	0.92	43.9	365
50 wt% Tailings	0.11	0.03	0.52	30.2	2.02	238	787	0.20	7.27	0.44	197	341

Table B.4. Biomass production (Total DW; g plant^{-1}) and plant chemistry (µg g^{-1}) of *Gomphrena canescens* grown on soil contaminated with mine tailings.

Substrate	DW (g)	Total DW (g)	Sample Conc. (µg g^{-1})					Plant Conc. (µg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.07	0.07	0.001	0.001	0.004	0.017	0.627	0.15	0.14	0.50	2.29	87.1
	0.06	0.06	0.001	<0.005	0.006	0.041	0.852	0.23	<0.01	0.95	6.53	137
	0.15	0.15	0.002	<0.005	0.007	0.027	0.992	0.12	<0.01	0.50	1.84	68.4
	ns											
5 wt%	0.78	0.78	0.003	0.340	0.029	0.279	5.95	0.03	4.36	0.37	3.58	76.3
Tailings	0.02	0.02	0.001	<0.005	<0.005	0.029	0.174	0.68	<0.01	<0.01	15.2	91.6
	ns											
	0.14	0.14	0.002	0.014	0.010	0.288	1.48	0.16	1.02	0.76	21.3	110
15 wt%	0.02	0.02	0.001	<0.005	0.005	0.049	0.507	0.43	<0.01	2.16	21.3	220
Tailings	0.04	0.04	0.002	0.045	0.015	0.284	0.828	0.51	10.3	3.39	64.6	188
	0.04	0.04	0.002	<0.005	0.022	0.124	1.24	0.39	<0.01	5.51	31.8	317
	ns											
25 wt%	0.03	0.03	0.002	0.047	0.038	0.123	1.25	0.56	17.3	14.1	45.6	463
Tailings	ns (x3)											

ns = no sample

Table B.4.1 Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) for *Gomphrena canescens* grown on soil contaminated with mine tailings.

Substrate	Biomass		Plant Chemistry (µg g^{-1})					Standard Deviation (µg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.09	0.05	0.17	0.14	0.65	3.56	97.6	0.06	<0.01	0.26	2.59	35.7
5 wt% Tailings	0.31	0.41	0.29	2.69	0.56	13.4	92.5	0.34	2.36	0.28	9.02	16.7
15 wt% Tailings	0.04	0.01	0.44	10.3	3.69	39.2	242	0.06	<0.01	1.70	22.6	67.6
25 wt% Tailings	0.03	<0.01	0.56	17.3	14.1	45.6	462	<0.01	<0.01	<0.01	<0.01	<0.01

Table B.5. Biomass production (Total DW; g plant^{-1}) and plant chemistry (µg g^{-1}) of *Cyperus victoriensis* grown on soil contaminated with mine tailings.

Substrate	DW (g)	Total DW (g)	Sample Conc. (µg g^{-1})					Plant Conc. (µg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.29	0.29	0.001	0.001	0.038	0.026	3.850	0.05	0.04	1.33	0.90	135
	0.54	0.54	0.001	<0.005	0.013	0.026	0.914	0.02	<0.01	0.24	0.48	16.9
	0.46	0.46	0.007	<0.005	0.045	0.082	7.810	0.15	<0.01	0.98	1.79	171
	0.61	0.61	0.003	<0.005	0.094	0.169	1.180	0.05	<0.01	1.55	2.79	19.5
5 wt%	0.08	0.08	0.003	<0.005	0.013	0.056	2.670	0.30	<0.01	1.58	6.76	322
Tailings	0.07	0.07	0.001	0.007	0.022	0.052	0.302	0.20	1.09	3.24	7.62	44.4
	0.07	0.07	0.003	<0.005	0.003	0.028	0.354	0.37	<0.01	0.42	4.17	52.4
	ns											
15 wt%	0.06	0.06	0.002	0.007	0.013	0.043	1.420	0.41	1.12	2.20	7.29	241
Tailings	0.02	0.02	0.002	0.009	0.003	0.054	0.627	1.01	5.34	1.88	33.6	392
	0.11	0.11	0.004	0.061	0.013	0.167	2.020	0.33	5.52	1.21	15.2	184
	0.04	0.04	0.001	0.004	0.004	0.084	1.000	0.39	1.16	1.14	23.4	278
25 wt%	0.05	0.05	0.002	0.015	0.004	0.234	7.060	0.37	2.94	0.81	45.9	1384
Tailings	0.18	0.18	0.003	0.064	0.013	0.063	6.430	0.16	3.54	0.73	3.48	357
	0.01	0.01	0.001	0.004	0.005	0.077	0.234	1.22	4.63	5.44	85.0	260
	0.06	0.06	0.002	0.017	0.018	0.163	1.340	0.41	3.02	3.25	29.1	239
35 wt%	0.02	0.02	0.003	0.021	0.023	0.978	1.010	1.35	11.0	12.1	514	532
Tailings	0.01	0.01	0.001	0.006	0.006	0.216	0.879	1.46	6.26	6.82	240	977
	ns (x2)											
50 wt%	0.02	0.02	0.002	0.113	0.007	0.438	0.560	1.33	75.3	4.74	292	373
Tailings	0.03	0.03	0.005	0.070	0.015	0.649	1.460	1.92	28.0	5.88	260	584
	ns (x2)											

ns = no sample

Table B.5.1. Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) for *Cyperus victoriensis* grown on soil contaminated with mine tailings.

Substrate	Biomass		Plant Chemistry (µg g^{-1})					Standard Deviation (µg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.47	0.14	0.07	0.04	1.03	1.49	85.6	0.06	<0.01	0.58	1.03	79.2
5 wt% Tailings	0.07	0.01	0.29	1.09	1.75	6.18	140	0.08	<0.01	1.41	1.79	158
15 wt% Tailings	0.07	0.04	0.54	3.29	1.61	19.9	273	0.32	2.48	0.52	11.3	87.9
25 wt% Tailings	0.01	0.07	0.54	3.53	2.56	40.9	560	0.47	0.78	2.25	34.2	552
35 wt% Tailings	0.01	0.01	1.40	8.60	9.46	377	754	0.08	3.32	3.74	194	315
50 wt% Tailings	0.02	0.01	1.63	51.7	5.31	276	479	0.41	33.4	0.81	22.9	149

Table B.6. Biomass production (Total DW; g plant^{-1}) and plant chemistry (µg g^{-1}) of *Crotalaria novae-hollandiae* grown on soil contaminated with mine tailings.

Substrate	DW (g)	Total DW (g)	Sample Conc. (µg g^{-1})					Plant Conc. (µg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns (x4)											
5 wt%	0.67	0.67	0.002	<0.005	0.066	0.724	4.130	0.03	<0.01	0.99	10.9	61.8
Tailings	0.51	0.51	0.002	0.015	0.038	0.326	4.240	0.05	0.29	0.74	6.34	82.5
	ns											
	0.29	0.29	0.003	<0.005	0.022	0.166	2.090	0.09	<0.01	0.75	5.68	71.6
15 wt%	0.40	0.40	0.003	0.816	0.059	0.576	7.560	0.07	20.4	1.48	14.4	189
Tailings	0.24	0.24	0.003	0.889	0.039	0.324	5.880	0.12	37.5	1.63	13.7	248
	0.36	0.36	0.002	0.816	0.063	0.453	7.820	0.06	22.9	1.77	12.7	219
	0.59	0.59	0.003	1.520	0.172	0.884	18.60	0.06	25.9	2.93	15.0	316
25 wt%	0.03	0.03	0.002	0.201	0.018	0.289	0.984	0.68	71.5	6.55	102	350
Tailings	0.17	0.17	0.002	1.150	0.100	1.160	5.160	0.13	66.2	5.76	66.8	297
	0.18	0.18	0.002	1.120	0.060	0.532	4.640	0.11	60.7	3.25	28.8	251
	0.09	0.09	0.003	0.499	0.045	0.523	3.120	0.30	55.4	5.01	58.1	346
35 wt%	0.12	0.12	0.002	0.540	0.044	0.798	3.760	0.21	45.2	3.67	66.7	314
Tailings	0.08	0.08	0.002	0.457	0.030	0.546	3.430	0.18	54.5	3.62	65.1	409
	0.06	0.06	0.002	0.382	0.032	0.499	2.240	0.33	65.2	5.53	85.2	382
	0.05	0.05	0.002	0.491	0.037	0.430	3.010	0.31	89.6	6.68	78.5	549
50 wt%	0.07	0.07	0.002	0.415	0.037	0.434	2.550	0.27	58.6	5.28	61.3	360
Tailings	0.09	0.09	0.002	0.373	0.018	1.220	3.310	0.19	39.5	1.87	129	350
	ns (x2)											

ns = no sample

Table B.6.1. Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) for *Crotalaria novae-hollandiae* grown on soil contaminated with mine tailings.

Substrate	Biomass		Plant Chemistry (µg g^{-1})					Standard Deviation (µg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns											
5 wt% Tailings	0.64	0.33	0.04	10.8	0.95	8.18	83.6	0.03	<0.01	0.14	2.80	10.3
15 wt% Tailings	0.40	0.15	0.08	26.7	1.95	14.0	243	0.03	7.6	0.66	1.01	54.6
25 wt% Tailings	0.12	0.07	0.30	63.5	5.14	64.1	311	0.27	7.0	1.41	30.5	46.7
35 wt% Tailings	0.08	0.03	0.26	63.6	4.88	73.9	414	0.07	19.2	1.49	9.61	98.8
50 wt% Tailings	0.08	0.02	0.23	49.0	3.58	95.2	355	0.05	13.5	2.41	47.9	7.00

ns = no sample

Table B.7. Plant chemistry (μg g^{-1}) and biomass production (Total DW, g $plant^{-1}$) of *Chloris gayana* Kunth cv. 'Pioneer' grown on soil contaminated with mine tailings.

Substrate	DW (g)	Total DW (g)	Sample Conc. (μg g^{-1})					Plant Conc. (μg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.12	0.12	<0.001	0.045	0.002	0.412	0.254	<0.01	3.85	0.17	35.0	21.6
	0.10	0.10	<0.001	0.042	0.001	0.248	0.394	<0.01	4.07	0.12	24.3	38.6
	0.10	0.10	<0.001	0.026	0.002	0.575	0.388	<0.01	2.55	0.21	56.4	38.0
	ns											
5 wt%	0.28	4.85	<0.001	0.055	0.016	0.547	2.26	<0.01	1.96	0.58	19.5	80.3
Tailings	0.52	4.82	<0.001	0.006	0.031	0.130	2.14	<0.01	0.12	0.60	2.50	41.1
	0.61	3.04	<0.001	0.014	0.018	0.169	2.28	<0.01	0.23	0.30	2.78	37.5
	0.31	2.82	<0.001	0.005	0.015	0.170	0.778	<0.01	0.18	0.49	5.52	25.3
15 wt%	0.31	0.95	<0.001	0.038	0.082	0.827	3.61	<0.01	1.22	2.62	26.5	116
Tailings	0.81	4.19	0.001	0.087	0.043	0.394	7.02	0.01	1.07	0.53	4.84	86.2
	0.53	4.40	<0.001	0.063	0.023	0.562	4.93	<0.01	1.18	0.43	10.5	92.2
	0.44	3.22	<0.001	0.106	0.027	0.604	4.46	<0.01	2.43	0.61	13.9	102
25 wt%	0.37	1.76	<0.001	0.006	0.041	0.293	2.34	<0.01	0.15	1.10	7.88	62.9
Tailings	0.59	1.37	0.005	0.153	0.654	2.94	58.2	0.09	2.60	11.1	60.0	990
	0.69	1.42	0.004	0.171	0.742	5.96	67.6	0.05	2.48	10.8	86.4	980
	0.43	1.11	0.002	0.064	0.452	2.70	42.3	0.05	1.49	10.5	62.8	984
35 wt%	0.52	0.52	0.001	0.065	1.240	4.60	18.2	0.03	1.26	24.0	89.0	352
Tailings	ns											
	0.84	0.84	0.003	0.134	2.080	8.82	11.6	0.04	1.60	24.9	105	139
	0.34	1.91	<0.001	0.031	0.784	2.28	18.6	<0.01	0.90	22.8	66.2	540
50 wt%	0.21	0.21	<0.001	0.072	0.757	4.35	40.0	<0.01	3.37	35.4	204	1870
Tailings	0.12	0.12	0.007	0.171	0.348	1.84	15.7	0.63	14.9	30.3	160	1365
	0.10	0.36	<0.001	0.042	0.389	0.559	3.45	<0.01	4.02	37.2	53.4	330
	0.24	0.24	0.007	0.465	0.580	3.65	42.7	0.29	19.0	23.7	149	1748
Tailings	0.03	0.03	<0.001	<0.005	0.137	0.327	2.67	<0.01	<0.01	39.6	94.5	772
	0.05	0.05	<0.001	0.048	0.234	1.23	11.2	<0.01	9.56	47.0	247	2249
	0.08	0.08	<0.001	0.018	0.085	0.528	3.93	<0.01	2.18	10.2	63.5	472
	0.04	0.04	<0.001	0.001	0.110	0.460	2.72	<0.01	0.26	28.8	120	712

ns = no sample

Table B.7.1. Mean and standard deviation (SD) of the biomass production (g $plant^{-1}$) and plant chemistry (μg g^{-1}) for *Chloris gayana* grown on soil contaminated with mine tailings.

Substrate	Biomass		Plant Chemistry (μg g^{-1})					Standard Deviation (μg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.11	0.01	<0.01	3.49	0.17	38.6	32.7	<0.01	0.82	0.04	16.3	9.68
5 wt% Tailings	3.88	1.10	<0.01	0.62	0.49	7.6	46.1	<0.01	0.89	0.14	8.04	23.8
15 wt% Tailings	3.19	1.58	0.01	1.47	1.05	13.9	99.1	<0.01	0.64	1.05	9.17	12.9
25 wt% Tailings	1.42	0.27	0.06	1.68	8.37	51.8	344	0.02	1.13	4.85	32.9	201
35 wt% Tailings	0.92	0.69	0.03	1.25	23.7	86.9	754	0.01	0.35	1.05	19.7	201
50 wt% Tailings	0.23	0.10	0.46	10.8	31.7	142	1328	0.24	7.85	6.04	63.2	700
Tailings	0.05	0.02	<0.01	4.00	31.4	131	1051	<0.01	4.91	16.0	80.5	809

APPENDIX C. PLANT CHEMISTRY AND BIOMASS PRODUCTION OF THREE NATIVE PASTURE PLANT SPECIES GROWN ON STL MIXTURES

The concentration of metals and arsenic present in the dry weight (DW) of each plant sample (P) was determined by multiplying the element concentration in the liquid sample (E) by the sample dilution factor (10) and expressing this concentration as a proportion of the plant biomass (DW) present in the sample. The following equation was used to calculate such DW element concentrations: [P = (E x 10)/DW] (Chapter 2, Section 2-11.1). Plants cultivated in substrates that died prior to harvesting have been annotated in the tables with 'ns' (no sample). Substrates that were not prepared for cultivation have been annotated in the tables with 'nt' (not tested).

Table C.1. Biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) of *Cymbopogon ambiguus* grown on soil contaminated with mine tailings and amended with 10 wt% limestone (e.g. 10L5T = 10 wt% limestone + 5 wt% tailings + 85 wt% soil).

Substrate	DW (g)	Total DW (g)	Sample Conc. (μg g^{-1})					Plant Conc. (μg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns (x4)											
10L	0.26	0.26	0.003	0.591	0.007	0.075	0.609	0.01	1.51	0.02	0.19	1.56
	0.16	0.16	0.002	0.492	0.003	0.050	0.402	<0.01	0.81	<0.01	0.08	0.66
	0.27	0.27	<0.001	0.063	0.001	0.007	0.051	<0.01	0.17	<0.01	0.02	0.14
	0.35	0.35	0.002	0.400	0.005	0.040	0.491	0.01	1.40	0.02	0.14	1.71
10L5T	0.13	0.13	0.002	0.544	0.009	0.080	0.425	<0.01	0.71	0.01	0.11	0.56
	0.05	0.05	0.002	0.529	0.005	0.067	0.269	<0.01	0.26	<0.01	0.03	0.13
	0.15	0.15	0.002	0.513	0.010	0.066	1.240	<0.01	0.79	0.02	0.10	1.90
	0.16	0.16	0.002	0.409	0.011	0.054	0.523	<0.01	0.64	0.02	0.09	0.82
10L15T	0.18	0.18	0.002	0.373	0.015	0.113	0.456	<0.01	0.66	0.03	0.20	0.80
	0.13	0.13	0.002	0.365	0.020	0.087	0.495	<0.01	0.49	0.03	0.12	0.66
	0.05	0.15	0.002	0.469	0.060	0.689	7.090	<0.01	0.21	0.03	0.32	3.24
	0.14	0.14	0.003	0.811	0.035	0.289	0.967	0.01	1.17	0.05	0.42	1.40
10L25T	0.11	0.11	0.002	0.559	0.020	0.205	0.648	<0.01	0.64	0.02	0.23	0.74
	0.08	0.08	0.002	0.475	0.017	0.264	0.641	<0.01	0.39	0.01	0.21	0.52
	0.09	0.09	0.002	0.599	0.019	0.156	0.762	<0.01	0.53	0.02	0.12	0.68
	0.15	0.15	0.003	0.799	0.019	0.534	0.821	0.01	1.18	0.03	0.79	1.21
10L50T	0.11	0.11	0.003	0.550	0.011	0.524	0.966	<0.01	0.61	0.01	0.59	1.08
	0.11	0.11	0.005	0.731	0.017	0.816	0.843	0.01	0.79	0.02	0.89	0.91
	0.10	0.10	0.004	0.638	0.011	0.466	0.604	<0.01	0.65	0.01	0.48	0.62
	0.11	0.11	0.003	0.416	0.017	0.269	0.530	<0.01	0.45	0.02	0.29	0.57

ns = no sample

Table C.1.1 Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) for *Cymbopogon ambiguus* grown on mine tailings contaminated soil amended with 10 wt% limestone.

	Biomass		Plant Chemistry (μg g^{-1})					Standard Deviation (μg g^{-1})				
Substrate	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns											
10L	0.26	0.08	<0.01	0.97	0.01	0.11	1.02	<0.01	0.62	0.01	0.08	0.75
10L5T	0.12	0.05	<0.01	0.60	0.01	0.08	0.85	<0.01	0.23	0.01	0.03	0.75
10L15T	0.15	0.02	<0.01	0.63	0.03	0.26	1.52	<0.01	0.40	0.01	0.13	1.19
10L25T	0.11	0.03	<0.01	0.68	0.02	0.34	0.79	<0.01	0.34	0.01	0.30	0.30
10L50T	0.11	0.00	<0.01	0.63	0.02	0.56	0.80	<0.01	0.14	0.00	0.25	0.24
Soil	ns											

ns = no sample

Table C.2. Biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) of *Cymbopogon ambiguus* for mine tailings contaminated amended with limestone (20 wt%) (e.g. 20L5T = 20 wt% limestone + 5 wt% tailings + 75 wt% soil).

Substrate	DW (g)	Total DW (g	Sample Conc. (μg g^{-1})					Plant Conc. (μg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	nt											
20L	ns (x4)											
20L5T	0.12	0.12	0.002	0.438	0.006	0.082	0.668	<0.01	0.52	0.01	0.10	0.79
	0.15	0.15	0.003	0.449	0.012	0.122	1.130	<0.01	0.69	0.02	0.19	1.73
	0.20	0.20	0.001	0.408	0.008	0.047	0.676	<0.01	0.84	0.02	0.10	1.38
	0.17	0.17	0.002	0.437	0.009	0.162	0.406	<0.01	0.76	0.02	0.28	0.70
20L15T	0.05	0.05	0.002	0.522	0.008	0.062	0.496	<0.01	0.24	<0.01	0.03	0.23
	0.08	0.08	0.002	0.414	0.011	0.091	0.532	<0.01	0.33	0.01	0.07	0.42
	0.12	0.12	0.002	0.393	0.018	0.113	0.899	<0.01	0.48	0.02	0.14	1.09
	0.10	0.10	0.002	0.341	0.019	0.122	0.657	<0.01	0.34	0.02	0.12	0.66
20L25T	0.09	0.09	0.003	0.352	0.006	0.136	0.906	<0.01	0.32	0.01	0.12	0.81
	0.09	0.09	0.002	0.328	0.006	0.077	0.601	<0.01	0.31	0.01	0.07	0.56
	0.12	0.12	0.002	0.349	0.010	0.119	0.691	<0.01	0.42	0.01	0.14	0.83
	0.08	0.08	0.002	0.531	0.020	0.089	0.600	<0.01	0.43	0.02	0.07	0.49
20L50T	0.08	0.08	0.002	0.509	0.006	0.120	0.366	<0.01	0.41	0.01	0.10	0.30
	0.16	0.16	0.003	0.480	0.029	0.321	0.630	<0.01	0.76	0.05	0.51	1.00
	0.10	0.10	0.002	0.672	0.014	0.072	2.320	<0.01	0.68	0.01	0.07	2.36
	0.10	0.10	0.002	0.392	0.012	0.104	0.338	<0.01	0.39	0.01	0.10	0.33

nt = not tested, ns = no sample

Table C.2.1 Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) for *Cymbopogon ambiguus* grown on mine tailings contaminated soil amended with 20 wt% limestone.

Substrate	Biomass		Plant Chemistry (μg g^{-1})					Standard Deviation (μg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	nt											
20L	ns											
20L5T	0.16	0.04	<0.01	0.70	0.01	0.17	1.15	<0.01	0.14	0.01	0.09	0.49
20L15T	0.09	0.03	<0.01	0.35	0.01	0.09	0.60	<0.01	0.10	0.01	0.05	0.37
20L25T	0.10	0.02	<0.01	0.37	0.01	0.10	0.67	<0.01	0.07	0.01	0.04	0.17
20L50T	0.11	0.03	<0.01	0.56	0.02	0.20	1.00	<0.01	0.19	0.02	0.21	0.96

nt = not tested, ns = no sample

Table C.3. Biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) of *Cymbopogon bombycinus* for mine tailings contaminated amended with limestone (10 wt%) (e.g. 10L5T = 10 wt% limestone + 5 wt% tailings + 85 wt% soil).

Substrate	DW (g)	Total DW (g)	Sample Conc. (µg g^{-1})					Plant Conc. (µg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns (x4)											
10L	0.37	0.37	0.002	0.364	0.007	0.119	1.050	0.01	1.35	0.03	0.44	3.89
	0.33	0.33	0.002	0.453	0.013	0.173	0.720	0.01	1.50	0.04	0.58	2.39
	0.38	0.38	0.002	0.395	0.011	0.272	4.720	0.01	1.50	0.04	1.03	17.9
	0.23	0.23	0.001	0.382	0.014	0.191	0.766	<0.01	0.89	0.03	0.44	1.78
10L5T	ns											
	0.23	0.23	0.002	0.453	0.013	0.173	0.720	0.01	1.06	0.03	0.41	1.68
	0.51	0.51	0.002	0.467	0.019	0.254	4.900	0.01	2.39	0.10	1.30	25.1
	0.53	0.53	0.002	0.530	0.023	0.302	4.530	0.01	2.82	0.12	1.61	24.1
10L15T	0.24	0.24	0.002	0.464	0.014	0.183	0.744	0.01	1.13	0.03	0.44	1.80
	0.30	0.30	0.003	0.584	0.025	0.526	3.950	0.01	1.78	0.08	1.60	12.0
	0.34	0.34	0.002	0.414	0.024	0.249	2.720	0.01	1.42	0.08	0.86	9.36
	0.34	0.34	0.003	0.621	0.030	0.556	2.890	0.01	2.12	0.10	1.90	9.85
10L25T	0.22	0.22	0.002	0.415	0.027	0.228	2.493	0.01	0.92	0.06	0.51	5.54
	0.29	0.29	0.003	0.462	0.026	0.407	2.705	0.01	1.34	0.08	1.16	7.81
	0.15	0.15	0.001	0.294	0.017	0.149	2.061	<0.01	0.45	0.03	0.23	3.12
	0.40	0.40	0.003	0.534	0.032	0.273	3.725	0.01	2.15	0.13	1.10	15.03

ns = no sample

Table C.3.1. Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) for *Cymbopogon bombycinus* grown on mine tailings contaminated soil amended with 10 wt% limestone.

Substrate	Biomass		Plant Chemistry (µg g^{-1})					Standard Deviation (µg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns											
10L	0.33	0.07	0.01	1.31	0.04	0.62	6.50	<0.01	0.29	0.01	0.28	7.67
10L5T	0.43	0.17	0.01	2.09	0.08	1.10	17.0	<0.01	0.92	0.05	0.63	13.3
10L15T	0.31	0.05	0.01	1.61	0.07	1.20	8.26	<0.01	0.43	0.03	0.67	4.46
10L25T	0.27	0.11	0.01	1.21	0.07	0.75	7.87	0.01	0.72	0.04	0.46	5.14

ns = no sampled

Table C.4. Biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) of *Cymbopogon bombycinus* for mine tailings contaminated amended with limestone (20 wt%) (e.g. 20L5T = 20 wt% limestone + 5 wt% tailings + 75 wt% soil).

Substrate	DW (g)	Total DW (g)	Sample Conc. (µg g^{-1})					Plant Conc. (µg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns (x4)											
20L	0.31	0.31	0.001	0.402	0.009	0.123	1.530	<0.01	1.24	0.03	0.38	4.70
	ns (x3)											
20L5T	0.24	0.24	0.003	0.356	0.012	0.344	1.300	0.01	0.87	0.03	0.84	3.17
	ns (x3)											
20L15T	0.15	0.15	0.002	0.403	0.028	0.332	0.324	<0.01	0.59	0.04	0.49	0.48
	0.40	0.40	0.006	0.651	0.042	0.735	1.770	0.02	2.60	0.17	2.94	7.07
	0.24	0.24	0.003	0.661	0.027	0.710	6.090	0.01	1.57	0.06	1.69	14.5
	0.26	0.26	0.003	0.489	0.025	0.371	2.870	0.01	1.28	0.07	0.97	7.51
20L25T	0.07	0.07	0.002	0.369	0.017	0.132	8.960	<0.01	0.25	0.01	0.09	5.94
	0.37	0.17	0.012	0.744	0.032	0.929	1.100	0.04	2.76	0.12	3.44	4.07
	0.11	0.11	0.001	0.406	0.014	0.129	1.310	<0.01	0.45	0.02	0.14	1.44
	0.13	0.13	0.002	0.453	0.017	0.167	1.710	<0.01	0.57	0.02	0.21	2.16
20L50T	0.22	0.22	0.002	0.444	0.020	0.367	5.740	<0.01	0.96	0.04	0.80	12.4
	0.20	0.20	0.002	0.465	0.023	0.351	2.780	<0.01	0.92	0.05	0.70	5.49
	0.23	0.23	0.012	0.744	0.032	0.929	1.100	0.03	1.71	0.07	2.14	2.53
	0.14	0.14	0.001	0.332	0.004	0.091	4.190	<0.01	0.46	0.01	0.13	5.77

ns = no sample

Table C.4.1. Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (µg g^{-1}) for *Cymbopogon bombycinus* grown on mine tailings contaminated soil amended with 20 wt% limestone.

Substrate	Biomass		Plant Chemistry (µg g^{-1})					Standard Deviation (µg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	ns											
20L	0.31	<0.01	0.00	1.23	0.03	0.38	4.70	<0.01	<0.01	<0.01	<0.01	<0.01
20L5T	0.24	<0.01	0.01	0.87	0.03	0.84	3.17	<0.01	<0.01	<0.01	<0.01	<0.01
20L15T	0.26	0.10	0.01	1.51	0.08	1.52	7.38	0.01	0.83	0.06	1.06	5.71
20L25T	0.12	0.04	0.01	1.00	0.04	0.97	3.40	0.02	1.17	0.05	1.65	2.02
20L50T	0.20	0.04	0.01	1.01	0.04	0.94	6.55	0.01	0.52	0.03	0.85	4.17

ns = no sample

Table C.5. Biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) of *Crotalaria novae-hollandiae* grown on soil contaminated with mine tailings and amended with 10 wt% limestone (e.g. 10L5T = 10 wt% limestone + 5 wt% tailings + 85 wt% soil).

Substrate	DW (g)	Total DW (g)	Sample Conc. (μg g^{-1})					Plant Conc. (μg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.54	0.54	0.004	0.925	0.019	0.199	4.130	0.02	5.00	0.10	1.08	22.3
	0.32	0.32	0.003	0.606	0.009	0.418	2.630	0.01	1.94	0.03	1.34	8.41
	0.53	0.53	0.003	0.798	0.017	0.174	4.460	0.02	4.21	0.09	0.92	23.5
	0.36	0.36	0.002	0.667	0.013	0.116	3.330	0.01	2.43	0.05	0.42	12.1
10L	0.11	0.11	0.001	0.408	0.008	0.047	0.676	<0.01	0.45	0.01	0.05	0.75
	0.20	0.20	0.001	0.662	0.008	0.041	1.150	<0.01	1.35	0.02	0.08	2.34
	0.16	0.16	0.002	0.480	0.008	0.026	0.716	<0.01	0.76	0.01	0.04	1.13
	0.14	0.14	0.002	0.794	0.016	0.059	1.730	<0.01	1.13	0.02	0.08	2.46
10L5T	0.26	0.26	0.002	0.672	0.014	0.072	2.320	0.01	1.74	0.04	0.19	6.02
	0.30	0.30	0.002	0.543	0.014	0.052	2.740	0.01	1.61	0.04	0.15	8.13
	0.66	0.66	0.002	0.715	0.020	0.111	4.130	0.02	4.69	0.13	0.73	27.1
	0.29	0.29	0.002	0.493	0.010	0.068	2.950	0.01	1.44	0.03	0.20	8.65
10L15T	0.37	0.37	0.003	0.797	0.020	0.190	3.350	0.01	2.93	0.07	0.70	12.3
	0.34	0.34	0.003	0.719	0.026	0.157	4.090	0.01	2.41	0.09	0.53	13.7
	0.17	0.17	0.002	0.553	0.011	0.079	2.140	<0.01	0.96	0.02	0.14	3.70
	0.32	0.32	0.003	0.965	0.027	0.160	4.710	0.01	3.10	0.09	0.51	15.1
10L25T	0.18	0.18	0.002	0.488	0.024	0.211	2.410	<0.01	0.90	0.04	0.39	4.46
	0.28	0.28	0.002	0.470	0.054	0.269	4.330	0.01	1.34	0.15	0.77	12.3
	0.30	0.30	0.002	0.650	0.047	0.328	4.180	0.01	1.93	0.14	0.98	12.4
	0.23	0.23	0.002	0.536	0.049	0.233	4.630	<0.01	1.24	0.11	0.54	10.7
10L50T	0.13	0.13	0.003	0.720	0.033	0.368	7.690	<0.01	0.93	0.04	0.48	10.0
	0.30	0.30	0.003	0.594	0.055	0.479	9.600	0.01	1.81	0.17	1.46	29.3
	0.12	0.12	0.003	0.554	0.040	0.456	6.010	<0.01	0.65	0.05	0.54	7.07
	0.21	0.21	0.003	0.640	0.024	0.344	6.430	0.01	1.37	0.05	0.74	13.8

ns = no sample

Table C.5.1. Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) for *Crotalaria novae-hollandiae* grown on mine tailings contaminated soil amended with 10 wt% limestone.

Substrate	Biomass		Plant Chemistry (μg g^{-1})					Standard Deviation (μg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.44	0.11	0.01	3.39	0.07	0.94	16.6	0.01	1.45	0.04	0.39	7.48
10L	0.15	0.04	<0.01	0.92	0.02	0.06	1.67	<0.01	0.40	0.01	0.02	0.86
10L5T	0.38	0.19	0.01	2.37	0.06	0.32	12.5	0.01	1.55	0.05	0.27	9.80
10L15T	0.30	0.09	0.01	2.35	0.07	0.47	11.2	<0.01	0.97	0.03	0.24	5.14
10L25T	0.25	0.05	0.01	1.35	0.11	0.67	10.0	<0.01	0.43	0.05	0.26	3.77
10L50T	0.19	0.09	0.01	1.19	0.08	0.80	15.0	<0.01	0.51	0.06	0.45	9.88

Table C.6. Biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) of *Crotalaria novae-hollandiae* for mine tailings contaminated amended with limestone (20 wt%) (e.g. 20L5T = 20 wt% limestone + 5 wt% tailings + 75 wt% soil).

Substrate	DW (g)	Total DW (g)	Sample Conc. (μg g^{-1})					Plant Conc. (μg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.24	0.24	0.002	<0.005	0.011	0.039	1.13	0.01	<0.01	0.03	0.09	2.77
	0.26	0.26	0.002	<0.005	0.006	0.014	2.13	0.01	<0.01	0.02	0.04	5.62
	0.08	0.08	0.001	<0.005	0.004	0.010	3.13	<0.01	<0.01	<0.01	0.01	2.35
	0.54	0.54	0.004	0.925	0.019	0.199	4.13	0.02	5.00	0.10	1.08	22.3
20L	Ns (x4)											
20L5T	0.62	0.62	0.005	1.830	0.058	0.247	8.68	0.03	11.4	0.36	1.54	54.2
	0.77	0.77	0.003	0.942	0.036	0.139	6.25	0.03	7.26	0.28	1.07	48.2
	0.57	0.57	0.004	1.430	0.058	0.175	7.48	0.02	8.21	0.33	1.01	43.0
	0.63	0.63	0.003	0.680	0.025	0.091	4.02	0.02	4.29	0.16	0.57	25.4
20L15T	0.35	0.35	0.004	0.661	0.035	0.675	4.71	0.01	2.31	0.12	2.36	16.4
	0.35	0.35	0.002	0.628	0.029	0.366	3.88	0.01	2.20	0.10	1.27	13.6
	0.47	0.47	0.004	0.755	0.052	0.903	5.01	0.02	3.57	0.25	4.27	23.7
	0.54	0.54	0.003	0.772	0.054	0.738	6.66	0.02	4.14	0.29	3.96	35.7
20L25T	0.41	0.41	0.003	0.627	0.028	0.165	5.49	0.01	2.60	0.12	0.68	22.8
	0.38	0.38	0.004	0.804	0.034	0.180	7.78	0.01	3.03	0.13	0.68	29.4
	0.48	0.48	0.005	0.893	0.049	0.314	9.80	0.02	4.27	0.23	1.50	46.9
	0.45	0.45	0.004	0.676	0.049	0.179	7.83	0.02	3.03	0.22	0.80	35.1
20L50T	0.22	0.22	0.003	0.776	0.037	0.211	7.30	0.01	1.74	0.08	0.47	16.3
	0.31	0.31	0.003	0.537	0.034	0.189	7.09	0.01	1.68	0.11	0.59	22.2
	0.17	0.17	0.003	0.472	0.026	0.178	3.92	<0.01	0.81	0.05	0.30	6.70
	0.24	0.24	0.003	0.660	0.059	0.272	10.1	0.01	1.60	0.14	0.66	24.5

ns = no sample

Table C.6.1. Mean and standard deviation (SD) of the biomass production (g plant^{-1}) and plant chemistry (μg g^{-1}) for *Crotalaria novae-hollandiae* grown on mine tailings contaminated soil amended with 20 wt% limestone.

Substrate	Biomass		Plant Chemistry (μg g^{-1})					Standard Deviation (μg g^{-1})				
	Mean (g)	SD (g)	Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Soil	0.29	0.23	0.01	5.00	0.04	0.30	8.26	0.01	<0.01	0.05	0.52	9.49
20L	ns											
20L5T	0.65	0.08	0.02	7.80	0.28	1.05	42.7	0.01	2.94	0.09	0.40	12.4
20L15T	0.43	0.09	0.01	3.05	0.19	2.96	22.4	<0.01	0.96	0.09	1.40	9.89
20L25T	0.43	0.04	0.02	3.23	0.17	0.92	33.5	<0.01	0.72	0.06	0.39	10.2
20L50T	0.24	0.06	0.01	1.46	0.09	0.51	17.4	<0.01	0.44	0.04	0.16	7.94

ns = no sample

APPENDIX D. SEQUENTIAL EXTRACTION OF THE SOIL CONTAMINATED WITH 25 WT% MINE TAILINGS.

Table D.1. The concentrations of metals and arsenic sequentially extracted from duplicate samples (Rep.) of the 25 wt% ST mixture. Concentrations (μg g^{-1}) of extracted elements are derived by multiplying the Sample Conc. of each extract (determined by ICP MS) by the dilution factor (Dil.) for each extraction procedure.

Extract	Rep.	Dil. (mL)	Sample Conc. (μg g^{-1})					Conc. (μg g^{-1})				
			Ag	As	Cd	Pb	Zn	Ag	As	Cd	Pb	Zn
Water Soluble	1	10	0.002	0.033	0.001	0.210	0.095	0.02	0.33	0.01	2.10	0.95
	2	10	0.002	0.029	0.001	0.186	0.094	0.02	0.29	0.01	1.86	0.94
Ion Exchangeable	1	10	0.002	0.454	0.232	21.9	3.44	0.02	4.54	2.32	219	34.4
	2	10	0.002	0.418	0.249	26.4	3.80	0.02	4.18	2.49	264	38.0
Carbonate	1	10	0.002	2.02	0.089	129	15.0	0.02	20.2	0.89	1290	150
	2	10	0.002	2.71	0.096	145	16.9	0.02	27.1	0.96	1450	169
Oxide	1	10	0.076	2.39	0.014	47.2	5.50	0.76	23.9	0.14	472	55.0
	2	10	0.071	5.46	0.017	53.0	6.20	0.71	54.6	0.17	530	62.0
Sulphide A	1	25	0.375	4.83	0.079	11.4	18.2	9.38	121	1.97	285	455
	2	25	0.313	3.75	0.059	11.2	14.9	7.83	93.8	1.48	280	373
Sulphide B	1	25	0.144	9.02	0.027	2.01	4.50	3.60	226	0.68	50.3	113
	2	25	0.215	8.75	0.052	3.54	10.1	5.38	219	1.30	88.5	253

Table D.1.1. Average concentrations of sequentially extracted metals and arsenic (μg g^{-1}) from the 25 wt% ST mixture. Total element concentrations for the substrate are included. The Residual/Silicate fraction has been determine arithmetically by subtracting the total quantity of each element dissolved by the extractions from the total metal concentration of the substrate.

Extract	Average Conc. (μg g^{-1})				
	Ag	As	Cd	Pb	Zn
Water Soluble	0.02	0.31	0.01	1.98	0.95
Ion Exchangeable	0.02	4.36	2.41	242	36.2
Carbonate	0.02	23.7	0.92	1370	160
Oxide	0.74	39.3	0.15	501	58.5
Sulphide	13.1	329	2.72	352	596
Residual/Silicate	1.13	118	1.80	544	240
Total	15	515	8	3010	1091

APPENDIX E. GEOCHEMICAL DATA - PARTIAL AND SEQUENTIAL EXTRACTION OF THE SOIL CONTAMINATED WITH 12.5 WT% MINE TAILINGS.

Table E.1. The concentrations of metals and metalloids (μg g^{-1}) dissolved using standards soil extractions of 0.01M EDTA and 0.005M DTPA, and for comparison 0.01M EDDS and deionised water (dH_2O), from the 12.5 wt% mine tailings contaminated soil.

Extract	Sample wt (g)	Sample Conc. (μg g^{-1})						Conc. (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
dH_2O	1.04	<0.001	0.083	0.008	0.009	0.036	0.215	<0.01	0.79	0.08	0.09	0.35	0.35
0.01M EDTA	0.99	0.001	3.020	0.229	149	0.247	35.5	0.01	30.4	2.31	1502	2.49	2.49
0.005M DTPA	1.06	0.001	1.300	0.241	136	0.155	37.3	0.01	12.3	2.27	1283	1.46	1.46
0.01M EDDS	1.11	0.001	0.777	0.198	146	0.276	27.2	0.01	6.98	1.78	1311	2.48	2.48

Table E.1.1. The concentrations of metals and arsenic sequentially extracted from the 12.5 wt% tailings contaminated soil. Concentrations (μg g^{-1}) of extracted elements are derived by multiplying the Sample Conc. of each extract (determined by ICP MS) by the dilution factor (Dil.) for each extraction procedure. The Residual/Silicate fraction has been determined arithmetically by subtracting the total quantity of each element dissolved by the extractions from the total metal concentration of the substrate.

Extract	Dil. (mL)	Sample Conc. (μg g^{-1})						Conc. (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Water Soluble	10	<0.001	0.009	0.010	0.034	0.041	0.372	<0.01	0.09	0.10	0.34	0.41	3.72
Ion Exchangeable	10	<0.001	0.166	0.128	17.29	0.212	4.006	<0.01	1.66	1.28	173	2.12	40.1
Carbonate	10	0.005	0.557	0.066	41.74	0.239	9.610	0.05	5.57	0.66	417	2.39	96.1
Oxide	10	0.008	1.047	0.066	51.12	0.025	18.05	0.08	10.5	0.66	511	0.25	181
Sulphide A	25	0.114	0.936	0.005	8.26	0.411	2.657	2.84	23.4	0.11	206	10.3	66.4
Sulphide B	25	0.172	8.626	0.040	7.45	0.954	7.162	4.30	216	1.00	186	23.9	179
Residual/Silicate								0.74	4.18	0.27	28.9	2.90	17.5

APPENDIX F. PLANT CHEMISTRY - CHEMICALLY-ASSISTED PHYTOEXTRACTION OF METALS AND METALLOIDS USING THREE NATIVE PASTURE PLANT SPECIES GROWN ON SOIL CONTAMINATED WITH 12.5 WT% MINE TAILINGS.

The concentration of metals and arsenic present in the dry weight (DW) of each plant sample (P) was determined by multiplying the element concentration in the liquid sample (E) by the sample dilution factor (10) and expressing this concentration as a proportion of the plant biomass (DW) present in the sample. The following equation was used to calculate such DW element concentrations: [P = (E x 10)/DW] (Chapter 2, Section 2-11.1). The chemical amendments used were EDTA (ethylene diamine tetraacetic acid), DTPA (diethylene triamine pentaacetic acid), EDDS (ethylene diaminedissuccinatic acid), TSP (ammonium thiosulphate), SCN (ammonium thiocyanate) and THIO (thiourea) at concentrations of 0.5M and 2.0M per kg soil. Plants cultivated in substrates that died prior to harvesting have been annotated in the tables with 'ns' (no sample). Substrates that were not prepared for cultivation have been annotated in the tables with 'nt' (not tested).

Table F.1. Soil amendment and plant sampling regime for the chemically-assisted phytoextraction pot trial experiment using *C. novae-hollandiae*, *C. bombycinus* and *C. victoriensis*. Chemical treatments (→) and vegetation sampling (#) was performed weekly and chemical treatments ceased when 50% of the plants in a treatment group had died.

Reagent	*C. novae-hollandiae*	*C. bombycinus*	*C. victoriensis*
	Treatments (weeks)	Treatments (weeks)	Treatments (weeks)
Control	5	5	5
2.0_EDTA	1	1 2 → 4	→ → → → 5
0.5 EDTA	→ → → → 5	1 2 3 4	→ → → → 5
2.0 DTPA	1	not tested	→ → → 4
2.0 EDDS	1	1 2	→ → → 4
0.5 EDDS	1	1 → → → 5	→ → → → 5
2.0 TSP	1	1 2	→ → → 4
0.5 TSP	→ → → → 5	1 2 3 4 5	→ → → → 5
2.0 SCN	1	1	1
0.5 SCN	1	1	1
2.0 THIO	1	1	1
0.5 THIO	1	1	1
2.0 EDDS	1	1 2	→ → → 4

Table F.2. Plant chemistry of *Crotalaria novae-hollandiae* grown on a soil contaminated with 12.5 wt% mine tailings after one weekly chemical treatment.

Reagent	DW (g)	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.26	0.001	0.118	0.129	53.41	0.012	16.0	0.04	4.57	4.99	2067	0.48	619
	0.20	0.001	0.444	0.273	53.93	0.026	32.8	0.05	22.7	14.0	2760	1.31	1679
	0.12	<0.001	0.119	0.047	20.71	0.004	5.75	<0.01	9.69	3.84	1686	0.35	468
	0.17	0.001	0.051	0.111	19.65	0.003	6.89	0.03	2.90	6.35	1125	0.18	394
2.0 DTPA	0.12	<0.001	0.014	0.050	1.50	0.002	3.33	<0.01	1.10	4.03	121	0.13	268
	0.11	<0.001	0.020	0.052	1.26	0.002	3.73	<0.01	1.88	4.86	117	0.20	346
	0.13	<0.001	0.038	0.101	2.48	0.004	6.39	<0.01	2.83	7.52	185	0.27	476
	0.30	0.001	0.033	0.127	0.961	0.002	6.83	0.02	1.11	4.25	32.2	0.07	229
2.0 TSP	0.16	0.045	0.089	0.051	0.511	0.005	3.52	2.79	5.50	3.13	31.5	0.34	217
	0.19	0.059	0.073	0.100	0.544	0.007	4.15	3.04	3.75	5.12	28.0	0.37	213
	0.08	0.019	0.037	0.035	0.258	0.003	2.29	2.35	4.69	4.49	32.7	0.40	290
	0.72	0.072	0.094	0.274	0.866	0.006	8.37	1.00	1.31	3.83	12.1	0.08	117
2.0 SCN	0.31	0.001	0.123	0.175	0.578	0.003	7.69	0.02	3.92	5.58	18.4	0.10	245
	0.24	0.005	0.047	0.137	0.384	0.003	5.45	0.21	1.93	5.64	15.8	0.13	225
	0.36	0.006	0.074	0.191	0.418	0.005	8.64	0.17	2.08	5.37	11.8	0.15	243
	0.43	0.011	0.092	0.251	0.695	0.003	8.06	0.25	2.11	5.78	16.0	0.07	186
0.5 SCN	0.65	0.001	0.062	0.230	0.402	0.004	8.28	0.01	0.95	3.54	6.18	0.06	127
	0.53	0.001	0.045	0.220	0.617	0.003	7.30	0.01	0.84	4.13	11.6	0.05	137
	0.15	<0.001	0.030	0.037	0.297	0.002	2.64	<0.01	1.98	2.49	19.9	0.13	177
	0.28	0.001	0.044	0.071	0.448	0.002	4.39	0.02	1.56	2.53	16.1	0.07	157
2.0 THIO	0.35	0.003	0.213	0.103	0.944	0.006	5.48	0.10	6.08	2.94	26.9	0.16	156
	0.25	0.004	0.278	0.074	0.378	0.008	3.55	0.17	10.9	2.91	14.9	0.32	140
	0.20	0.006	0.167	0.094	0.424	0.007	3.23	0.30	8.34	4.72	21.2	0.36	161
	0.19	0.009	0.160	0.092	0.459	0.008	3.33	0.50	8.43	4.83	24.2	0.42	175
0.5 THIO	0.14	0.001	0.024	0.089	0.496	0.002	3.49	0.05	1.79	6.55	36.5	0.17	257
	0.22	0.001	0.033	0.067	0.640	0.002	4.97	0.03	1.48	3.01	29.0	0.09	225
	0.25	0.002	0.104	0.147	0.715	0.005	5.51	0.08	4.09	5.78	28.1	0.22	217
	0.15	0.002	0.065	0.086	0.395	0.003	3.13	0.11	4.42	5.84	26.9	0.22	213
2.0 EDDS	0.16	<0.001	0.019	0.055	0.832	0.002	5.61	<0.01	1.21	3.45	52.5	0.12	354
	0.16	0.001	0.019	0.049	0.493	0.002	4.85	0.05	1.21	3.12	31.2	0.13	307
	0.17	<0.001	0.021	0.090	0.826	0.002	5.43	<0.01	1.24	5.34	49.1	0.12	322
	0.35	0.001	0.036	0.162	2.410	0.003	9.03	0.04	1.03	4.64	69.0	0.10	258
0.5 EDDS	0.08	<0.001	0.035	0.036	0.175	0.002	2.72	<0.01	4.53	4.55	22.4	0.30	348
	0.15	<0.001	0.032	0.097	0.340	0.003	4.75	<0.01	2.18	6.56	23.1	0.18	322
	0.18	0.001	0.018	0.083	0.512	0.002	5.56	0.06	1.00	4.60	28.3	0.10	307
	0.25	0.001	0.023	0.088	0.769	0.002	6.81	0.03	0.92	3.51	30.7	0.07	272

Table F.2.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Crotalaria novae-hollandiae* after one weekly chemical treatment.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.04	9.97	7.29	1909	0.58	790	0.01	8.98	4.57	686	0.50	608
0.5 EDTA	nt											
2.0 DTPA	0.02	1.73	5.16	114	0.16	330		0.82	1.61	62.6	0.09	109
2.0 TSP	2.29	3.81	4.14	26.1	0.30	209	0.91	1.81	0.86	9.52	0.15	71.1
0.5 TSP	nt											
2.0 SCN	0.16	2.51	5.59	15.5	0.11	225	0.10	0.95	0.17	2.77	0.03	27.6
0.5 SCN	0.01	1.33	3.17	13.4	0.08	150	<0.01	0.53	0.80	5.91	0.03	22.0
2.0 THIO	0.26	8.44	3.85	21.8	0.32	158	0.17	1.98	1.07	5.18	0.11	14.8
0.5 THIO	0.07	2.94	5.29	30.1	0.17	228	0.04	1.53	1.56	4.34	0.06	2.00
2.0 EDDS	0.04	1.17	4.14	50.4	0.12	310	0.01	0.09	1.03	15.5	0.01	39.9
0.5 EDDS	0.05	2.16	4.80	26.1	0.16	312	0.02	1.69	1.27	4.02	0.10	32.0

nt = not tested

Table F.3. Plant chemistry of *Crotalaria novae-hollandiae* grown on a soil contaminated with 12.5 wt% mine tailings after five weekly chemical treatments.

Reagent	DW	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
	(g)	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Control	0.39	0.001	0.053	0.113	0.721	0.003	7.35	0.02	1.37	2.89	18.4	0.09	188
	0.55	0.001	0.077	0.118	0.465	0.004	8.71	0.02	1.40	2.16	8.52	0.07	160
	0.44	0.001	0.061	0.096	0.503	0.003	7.29	0.02	1.39	2.21	11.5	0.06	167
	0.85	0.001	0.274	0.424	1.26	0.009	11.4	0.01	3.22	4.98	14.8	0.11	134
0.5 EDTA	0.53	0.001	0.111	0.250	55.0	0.008	95.3	0.01	2.08	4.68	1030	0.14	1785
	0.72	0.001	0.220	0.435	85.0	0.014	179	0.02	3.06	6.04	1180	0.20	2482
	0.95	0.001	0.184	0.290	61.4	0.012	181	0.01	1.94	3.05	646	0.13	1906
	0.47	0.001	0.114	0.147	35.6	0.008	78.0	0.01	2.41	3.11	754	0.16	1652
0.5 TSP	0.70	0.015	0.053	0.243	0.879	0.005	11.8	0.21	0.75	3.46	12.5	0.07	168
	1.06	0.015	0.069	0.265	1.200	0.006	18.0	0.14	0.65	2.51	11.4	0.05	170
	0.76	0.010	0.065	0.251	0.740	0.006	11.6	0.14	0.86	3.31	9.78	0.07	153
	0.22	0.005	0.031	0.030	0.144	0.004	2.80	0.24	1.39	1.37	6.50	0.19	126

Table F.3.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Crotalaria novae-hollandiae* after five weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Control	0.02	1.84	3.06	13.3	0.08	162	<0.01	0.91	1.32	4.27	0.02	22.4
0.5 EDTA	0.01	2.37	4.22	903	0.16	1956	<0.01	0.50	1.43	246	0.03	366
0.5 TSP	0.18	0.91	2.66	10.0	0.10	154	0.05	0.33	0.96	2.61	0.06	20.2

Table F.4. Plant chemistry of *Cymbopogon bombycinus* grown on a soil contaminated with 12.5 wt% mine tailings after one weekly chemical treatment.

Reagent	DW (g)	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.02	<0.001	<0.001	0.003	0.397	0.002	0.451	<0.01	<0.01	1.43	196	0.99	222
	0.04	0.001	<0.001	0.034	0.480	0.004	0.571	0.24	<0.01	8.94	125	1.14	148
	0.03	<0.001	<0.001	0.031	0.438	0.003	0.403	<0.01	<0.01	10.3	144	0.98	132
	0.06	<0.001	<0.001	0.073	1.210	0.005	1.100	<0.01	<0.01	12.5	208	0.86	189
0.5 EDTA	0.05	<0.001	<0.001	0.032	0.155	0.001	0.484	<0.01	<0.01	7.01	33.6	0.18	105
	0.03	<0.001	<0.001	0.039	0.088	0.001	0.312	<0.01	<0.01	15.6	35.1	0.20	124
	0.02	<0.001	<0.001	0.012	0.236	0.001	0.293	<0.01	<0.01	7.47	149	0.34	185
	0.06	<0.001	<0.001	0.065	0.348	0.001	0.814	<0.01	<0.01	11.2	59.6	0.11	139
2.0 EDDS	0.07	<0.001	<0.001	0.038	0.068	0.001	0.942	<0.01	<0.01	5.48	9.74	0.07	135
	0.01	<0.001	<0.001	0.004	0.019	<0.001	0.158	<0.01	<0.01	2.96	13.6	<0.01	112
	0.04	<0.001	<0.001	0.024	0.036	<0.001	0.877	<0.01	<0.01	6.06	8.98	<0.01	219
	0.04	<0.001	<0.001	0.020	0.063	0.001	1.490	<0.01	<0.01	4.51	14.4	0.13	341
0.5 EDDS	0.02	<0.001	<0.001	0.003	0.023	<0.001	0.104	<0.01	<0.01	1.30	11.3	<0.01	50.5
	0.02	<0.001	<0.001	0.004	0.012	<0.001	0.175	<0.01	<0.01	2.32	6.43	<0.01	94.6
	0.05	<0.001	<0.001	0.019	0.017	0.001	0.809	<0.01	<0.01	3.43	3.12	0.12	150
	0.06	<0.001	<0.001	0.011	0.015	<0.001	0.582	<0.01	<0.01	1.93	2.69	<0.01	106
2.0 TSP	0.03	0.001	<0.001	0.043	0.024	0.001	0.219	0.25	<0.01	16.8	9.25	0.20	85.9
	0.03	0.001	0.017	0.050	0.043	0.001	0.307	0.35	5.00	14.6	12.8	0.21	90.8
	0.03	<0.001	<0.001	0.022	0.021	0.001	0.283	<0.01	<0.01	6.49	6.13	0.16	84.2
	0.06	<0.001	0.008	0.016	0.055	0.001	0.338	<0.01	1.53	2.89	10.0	0.09	61.1
0.5 TSP	0.05	<0.001	<0.001	0.009	0.011	<0.001	0.372	<0.01	<0.01	1.85	2.44	<0.01	80.2
	0.07	<0.001	<0.001	0.026	0.011	<0.001	0.549	<0.01	<0.01	3.49	1.52	<0.01	74.6
	0.01	<0.001	<0.001	0.009	0.010	<0.001	0.205	<0.01	<0.01	7.08	7.80	<0.01	155
	0.02	<0.001	<0.001	0.019	0.014	<0.001	0.273	<0.01	<0.01	8.87	6.34	<0.01	128
2.0 SCN	0.18	0.008	0.029	0.145	0.113	0.010	2.410	0.45	1.61	7.99	6.23	0.54	133
	0.15	0.006	<0.001	0.300	0.101	0.003	1.730	0.38	<0.01	19.6	6.59	0.20	113
	0.34	0.002	0.009	0.093	0.135	0.002	4.310	0.05	0.26	2.73	3.96	0.05	127
	0.21	0.002	0.023	0.157	0.068	0.002	3.230	0.08	1.10	7.43	3.19	0.09	153
0.5 SCN	0.17	0.001	<0.001	0.062	0.044	0.001	1.980	0.06	<0.01	3.60	2.59	0.07	115
	0.12	<0.001	<0.001	0.054	0.023	0.001	1.450	<0.01	<0.01	4.35	1.85	0.05	117
	0.18	<0.001	<0.001	0.039	0.040	0.001	1.160	<0.01	<0.01	2.13	2.15	0.05	63.1
	0.20	0.001	0.006	0.045	0.043	0.002	1.680	0.04	0.29	2.31	2.20	0.08	85.5
2.0 THIO	0.17	0.001	<0.001	0.301	0.083	0.001	1.820	0.03	<0.01	18.0	4.95	0.05	106
	0.21	0.003	0.025	0.411	0.145	0.002	2.400	0.13	1.20	19.8	6.98	0.10	116
	0.20	0.001	<0.001	0.154	0.123	0.001	1.740	0.05	<0.01	7.74	6.18	0.07	87.4
	0.17	0.001	<0.001	0.255	0.076	0.001	2.110	0.05	<0.01	15.2	4.52	0.08	126
0.5 THIO	ns												
	0.14	0.002	<0.001	0.027	0.124	0.002	0.915	0.16	<0.01	1.97	8.91	0.11	65.8
	0.06	0.001	<0.001	0.037	0.068	0.000	0.353	0.20	<0.01	6.42	11.9	0.07	61.9
	0.08	0.001	<0.001	0.075	0.052	0.001	0.693	0.08	<0.01	9.28	6.44	0.14	85.9

ns = no sample

Table F.4.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Cymbopogon bombycinus* after one weekly chemical treatment.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.24	<0.01	8.27	168	0.99	173	<0.01	<0.01	4.78	40.1	0.12	40.6
0.5 EDTA	<0.01	<0.01	10.3	69.4	0.21	138	<0.01	<0.01	3.98	54.6	0.10	34.4
2.0 TSP	0.30	3.27	10.2	9.54	0.17	80.5	0.07	2.45	6.59	2.72	0.05	13.2
0.5 TSP	<0.01	<0.01	5.32	4.52	<0.01	110	<0.01	<0.01	3.22	3.02	<0.01	38.8
2.0 SCN	0.24	0.99	9.43	4.99	0.22	131	0.20	0.68	7.16	1.67	0.23	16.6
0.5 SCN	0.05	0.29	3.10	2.20	0.06	95.2	0.01	<0.01	1.06	0.30	0.01	25.8
2.0 THIO	0.07	1.20	15.2	5.66	0.08	109	0.04	<0.01	5.30	1.13	0.02	16.2
0.5 THIO	0.14	<0.01	5.89	9.08	0.11	71.2	0.06	<0.01	3.68	2.72	0.03	12.9
2.0 EDDS	<0.01	<0.01	4.75	11.7	0.10	202	<0.01	<0.01	1.36	2.71	0.04	104
0.5 EDDS	<0.01	<0.01	2.25	5.88	0.12	100	<0.01	<0.01	0.90	3.96	<0.01	40.9

Table F.5. Plant chemistry of *Cymbopogon bombycinus* grown on a soil contaminated with 12.5 wt% mine tailings after two weekly chemical treatments.

Reagent	DW	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
	(g)	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.05	<0.001	<0.001	0.046	2.704	0.002	3.560	<0.01	<0.01	19.5	1141	0.84	1502
	0.05	<0.001	<0.001	0.033	4.100	0.004	4.100	<0.01	<0.01	6.87	851	0.83	851
	0.05	<0.001	<0.001	0.033	5.137	0.004	2.740	<0.01	<0.01	6.45	997	0.93	532
	ns												
0.5 EDTA	0.04	<0.001	<0.001	0.026	2.031	0.002	3.490	<0.01	<0.01	6.02	476	0.37	817
	0.04	<0.001	<0.001	0.048	2.005	0.001	4.140	<0.01	<0.01	11.6	489	0.32	1010
	0.03	<0.001	<0.001	0.019	0.307	0.001	0.749	<0.01	<0.01	5.65	90.8	0.40	222
	0.05	<0.001	<0.001	0.019	2.075	0.001	1.070	<0.01	<0.01	3.71	407	0.11	210
2.0 TSP	0.13	0.003	0.007	0.009	0.041	0.001	1.290	0.20	0.51	0.71	3.20	0.05	100
	0.25	0.016	0.030	0.023	0.098	0.001	2.210	0.62	1.21	0.92	3.87	0.03	87.7
	0.24	0.006	0.017	0.019	0.048	0.001	2.450	0.27	0.71	0.79	2.05	0.04	104
	ns												
0.5 TSP	0.04	0.001	<0.001	0.027	0.041	<0.001	0.404	0.16	<0.01	6.63	10.1	<0.01	99.3
	0.04	0.001	<0.001	0.058	0.031	<0.001	0.473	0.13	<0.01	13.3	7.00	<0.01	108
	0.09	0.001	<0.001	0.052	0.042	<0.001	0.744	0.10	<0.01	5.60	4.56	<0.01	80.2
	0.06	0.001	<0.001	0.034	0.028	<0.001	0.384	0.12	<0.01	5.99	4.94	<0.01	68.7
0.5 EDDS	0.05	<0.001	<0.001	0.017	0.031	<0.001	0.697	<0.01	<0.01	3.51	6.39	<0.01	145
	0.01	<0.001	<0.001	0.004	0.035	<0.001	0.204	<0.01	<0.01	2.77	25.9	<0.01	152
	0.07	<0.001	<0.001	0.034	0.034	0.002	3.220	<0.01	<0.01	5.12	5.14	0.28	4860
	ns												

ns = no sample

Table F.5.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Cymbopogon bombycinus* after two weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	<0.01	<0.01	11.0	996	0.87	962	<0.01	<0.01	7.44	145	0.06	494
0.5 EDTA	<0.01	<0.01	6.75	366	0.30	565	<0.01	<0.01	3.40	187	0.13	411
2.0 TSP	0.36	0.81	0.81	3.04	0.04	97.3	0.22	0.36	0.10	0.92	0.01	8.43
0.5 TSP	0.13	<0.01	7.89	6.65	<0.01	89.2	0.02	<0.01	3.65	2.54	<0.01	18.1
0.5 EDDS	<0.01	<0.01	3.80	12.5	0.28	261	<0.01	<0.01	1.20	11.6	<0.01	195

Table F.6. Plant chemistry of *Cymbopogon bombycinus* grown on a soil contaminated with 12.5 wt% mine tailings after three weekly chemical treatments.

Reagent	DW (g)	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
0.5 EDTA	0.07	<0.001	<0.001	0.010	0.263	0.002	2.060	<0.01	<0.01	1.46	36.9	0.21	289
	0.02	<0.001	<0.001	0.017	1.020	0.001	1.280	<0.01	<0.01	10.8	650	0.39	815
	0.02	<0.001	<0.001	0.023	2.050	0.002	4.900	<0.01	<0.01	9.27	830	0.69	1984
	0.08	<0.001	<0.001	0.012	0.976	0.002	4.110	<0.01	<0.01	1.65	130	0.22	547
0.5 TSP	0.14	0.001	<0.001	0.051	0.070	<0.001	0.766	0.06	<0.01	3.59	4.93	<0.01	54.1
	0.08	<0.001	<0.001	0.024	0.149	0.001	0.284	<0.01	<0.01	2.83	17.6	0.15	33.5
	0.05	0.001	<0.001	0.023	0.034	0.001	0.211	0.16	<0.01	4.80	6.93	0.11	43.2
	ns												

ns = no sample

Table F.6.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Cymbopogon bombycinus* after three weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
0.5 EDTA	<0.01	<0.01	5.79	412	0.38	909	<0.01	<0.01	4.92	388	0.22	748
0.5 TSP	0.07	<0.01	3.74	9.80	0.13	43.6	0.08	<0.01	0.99	6.78	0.03	10.3

Table F.7. Plant chemistry of *Cymbopogon bombycinus* grown on a soil contaminated with 12.5 wt% mine tailings after four weekly chemical treatments.

Reagent	DW	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
	(g)	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.09	0.036	0.012	0.023	3.841	0.002	3.950	3.85	1.31	2.43	411	0.20	423
	0.32	0.045	0.020	0.036	11.84	0.003	10.10	1.43	0.65	1.13	375	0.11	320
	0.25	0.054	0.026	0.052	8.350	0.006	13.70	2.15	1.05	2.08	333	0.23	546
	0.07	0.017	0.014	0.059	4.438	0.002	7.920	2.27	1.95	8.16	610	0.30	1088
0.5 EDTA	ns												
	0.13	<0.001	0.061	0.036	5.610	0.005	12.80	<0.01	4.58	2.72	420	0.39	957
	0.42	0.001	0.054	0.064	5.710	0.005	18.00	0.02	1.27	1.51	135	0.11	425
	0.21	0.001	0.013	0.035	1.570	0.002	6.300	0.02	0.61	1.64	74.4	0.11	298
0.5 TSP	0.12	0.001	<0.001	0.137	0.081	<0.001	0.738	0.06	<0.01	11.3	6.69	<0.01	61.1
	0.13	0.001	<0.001	0.066	0.044	<0.001	0.652	0.05	<0.01	4.96	3.31	<0.01	49.2
	0.13	0.001	<0.001	0.089	0.059	<0.001	0.604	0.08	<0.01	6.74	4.51	<0.01	45.9
	0.07	<0.001	<0.001	0.022	0.020	<0.001	0.227	<0.01	<0.01	3.27	2.91	<0.01	33.2

ns = no sample

Table F.7.1. The mean and standard deviation of the plant chemistry (μg g^{-1}) for *Cymbopogon bombycinus* after four weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	2.43	1.24	3.45	432	0.21	594	1.02	0.55	3.19	122	0.08	342
0.5 EDTA	0.02	2.16	1.96	210	0.20	560	<0.01	2.13	0.66	185	0.16	350
0.5 TSP	0.07	<0.01	6.58	4.36	<0.01	47.4	0.02	<0.01	3.48	1.70	<0.01	11.5

Table F.8. Plant chemistry of *Cymbopogon bombycinus* grown on a soil contaminated with 12.5 wt% mine tailings after five weekly chemical treatments.

Reagent	DW	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
	(g)	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Control	0.99	0.001	0.518	0.165	0.442	0.002	12.90	0.01	5.23	1.67	4.47	0.02	130
	0.19	<0.001	0.065	0.047	0.095	<0.001	0.858	<0.01	3.38	2.48	4.97	<0.01	44.9
	0.90	0.001	0.233	0.140	0.469	0.001	9.430	0.01	2.59	1.56	5.21	0.01	105
	ns												
0.5 TSP	0.08	<0.001	<0.001	0.053	0.049	<0.001	0.443	<0.01	<0.01	6.62	6.14	<0.01	55.7
	0.57	0.005	0.067	0.286	0.256	0.004	2.940	0.09	1.17	5.04	4.51	0.07	51.8
	0.10	0.002	<0.001	0.055	0.102	<0.001	0.532	0.18	<0.01	5.61	10.3	<0.01	53.9
	0.37	0.001	0.025	0.153	0.179	0.001	1.510	0.03	0.68	4.19	4.90	0.03	41.3
0.5 EDDS	0.33	<0.001	0.019	0.081	0.313	0.003	6.740	<0.01	0.58	2.44	9.43	0.09	203
	0.25	<0.001	<0.001	0.079	0.307	0.005	10.80	<0.01	<0.01	3.13	12.2	0.20	428
	0.15	<0.001	<0.001	0.024	0.318	0.001	3.550	<0.01	<0.01	1.58	21.0	0.08	234
	0.16	<0.001	<0.001	0.034	0.152	0.001	2.830	<0.01	<0.01	2.13	9.39	0.07	175

ns = no sample

Table F.8.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Cymbopogon bombycinus* after five weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Control	0.01	3.73	1.90	4.88	0.02	93.4	<0.01	1.36	0.50	0.38	0.01	43.8
0.5 TSP	0.10	0.93	5.36	6.47	0.05	50.7	0.08	0.35	1.02	2.66	0.03	6.44
0.5 EDDS	<0.01	0.58	2.32	13.0	0.11	260	<0.01	<0.01	0.65	5.47	0.06	115

Table F.9. Plant chemistry of *Cyperus victoriensis* grown on a soil contaminated with 12.5 wt% mine tailings after one weekly chemical treatment.

Reagent	DW	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
	(g)	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 SCN	0.06	0.003	0.008	0.109	0.024	0.003	2.48	0.50	1.38	18.3	3.95	0.50	417
	0.09	0.008	0.028	0.116	0.023	0.002	2.56	0.86	3.19	13.3	2.68	0.21	294
	0.14	0.027	0.025	0.246	0.138	0.003	5.74	1.98	1.84	18.1	10.1	0.22	421
	0.09	0.011	<0.001	0.126	0.064	0.002	1.99	1.22	<0.01	14.0	7.05	0.23	220
0.5 SCN	0.06	0.001	0.021	0.030	0.020	0.002	1.61	0.12	3.84	5.32	3.60	0.31	289
	0.02	0.001	<0.001	0.008	0.012	0.001	1.06	0.30	<0.01	3.50	5.11	0.34	467
	0.16	0.006	0.024	0.114	0.092	0.002	5.26	0.36	1.49	7.06	5.72	0.11	326
	ns												
2.0 THIO	0.08	0.005	0.024	0.119	0.067	0.002	3.18	0.66	3.08	15.2	8.57	0.31	407
	0.17	0.002	0.046	0.086	0.055	0.005	5.73	0.12	2.70	5.01	3.21	0.27	333
	0.05	0.007	<0.001	0.055	0.056	0.001	3.07	1.33	<0.01	10.5	10.8	0.28	590
	0.09	0.006	<0.001	0.050	0.033	0.002	5.87	0.68	<0.01	5.56	3.72	0.22	653
0.5 THIO	0.10	0.005	<0.001	0.223	0.102	0.001	5.50	0.50	<0.01	23.0	10.5	0.12	567
	0.07	0.006	0.010	0.171	0.092	0.001	2.32	0.83	1.33	23.7	12.7	0.16	322
	0.02	0.003	<0.001	0.046	0.026	0.001	0.775	1.03	<0.01	18.7	10.5	0.29	316
	0.03	0.001	<0.001	0.058	0.058	0.001	2.02	0.25	<0.01	22.2	22.1	0.28	772

ns = no sample

Table F.9.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Cyperus victoriensis* after one weekly chemical treatment.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 SCN	1.14	1.60	15.9	5.95	0.29	338	0.63	1.32	2.64	3.34	0.14	98.2
0.5 SCN	0.26	1.77	5.29	4.81	0.25	360	0.13	1.93	1.78	1.09	0.13	94.1
2.0 THIO	0.70	1.44	9.07	6.57	0.27	496	0.50	1.68	4.78	3.71	0.04	150
0.5 THIO	0.65	0.33	21.9	14.0	0.21	496	0.35	0.66	2.21	5.53	0.08	221

Table F.10. Plant chemistry of *Cyperus victoriensis* grown on a soil contaminated with 12.5 wt% mine tailings after four weekly chemical treatments.

Reagent	DW (g)	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 DTPA	0.06	0.002	0.063	0.083	3.520	0.002	5.44	0.29	10.6	13.9	590	0.33	911
	0.26	0.007	0.266	0.348	3.870	0.007	14.7	0.25	10.2	13.3	148	0.28	562
	0.03	0.002	0.077	0.052	2.990	0.002	4.14	0.50	24.7	16.7	961	0.49	1331
	0.04	0.003	0.040	0.084	4.180	0.002	5.60	0.84	10.8	22.6	1124	0.59	1505
2.0 TSP	0.23	0.052	0.057	0.410	3.410	0.002	8.28	2.29	2.50	18.0	149	0.07	363
	0.16	0.046	0.073	0.293	3.510	0.002	6.12	2.92	4.62	18.7	223	0.10	390
	0.18	0.089	0.050	0.473	4.230	0.004	9.61	5.06	2.85	27.0	241	0.23	548
	0.22	0.141	0.061	0.576	4.920	0.002	9.56	6.42	2.78	26.2	224	0.07	435
2.0 EDDS	0.17	0.017	0.093	0.351	15.98	0.013	18.8	0.99	5.62	21.1	962	0.76	1133
	0.17	0.017	0.058	0.343	13.15	0.008	17.7	0.98	3.34	19.7	755	0.49	1017
	0.19	0.010	0.061	0.338	16.64	0.007	19.6	0.53	3.20	17.8	877	0.38	1033
	0.05	0.007	<0.001	0.145	10.40	0.004	11.2	1.51	<0.01	30.8	2209	0.78	2378

ns = no sample

Table F.10.1 Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Cyperus victoriensis* after four weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 DTPA	0.47	14.1	16.7	706	0.42	1078	0.27	7.09	4.26	434	0.14	424
2.0 TSP	4.17	3.19	22.4	209	0.12	434	1.91	0.97	4.80	41	0.08	82
2.0 EDDS	1.00	4.05	22.4	1201	0.60	1390	0.40	1.36	5.78	677	0.20	661

Table F.11. Plant chemistry of *Cyperus victoriensis* grown on a soil contaminated with 12.5 wt% mine tailings after five weekly chemical treatments.

Reagent	DW	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
	(g)	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Control	0.31	0.006	0.202	0.265	0.176	0.001	14.2	0.20	6.48	8.50	5.64	0.03	455
	0.11	0.004	0.026	0.077	0.069	0.001	3.52	0.40	2.46	7.22	6.48	0.07	329
	0.06	0.002	0.018	0.067	0.040	<0.001	1.90	0.35	3.05	11.6	7.00	<0.01	332
	0.11	0.007	0.030	0.075	0.084	0.001	3.53	0.65	2.83	7.11	7.98	0.06	334
2.0 EDTA	0.11	0.005	0.027	0.061	6.05	0.001	7.60	0.47	2.48	5.62	555	0.13	697
	0.16	0.005	0.057	0.086	7.51	0.001	10.9	0.30	3.64	5.50	479	0.08	695
	0.18	0.004	0.093	0.129	10.1	0.002	9.58	0.20	5.07	7.04	550	0.12	523
	0.16	0.011	0.051	0.179	13.3	0.002	15.5	0.69	3.22	11.4	844	0.11	985
0.5 EDTA	0.10	0.001	0.182	0.042	8.100	0.004	16.8	0.07	18.8	4.34	838	0.37	1737
	0.12	0.006	0.099	0.083	14.50	0.002	15.1	0.53	8.44	7.07	1241	0.13	1293
	0.06	0.008	0.024	0.077	8.125	0.008	6.04	1.27	3.77	12.0	1266	1.24	941
	0.03	0.002	0.022	0.050	4.768	0.002	4.68	0.69	8.52	19.5	1855	0.83	1821
0.5 TSP	0.82	0.023	0.344	0.359	0.804	0.003	22.0	0.28	4.19	4.37	9.79	0.04	268
	0.61	0.015	0.265	0.313	0.338	0.002	16.2	0.25	4.36	5.15	5.56	0.03	266
	0.15	0.019	0.093	0.156	0.093	0.001	4.66	1.29	6.41	10.7	6.41	0.04	320
	0.08	0.005	0.046	0.027	0.063	<0.001	2.79	0.60	5.75	3.40	7.91	<0.01	348
0.5 EDDS	0.36	0.008	0.213	0.167	1.14	0.004	21.5	0.22	5.95	4.67	31.8	0.11	601
	0.28	0.006	0.168	0.267	3.16	0.009	22.7	0.20	5.92	9.42	111	0.30	800
	0.37	0.008	0.205	0.110	0.410	0.005	21.8	0.22	5.58	3.00	11.2	0.14	594
	0.02	0.001	<0.001	0.036	0.351	0.001	2.62	0.23	<0.01	15.9	154	0.40	1149

Table F.11.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Cyperus victoriensis* after five weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Control	0.40	3.70	8.62	6.78	0.04	362	0.19	1.86	2.11	0.98	0.03	61.9
2.0 EDTA	0.41	3.60	7.38	607	0.11	725	0.21	1.09	2.75	162	0.02	192
0.5 EDTA	0.64	9.89	10.7	1300	0.64	1448	0.50	6.36	6.65	419	0.49	410
0.5 TSP	0.61	5.18	5.91	7.42	0.04	301	0.48	1.08	3.29	1.86	0.02	40.2
0.5 EDDS	0.22	5.82	8.24	77.1	0.24	786	0.01	0.20	5.77	67.0	0.13	260

APPENDIX G. PLANT CHEMISTRY – CHEMICALLY-ASSISTED PHYTOEXTRACTION METALS AND METALLOIDS USING *CHLORIS GAYANA* GROWN ON THE SOIL CONTAMINATED WITH 12.5 WT% MINE TAILINGS

The concentration of metals and arsenic present in the dry weight (DW) of each plant sample (P) was determined by multiplying the element concentration in the liquid sample (E) by the sample dilution factor (10) and expressing this concentration as a proportion of the plant biomass (DW) present in the sample. The following equation was used to calculate such DW element concentrations: [P = (E x 10)/DW] (Chapter 2, Section 2-11.1). The chemical amendments used were EDTA (ethylene diamine tetraacetic acid), DTPA (diethylene triamine pentaacetic acid), EDDS (ethylene diaminedissuccinatic acid), TSP (ammonium thiosulphate), SCN (ammonium thiocyanate) and THIO (thiourea) at concentrations of 0.5M and 2.0M per kg soil. Plants cultivated in substrates that died prior to harvesting have been annotated in the tables with 'ns' (no sample).

Table G.1. Plant chemistry of *Chloris gayana* grown on a soil contaminated with 12.5 wt% mine tailings after one weekly chemical treatment.

Reagent	DW (g)	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.26	0.001	0.076	0.027	0.516	0.007	1.900	0.02	2.91	1.04	19.7	0.28	72.4
	0.08	<0.001	0.020	0.019	0.236	0.002	1.540	<0.01	2.40	2.21	28.1	0.19	183
	0.16	<0.001	0.050	0.054	0.315	0.001	2.930	<0.01	3.01	3.28	19.2	0.08	178
	0.13	<0.001	0.028	0.024	0.295	0.001	2.020	<0.01	2.25	1.89	23.4	0.10	160
0.5 EDTA	0.08	<0.001	<0.001	0.007	0.038	0.001	1.480	<0.01	<0.01	0.90	4.90	0.07	190
	0.15	<0.001	0.027	0.035	0.244	0.001	2.250	<0.01	1.79	2.32	16.3	0.10	150
	0.10	<0.001	0.040	0.019	0.043	<0.001	1.250	<0.01	3.97	1.88	4.31	<0.01	124
	0.19	<0.001	0.055	0.013	0.294	0.001	3.030	<0.01	2.89	0.68	15.4	0.05	159
2.0 EDDS	0.05	<0.001	<0.001	0.007	0.086	<0.001	0.455	<0.01	<0.01	1.39	17.6	<0.01	93.0
	0.03	<0.001	<0.001	0.007	0.037	<0.001	0.838	<0.01	<0.01	1.98	10.7	<0.01	247
	0.06	<0.001	<0.001	0.015	0.056	<0.001	2.550	<0.01	<0.01	2.52	9.57	<0.01	437
	0.09	<0.001	<0.001	0.070	0.124	0.001	1.790	<0.01	<0.01	7.92	14.1	0.06	204
0.5 EDDS	0.16	<0.001	0.020	0.040	0.098	0.001	1.610	<0.01	1.28	2.52	6.18	0.04	102
	0.23	0.001	0.046	0.079	0.156	0.001	3.340	0.02	2.02	3.48	6.87	0.03	147
	0.19	<0.001	0.011	0.038	0.066	0.001	3.870	<0.01	0.61	2.02	3.53	0.03	207
	0.18	0.001	0.023	0.047	0.086	0.001	1.470	0.03	1.32	2.68	4.93	0.04	83.9
2.0 TSP	0.22	0.004	0.019	0.022	0.058	0.001	2.250	0.17	0.87	1.01	2.70	0.04	104
	0.19	0.002	0.023	0.019	0.061	0.001	2.710	0.09	1.23	0.98	3.22	0.06	143
	0.06	0.001	<0.001	0.004	0.014	<0.001	0.940	0.10	<0.01	0.70	2.25	<0.01	154
	0.12	0.001	<0.001	0.030	0.039	<0.001	0.770	0.09	<0.01	2.60	3.37	<0.01	67.0
0.5 TSP	0.01	<0.001	<0.001	0.002	0.025	<0.001	0.149	<0.01	<0.01	1.34	20.3	<0.01	122
	0.05	<0.001	<0.001	0.009	0.099	<0.001	0.501	<0.01	<0.01	1.62	18.6	<0.01	94.2
	0.10	0.001	0.009	0.014	0.041	<0.001	1.500	0.06	0.85	1.38	4.01	<0.01	148
	0.13	0.003	0.016	0.017	0.061	<0.001	1.080	0.19	1.22	1.29	4.71	<0.01	83.7
2.0 SCN	0.44	<0.001	0.038	0.057	0.116	0.001	2.710	0.00	0.87	1.30	2.64	0.02	61.7
	0.30	0.001	0.036	0.069	0.158	0.001	10.60	0.02	1.20	2.26	5.21	0.02	350
	0.18	<0.001	0.009	0.019	0.089	0.001	1.810	<0.01	0.48	1.05	4.96	0.07	101
	0.38	<0.001	0.038	0.021	0.118	0.001	2.250	<0.01	0.98	0.54	3.08	0.03	58.7
0.5 SCN	0.22	<0.001	0.020	0.069	0.117	0.001	0.599	<0.01	0.90	3.19	5.39	0.03	27.6
	0.08	<0.001	<0.001	0.018	0.038	<0.001	1.650	<0.01	<0.01	2.23	4.62	<0.01	200
	0.06	<0.001	<0.001	0.014	0.090	<0.001	0.835	<0.01	<0.01	2.20	14.3	<0.01	133
	0.06	<0.001	<0.001	0.010	0.018	<0.001	0.512	<0.01	<0.01	1.59	2.85	<0.01	80.8
2.0 THIO	0.26	0.003	0.023	0.136	0.489	0.001	4.110	0.13	0.88	5.18	18.6	0.04	157
	0.52	0.001	0.064	0.055	0.149	0.001	7.020	0.01	1.24	1.07	2.88	0.02	136
	0.22	0.001	<0.001	0.050	0.186	0.001	2.330	0.06	<0.01	2.33	8.65	0.03	108
	0.35	0.001	0.028	0.090	0.146	0.001	4.640	0.02	0.81	2.55	4.15	0.02	132
0.5 THIO	0.04	<0.001	<0.001	0.009	0.033	<0.001	0.781	<0.01	<0.01	2.01	7.45	<0.01	178
	0.07	<0.001	<0.001	0.015	0.036	<0.001	0.660	<0.01	<0.01	2.08	5.07	<0.01	93.0
	0.09	<0.001	<0.001	0.015	0.051	<0.001	1.690	<0.01	<0.01	1.73	5.76	<0.01	193
	0.17	<0.001	0.028	0.083	0.159	0.001	3.100	<0.01	1.63	4.75	9.12	0.05	178
2.0 DTPA	0.17	<0.001	0.042	0.101	0.333	0.005	7.090	<0.01	2.50	6.07	20.0	0.28	426
	0.18	<0.001	0.040	0.050	0.938	<0.001	3.690	<0.01	2.25	2.83	53.1	<0.01	209
	0.28	<0.001	0.125	0.084	2.570	0.011	10.60	<0.01	4.43	2.98	91.1	0.39	376
	0.05	<0.001	0.008	0.007	0.148	0.002	0.927	<0.01	1.57	1.37	30.2	0.31	189

Table G.1.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Chloris gayana* after one weekly chemical treatment.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.02	2.64	2.11	22.6	0.16	149	<0.01	0.37	0.93	4.12	0.09	51.7
0.5 EDTA	<0.01	2.88	1.45	10.2	0.07	156	<0.01	1.09	0.78	6.51	0.02	26.9
2.0 EDDS	<0.01	<0.01	3.45	13.0	0.06	245	<0.01	0.58	0.60	1.47	<0.01	54.9
0.5 EDDS	0.03	1.31	2.67	5.38	0.04	135	<0.01	0.58	0.60	1.47	<0.01	54.9
2.0 TSP	0.11	1.05	1.32	2.88	0.05	117	0.04	0.25	0.86	0.51	0.01	39.7
0.5 TSP	0.13	1.04	1.40	11.9	<0.01	112	0.09	0.27	0.15	8.72	<0.01	28.9
2.0 SCN	0.01	0.88	1.29	3.97	0.04	143	0.01	0.30	0.72	1.30	0.02	139
0.5 SCN	<0.01	0.90	2.30	6.80	0.03	110	<0.01	<0.01	0.66	5.13	<0.01	73.6
2.0 THIO	0.05	0.98	2.78	8.57	0.03	133	0.05	0.23	1.72	7.14	0.01	19.7
0.5 THIO	<0.01	1.63	2.64	6.85	0.05	160	<0.01	<0.01	1.41	1.82	<0.01	45.4
2.0 DTPA	<0.01	2.69	3.31	48.6	0.32	300	<0.01	1.23	1.97	31.5	0.06	118

Table G.2. Plant chemistry of *Chloris gayana* grown on a soil contaminated with 12.5 wt% mine tailings after two weekly chemical treatments.

Reagent	DW (g)	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.06	0.001	0.006	0.010	0.467	0.002	1.350	0.11	1.03	1.56	74.2	0.25	215
	0.38	<0.001	0.191	0.072	0.332	0.002	6.720	<0.01	4.99	1.88	8.67	0.04	175
	0.17	<0.001	0.049	0.018	0.573	0.005	1.200	<0.01	2.94	1.08	34.5	0.31	72.3
	0.51	0.005	0.199	0.040	1.550	0.002	7.910	0.09	3.89	0.79	30.3	0.03	155
0.5 EDTA	0.13	<0.001	0.092	0.009	0.105	0.001	0.712	<0.01	7.03	0.69	8.05	0.10	54.6
	0.15	<0.001	0.031	0.029	0.368	0.002	1.560	<0.01	2.11	1.95	25.0	0.10	106
	0.26	<0.001	0.034	0.018	0.238	0.002	1.230	<0.01	1.32	0.69	9.26	0.07	47.8
	0.30	<0.001	0.087	0.021	0.115	0.002	3.190	<0.01	2.90	0.71	3.85	0.06	107
2.0 EDDS	0.03	<0.001	0.008	0.007	0.040	<0.001	0.909	<0.01	2.79	2.21	13.2	<0.01	302
	0.18	<0.001	0.050	0.065	0.328	0.001	3.010	<0.01	2.74	3.53	17.9	0.04	165
	0.08	<0.001	0.022	0.080	0.139	0.001	0.987	<0.01	2.78	9.90	17.3	0.08	122
	0.19	<0.001	0.041	0.030	0.134	0.001	8.510	<0.01	2.16	1.58	6.99	0.04	444
0.5 EDDS	0.29	0.001	0.062	0.072	0.137	0.001	1.080	0.03	2.14	2.47	4.72	0.03	37.2
	0.26	<0.001	0.066	0.043	0.088	0.001	9.690	<0.01	2.54	1.67	3.43	0.03	376
	0.16	<0.001	0.049	0.015	0.063	<0.001	1.360	<0.01	3.14	0.98	4.01	<0.01	87.3
	0.29	<0.001	0.106	0.111	0.209	0.001	9.360	<0.01	3.61	3.78	7.11	0.03	318
0.5 TSP	0.18	0.001	0.057	0.007	0.043	0.005	1.760	0.03	3.09	0.36	2.37	0.25	96.2
	0.03	<0.001	<0.001	0.008	0.023	<0.001	1.090	<0.01	<0.01	2.68	7.72	<0.01	360
	0.15	0.002	0.035	0.011	0.053	<0.001	1.070	0.11	2.36	0.73	3.56	<0.01	72.1
	0.35	0.006	0.099	0.068	0.388	0.001	4.370	0.16	2.83	1.96	11.2	0.02	126
0.5 SCN	0.36	0.001	0.048	0.316	0.396	0.007	7.560	0.02	1.34	8.77	11.0	0.21	210
	0.49	<0.001	0.079	0.083	0.148	0.002	9.220	<0.01	1.60	1.69	3.00	0.05	187
	0.28	0.005	0.028	0.114	0.158	0.001	1.770	0.17	1.01	4.11	5.70	0.04	63.8
	0.54	0.001	0.129	0.329	0.296	0.002	13.50	0.01	2.38	6.08	5.47	0.03	250
0.5 THIO	0.62	0.001	0.218	0.081	0.236	0.002	10.20	0.02	3.54	1.31	3.83	0.04	166
	0.53	0.001	0.142	0.353	0.256	0.001	5.770	0.01	2.67	6.64	4.81	0.03	109
	0.55	0.004	0.031	0.021	0.051	0.001	1.330	0.07	0.57	0.38	0.92	0.03	24.1
	0.14	0.005	0.021	0.090	0.214	0.001	5.060	0.34	1.53	6.62	15.7	0.04	372
2.0 DTPA	0.15	<0.001	0.094	0.051	1.300	0.004	4.310	<0.01	6.26	3.37	86.4	0.27	287
	0.11	<0.001	0.022	0.030	0.780	0.002	1.370	<0.01	1.96	2.69	69.3	0.18	122
	0.14	<0.001	0.050	0.046	0.883	0.003	2.970	<0.01	3.72	3.39	65.2	0.20	219
	0.15	<0.001	0.077	0.026	0.750	0.004	3.870	<0.01	5.29	1.77	51.4	0.27	265

Table G.2.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Chloris gayana* after two weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.10	3.21	1.33	36.9	0.16	154	0.01	1.68	0.49	27.3	0.14	60.0
0.5 EDTA	<0.01	3.34	1.01	11.5	0.09	78.8	<0.01	2.54	0.63	9.27	0.02	31.9
2.0 EDDS	<0.01	2.62	4.30	13.8	0.05	258	<0.01	0.31	3.82	5.03	0.02	146
0.5 EDDS	0.03	2.86	2.22	4.82	0.03	205	<0.01	0.65	1.20	1.62	<0.01	168
0.5 TSP	0.10	2.76	1.43	6.20	0.14	164	0.07	0.37	1.08	4.02	0.17	133
0.5 SCN	0.07	1.58	5.16	6.29	0.08	178	0.09	0.58	3.00	3.36	0.08	80.1
0.5 THIO	0.11	2.08	3.74	6.32	0.03	167	0.16	1.30	3.36	6.48	0.01	148
2.0 DTPA	<0.01	4.31	2.81	68.1	0.23	223	<0.01	1.88	0.76	14.4	0.05	73.3

Table G.3. Plant chemistry of *Chloris gayana* grown on a soil contaminated with 12.5 wt% mine tailings after three weekly chemical treatments.

Reagent	DW	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
	(g)	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.09	<0.001	0.051	0.016	0.156	0.001	1.760	<0.01	5.41	1.72	16.6	0.14	187
	0.08	<0.001	0.042	0.006	0.317	0.001	0.741	<0.01	5.07	0.72	38.2	0.09	89.2
	0.04	<0.001	<0.001	0.012	0.291	<0.001	0.365	<0.01	<0.01	2.68	65.5	<0.01	82.2
	0.07	<0.001	0.028	0.004	0.277	0.001	0.622	<0.01	4.29	0.66	42.6	0.12	95.5
0.5 EDTA	0.06	<0.001	<0.001	0.006	0.099	<0.001	0.170	<0.01	<0.01	1.07	17.0	<0.01	29.4
	0.13	<0.001	0.042	0.017	0.181	<0.001	1.250	<0.01	3.31	1.34	14.1	<0.01	97.5
	0.17	<0.001	0.073	0.019	0.429	0.001	1.480	<0.01	4.28	1.14	25.3	0.04	87.2
	0.15	<0.001	0.035	0.011	0.145	0.004	0.814	<0.01	2.36	0.71	9.77	0.25	54.9
2.0 DTPA	0.21	<0.001	0.097	0.039	0.735	0.004	3.540	<0.01	4.60	1.84	34.9	0.19	168
	0.10	<0.001	0.048	0.017	0.413	0.002	1.110	<0.01	4.87	1.71	41.7	0.24	112
	0.17	<0.001	0.072	0.028	0.418	0.003	2.050	<0.01	4.19	1.61	24.3	0.16	119
	ns												
2.0 TSP	0.09	0.000	0.014	0.035	0.100	0.001	1.300	0.06	1.61	3.94	11.3	0.09	146
	0.14	0.006	0.009	0.013	0.417	0.001	5.150	0.42	0.62	0.89	29.4	0.05	363
	0.24	0.009	0.021	0.057	0.865	0.002	1.240	0.36	0.86	2.37	36.1	0.08	51.7
	0.07	0.001	0.026	0.037	0.273	0.001	1.900	0.12	3.89	5.61	41.3	0.15	287
0.5 TSP	0.05	<0.001	<0.001	0.003	0.029	0.001	0.832	<0.01	<0.01	0.69	6.32	0.28	180
	0.13	<0.001	0.071	0.010	0.074	0.001	0.843	<0.01	5.55	0.80	5.75	0.11	65.9
	0.33	0.003	0.110	0.017	0.013	0.002	2.100	0.08	3.29	3.29	0.50	0.40	62.9
	0.15	0.001	0.043	0.010	0.054	0.001	1.440	0.09	2.81	0.67	3.51	0.09	93.1
2.0 EDDS	0.04	<0.001	0.038	0.023	0.104	0.001	1.940	<0.01	9.52	5.82	26.3	0.30	491
	0.08	<0.001	0.020	0.010	0.350	0.001	1.740	<0.01	2.61	1.29	46.2	0.13	230
	0.11	0.000	0.028	0.080	0.412	0.002	1.970	<0.01	2.45	7.07	36.3	0.14	174
	ns												
0.5 EDDS	0.33	0.001	0.128	0.114	0.155	0.002	7.850	0.02	3.89	3.46	4.71	0.05	239
	0.35	<0.001	0.153	0.036	0.131	0.001	3.920	<0.01	4.38	1.02	3.75	0.04	112
	0.29	<0.001	0.052	0.035	0.096	0.001	4.750	<0.01	1.78	1.20	3.27	0.04	163
	0.10	<0.001	0.025	0.019	0.116	0.001	1.330	<0.01	2.51	1.95	11.7	0.10	134

ns = no sample

Table G.3.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Chloris gayana* after three weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	<0.01	4.92	1.44	40.7	0.12	113	<0.01	0.58	0.96	20.1	0.03	49.2
0.5 EDTA	<0.01	3.31	1.07	16.6	0.14	67.2	<0.01	0.96	0.26	6.54	0.15	31.1
2.0 EDDS	<0.01	4.86	4.73	36.3	0.19	299	<0.01	4.04	3.04	9.92	0.09	169.5
0.5 EDDS	0.02	3.14	1.91	5.86	0.06	162	<0.01	1.20	1.11	3.94	0.03	55.1
2.0 TSP	0.24	1.74	3.21	29.5	0.09	213	0.18	1.49	2.03	13.1	0.04	140
0.5 TSP	0.08	3.89	1.36	4.02	0.22	101	0.01	1.47	1.29	2.64	0.15	54.8
2.0 DTPA	<0.01	4.56	1.72	33.6	0.20	133	<0.01	0.34	0.12	8.77	0.04	30.6

Table G.4. Plant chemistry of *Chloris gayana* grown on a soil contaminated with 12.5 wt% mine tailings after four weekly chemical treatments.

Reagent	DW	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
	(g)	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.13	0.001	0.083	0.036	2.523	0.003	5.640	0.08	6.27	2.68	190	0.22	424
	0.11	<0.001	0.032	0.010	2.001	0.002	1.650	<0.01	3.02	0.92	189	0.16	156
	0.28	0.001	0.065	0.008	4.100	0.002	2.170	0.02	2.36	0.29	148	0.07	78.5
	0.35	0.001	0.185	0.020	2.040	0.002	2.360	0.03	5.36	0.57	59.1	0.07	68.3
2.0 TSP	0.17	0.046	0.017	0.033	2.990	0.003	6.010	2.73	1.03	1.97	179	0.15	359
	0.56	0.092	0.127	0.437	4.940	0.004	10.00	1.64	2.26	7.79	88.0	0.07	178
	1.27	0.168	0.290	0.898	5.030	0.017	22.00	1.32	2.28	7.06	39.6	0.13	173
	0.99	0.132	0.159	0.623	5.620	0.009	16.2	1.33	1.60	6.27	56.6	0.10	163
0.5 TSP	0.19	<0.001	0.062	0.081	0.091	0.002	4.030	<0.01	3.30	4.34	4.84	0.12	216
	0.26	0.002	0.074	0.017	0.071	0.002	2.710	0.07	2.89	0.65	2.74	0.09	105
	0.11	<0.001	0.082	0.016	0.035	0.002	0.549	<0.01	7.52	1.50	3.23	0.15	50.2
	0.35	0.005	0.066	0.015	0.093	0.002	1.760	0.14	1.85	0.42	2.61	0.06	49.7
2.0 EDDS	0.16	<0.001	0.054	0.028	0.387	0.002	2.460	<0.01	3.45	1.81	24.9	0.13	158
	0.08	<0.001	0.016	0.027	0.280	0.002	7.620	<0.01	1.95	3.32	34.2	0.22	929
	0.17	<0.001	0.033	0.069	0.220	0.002	5.150	<0.01	1.91	4.04	12.9	0.13	301
	0.25	<0.001	0.081	0.039	0.287	0.003	6.960	<0.01	3.26	1.57	11.6	0.10	280
0.5 EDDS	0.26	<0.001	0.133	0.029	0.258	0.002	6.630	<0.01	5.06	1.10	9.82	0.09	252
	0.14	<0.001	0.031	0.006	0.023	0.001	1.590	<0.01	2.16	0.39	1.65	0.10	112
	0.12	<0.001	0.037	0.009	0.063	0.001	1.060	<0.01	3.21	0.77	5.47	0.13	91.9
	0.16	<0.001	0.050	0.012	0.045	0.002	4.600	<0.01	3.03	0.71	2.74	0.11	280

Table G.4.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Chloris gayana* after four weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
2.0 EDTA	0.04	4.25	1.12	146	0.13	182	0.03	1.86	1.08	61.3	0.07	166
2.0 EDDS	<0.01	2.64	2.69	20.9	0.15	417	<0.01	0.83	1.19	10.7	0.05	347
0.5 EDDS	<0.01	3.37	0.74	4.92	0.11	184	<0.01	1.22	0.29	3.64	0.02	95.9
2.0 TSP	1.76	1.79	5.77	90.7	0.11	218	0.67	0.60	2.61	62.0	0.04	94.0
0.5 TSP	0.05	3.89	1.73	3.35	0.10	105	0.07	2.50	1.80	1.03	0.04	78.1

Table G.5. Plant chemistry of *Chloris gayana* grown on a soil contaminated with 12.5 wt% mine tailings after five weekly chemical treatments.

Reagent	DW (g)	Solution Concentration (μg g^{-1}) (E)						Plant Concentration (μg g^{-1})					
		Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Control	0.59	0.001	0.110	0.084	0.117	0.003	10.60	0.02	1.86	1.42	1.98	0.05	180
	0.67	0.001	0.088	0.176	0.217	0.003	10.10	0.01	1.32	2.65	3.26	0.04	152
	0.43	<0.001	0.036	0.109	0.130	0.001	8.740	<0.01	0.83	2.56	3.05	0.03	205
	0.75	<0.001	0.384	0.075	0.257	0.002	8.300	<0.01	5.11	1.00	3.42	0.03	110
2.0 EDTA	0.58	0.001	0.321	0.040	4.214	0.003	4.760	0.01	5.54	0.68	72.7	0.05	82.2
	0.73	0.001	0.177	0.013	6.355	0.002	6.860	0.01	2.42	0.17	86.8	0.03	93.7
	1.00	0.001	0.614	0.036	14.27	0.004	12.90	0.01	6.15	0.36	143	0.04	129
	0.56	0.001	0.611	0.078	5.011	0.004	13.40	0.01	11.0	1.40	90.0	0.08	241
0.5 EDTA	0.79	0.001	0.206	0.121	1.390	0.002	6.190	0.02	2.60	1.53	17.5	0.02	78.1
	0.46	<0.001	0.262	0.022	2.860	0.003	4.480	<0.01	5.68	0.47	62.0	0.06	97.1
	0.71	0.001	0.431	0.036	0.898	0.002	5.080	0.01	6.05	0.50	12.6	0.03	71.3
	0.55	0.001	0.180	0.032	0.781	0.002	1.910	0.02	3.30	0.59	14.3	0.03	35.0
2.0 DTPA	0.81	0.001	0.427	0.056	1.510	0.009	12.10	0.01	5.30	0.69	18.7	0.11	150
	0.85	<0.001	0.517	0.044	1.370	0.007	9.460	<0.01	6.11	0.52	16.2	0.08	112
	0.87	<0.001	0.419	0.040	1.210	0.006	8.560	<0.01	4.84	0.46	14.0	0.07	98.9
	0.68	<0.001	0.382	0.053	1.170	0.007	9.390	<0.01	5.58	0.78	17.1	0.10	137
0.5 TSP	0.85	0.003	0.584	0.188	0.505	0.002	9.390	0.03	6.91	2.22	5.97	0.03	111
	0.90	0.014	0.287	0.230	0.589	0.003	6.820	0.16	3.20	2.57	6.57	0.04	76.1
	0.35	0.001	0.169	0.043	0.159	0.002	7.520	0.02	4.76	1.20	4.48	0.05	212
	0.92	0.008	0.266	0.245	0.256	0.002	6.960	0.09	2.89	2.66	2.78	0.02	75.7
2.0 EDDS	0.80	0.001	0.422	0.067	3.690	0.003	21.40	0.01	5.31	0.84	46.4	0.04	269
	0.51	0.005	0.080	0.072	3.502	0.004	7.580	0.09	1.56	1.41	68.5	0.08	148
	0.39	<0.001	0.128	0.100	1.500	0.003	7.790	<0.01	3.29	2.57	38.5	0.07	200
	0.08	<0.001	0.009	0.008	0.128	0.002	7.210	<0.01	1.13	1.00	16.8	0.24	947
0.5 EDDS	0.65	<0.001	0.293	0.036	0.305	0.002	6.590	<0.01	4.49	0.55	4.68	0.04	101
	1.10	<0.001	0.273	0.076	0.180	0.003	23.80	<0.01	2.49	0.69	1.64	0.03	217
	1.12	0.001	0.474	0.100	0.203	0.003	19.30	0.01	4.25	0.90	1.82	0.02	173
	0.51	0.001	0.184	0.039	0.273	0.002	9.360	0.02	3.64	0.77	5.39	0.05	185

Table G.5.1. Mean and standard deviation of the plant chemistry (μg g^{-1}) for *Chloris gayana* after five weekly chemical treatments.

Reagent	Average Plant Concentration (μg g^{-1})						Standard Deviation (μg g^{-1})					
	Ag	As	Cd	Pb	Sb	Zn	Ag	As	Cd	Pb	Sb	Zn
Control	0.01	2.28	1.90	2.93	0.04	162	0.01	1.93	0.82	0.65	0.01	40.5
2.0 EDTA	0.01	6.27	0.65	98.1	0.05	136	<0.01	3.53	0.54	30.8	0.02	72.3
0.5 EDTA	0.02	4.41	0.77	26.6	0.03	70.4	0.01	1.71	0.51	23.7	0.02	26.0
2.0 EDDS	0.05	2.82	1.46	42.6	0.11	391	0.06	1.90	0.78	21.3	0.09	374
0.5 EDDS	0.01	3.72	0.73	3.38	0.03	169	0.01	0.89	0.14	1.93	0.01	49.0
0.5 TSP	0.07	4.44	2.16	4.95	0.04	119	0.06	1.84	0.67	1.69	0.01	64.4
2.0 DTPA	0.01	5.46	0.61	16.5	0.09	124	<0.01	0.53	0.15	1.98	0.02	23.3

APPENDIX H. PLANT CHEMISTRY - CHEMICALLY-ASSISTED PHYTOEXTRACTION METALS USING *CHLORIS GAYANA* GROWN ON FERTILISED MINE TAILINGS

The concentration of metals and arsenic present in the dry weight (DW) of each plant sample (P) was determined by multiplying the element concentration in the liquid sample (E) by the sample dilution factor (10) and expressing this concentration as a proportion of the plant biomass (DW) present in the sample. The following equation was used to calculate such DW element concentrations: [P = (E x 10)/DW] (Chapter 2, Section 2-11.1). The chemical amendments used were SCN (ammonium thiocyanate), EDDS (ethylene diaminedissuccinatic acid) and EDTA (ethylene diamine tetraacetic acid) applied at concentrations of 2.0 g per kg soil. Plants cultivated in substrates that died prior to harvesting have been annotated in the tables with 'ns' (no sample). Substrates that were not prepared for cultivation have been annotated in the tables with 'nt' (not tested).

Table H.1. Plant chemistry of *Chloris gayana* cultivated on fertilised mine tailings after one amendment (week 12) of the chemical reagents.

Fertiliser	Treatment	DW (g)	Sample Conc. (µg g^{-1}) (E)			Plant Concentration (µg g^{-1})		
			Ag	Pb	Zn	Ag	Pb	Zn
No Fertiliser	None	0.78	0.017	1.29	17.2	0.22	16.5	219
300 kg ha^{-1} OSM	2 g kg^{-1} SCN	0.70	0.015	1.92	34.8	0.22	27.6	501
	2 g kg^{-1} EDDS	0.87	0.002	0.84	67.6	0.02	9.6	773
	2 g kg^{-1} EDTA	0.95	0.009	2.35	66.3	0.10	24.7	695
No Fertiliser	None	0.51	0.008	3.53	7.21	0.16	69.0	141
300 kg ha^{-1} OSM	2 g kg^{-1} SCN	0.64	0.157	2.27	22.4	2.45	35.4	349
+ 300 kg ha^{-1} TPP	2 g kg^{-1} EDDS	0.81	0.002	1.21	87.7	0.02	14.9	1079
	2 g kg^{-1} EDTA	1.17	0.001	4.27	224	0.01	36.4	1908

Table H.2. Plant chemistry of *Chloris gayana* cultivated on fertilised mine tailings after three amendments (week 32) of the chemical reagents.

Fertiliser	Treatment	DW (g)	Sample Conc. (µg g^{-1}) (E)			Plant Concentration (µg g^{-1})		
			Ag	Pb	Zn	Ag	Pb	Zn
No Fertiliser	None	nt						
300 kg ha^{-1} OSM	2 g kg^{-1} SCN	ns						
	2 g kg^{-1} EDDS	0.59	0.001	0.366	64.1	0.02	6.20	1085
	2 g kg^{-1} EDTA	0.36	0.002	0.871	20.5	0.06	24.5	577
No Fertiliser	None	nt						
300 kg ha^{-1} OSM	2 g kg^{-1} SCN	ns						
+ 300 kg ha^{-1} TPP	2 g kg^{-1} EDDS	0.44	0.001	0.230	33.5	0.02	5.21	758
	2 g kg^{-1} EDTA	0.49	0.001	0.156	21.2	0.02	3.18	432

nt = not tested, ns = no sample

Table H.3. Plant chemistry of *Chloris gayana* cultivated on fertilised mine tailings after four amendments (week 42) of the chemical reagents.

Fertiliser	Treatment	DW (g)	Sample Conc. (µg g^{-1}) (E)			Plant Concentration (µg g^{-1})		
			Ag	Pb	Zn	Ag	Pb	Zn
No Fertiliser	None	nt						
300 kg ha^{-1} OSM	2 g kg^{-1} SCN	ns						
	2 g kg^{-1} EDDS	0.53	<0.001	0.224	43.8	0.02	4.25	831
	2 g kg^{-1} EDTA	0.62	<0.001	0.982	24.6	0.02	15.9	399
No Fertiliser	None	nt						
300 kg ha^{-1} OSM	2 g kg^{-1} SCN	ns						
+ 300 kg ha^{-1} TPP	2 g kg^{-1} EDDS	0.30	<0.001	0.061	7.98	0.03	2.01	263
	2 g kg^{-1} EDTA	0.49	<0.001	0.320	18.6	0.02	6.59	383

nt = not tested, ns = no sample

APPENDIX I. GEOCHEMICAL DATA - PARTIAL EXTRACTION OF THE FERTILISED AND UNFERTILISED MINE TAILINGS

Table I.1. The concentrations of Pb and Zn (μg g^{-1}) dissolved using soil extractions of 0.01M EDTA and 0.005M DTPA, and for comparison 0.01M EDDS and deionised water (dH_2O), from the fertilised (OSM = 300 kg^{-1} ha^{-1} Osmocote, TPP = 300 kg^{-1} ha^{-1} Triphosphate) unfertilised mine tailings.

Extract	Treatment	Sample wt (g)	Extract Conc. (μg g^{-1})			Conc. (μg g^{-1})		
			Ag	Pb	Zn	Ag	Pb	Zn
dH_2O	Unfertilised	1.04	<0.001	0.30	1.62	<0.01	0.3	1.7
	OSM	0.97	<0.001	1.94	34.0	<0.01	1.9	33.1
	OSM+TPP	1.02	<0.001	0.05	2.74	<0.01	0.1	2.8
0.01M EDTA	Unfertilised	1.05	<0.001	199	129	<0.01	209	135
	OSM	0.96	<0.001	37.1	102	<0.01	35.6	98.0
	OSM+TPP	1.02	<0.001	99.5	95.7	<0.01	101	97.2
0.005M DTPA	Unfertilised	1.04	<0.001	856	151	<0.01	892	157
	OSM	0.95	<0.001	889	112	<0.01	843	106
	OSM+TPP	0.97	<0.001	776	104	<0.01	749	100
0.01M EDDS	Unfertilised	1.09	<0.001	830	87.4	<0.01	902	95.0
	OSM	1.03	<0.001	535	71.5	<0.01	551	73.6
	OSM+TPP	1.07	<0.001	324	47.7	<0.01	346	51.0

APPENDIX J. GEOCHEMICAL DATA - THE CONCENTRATIONS OF AG, PB AND ZN LEACHED FROM THE CANNINGTON MINE TAILINGS USING VARIOUS CHEMICAL AMENDMENTS.

The chemical reagents used to leach Ag, Pb and Zn from the mine tailings were EDTA (ethylene diamine tetraacetic acid), TSP (ammonium thiosulphate), SCN (ammonium thiocyanate), THIO (thiourea) and NaCN (sodium cyanide) at the concentrations indicated per kg soil. Samples lost prior to analysis have been annotated in the tables with 'ns' (no sample).

Table J.1. The concentrations of Ag (μg g^{-1}) leached from Cannington mine tailings over a twelve-week period.

Period (wks)	Ag Concentrations (μg g^{-1})								
	dH_2O	1M EDTA	0.1M EDTA	1M TSP	0.1M TSP	1M SCN	0.1M SCN	1M THIO	0.05% NaCN
1	<0.001	0.001	<0.001	<0.001	3.16	<0.001	<0.001	<0.001	<0.001
2	<0.001	0.001	<0.001	1.91	1.09	1.68	0.00	0.03	0.04
3	<0.001	0.004	<0.001	4.57	0.27	322	0.04	4.17	0.13
4	<0.001	0.003	<0.001	2.23	0.41	128	0.97	4.73	0.11
5	<0.001	0.006	ns	2.22	0.73	15.2	0.45	4.62	0.92
6	<0.001	0.008	0.005	1.48	1.19	9.58	0.20	4.20	0.74
7	<0.001	0.001	0.002	1.38	0.70	5.57	0.10	4.45	1.92
8	<0.001	0.001	<0.001	1.44	3.43	3.88	0.20	4.53	2.23
9	<0.001	0.001	<0.001	3.20	1.01	5.57	0.13	4.80	
10	<0.001	0.004	<0.001	2.35	0.68	3.88	0.25	4.72	
11	<0.001	0.004	<0.001	1.37	1.05	5.19	0.19	5.22	
12	<0.001	0.004	<0.001	1.23	2.56	1.64	0.26	8.02	

ns = no sample

Table J.2. The concentrations of Pb (μg g^{-1}) leached from Cannington mine tailings over a twelve-week period. Sodium concentrations for 1M and 0.1M EDTA leachates are included in brackets.

Period (wks)	Pb Concentrations (μg g^{-1})								
	dH_2O	1M EDTA	0.1M EDTA	1M TSP	0.1M TSP	1M SCN	0.1M SCN	1M THIO	0.05% NaCN
1	0.01	0.01 (1770)	0.19 (411)	0.03	120	0.24	0.02	0.04	3.49
2	0.03	162 (274)	0.29 (42.2)	59.6	121	0.46	0.07	0.03	0.24
3	0.08	15200 (7840)	2420 (1200)	1960	538	1.20	0.24	0.94	0.06
4	0.12	12400 (11322)	8460 (3270)	1500	2180	0.70	0.31	2.82	1.64
5	0.13	4280 (11200)	ns	1240	451	0.56	0.32	3.10	0.99
6	0.12	1570 (8790)	8230 (3490)	835	114	0.45	0.19	2.33	2.15
7	0.13	71.4 (1070)	7070 (3880)	684	117	0.53	0.21	2.52	2.14
8	0.12	7.28 (361)	1130 (3970)	494	83.6	0.51	0.16	2.76	2.05
9	0.19	3.17 (241)	2690 (3960)	131	77.4	0.53	0.16	3.09	
10	0.21	4.99 (178)	762 (3910)	367	54.8	0.51	0.15	3.28	
11	0.24	6.32 (131)	579 (3900)	132	35.3	0.56	0.16	3.07	
12	0.24	3.37 (80.7)	478 (4040)	20.7	34.8	0.03	0.00	2.16	

ns = no sample

Table J.3. The concentrations of Zn (μg g^{-1}) leached from Cannington mine tailings over a twelve-week period.

Period (wks)	Zn Concentrations (μg g^{-1})								
	dH_2O	1M EDTA	0.1M EDTA	1M TSP	0.1M TSP	1M SCN	0.1M SCN	1M THIO	0.05% NaCN
1	0.38	0.59	5.60	3.60	4.47	7.33	1.52	9.84	17.9
2	4.78	75.7	3.94	48.0	3.48	12.8	3.49	5.49	4.40
3	8.79	2860	908	1930	263	254	7.72	1.78	4.41
4	4.81	1190	1770	1110	710	513	10.1	1.35	5.46
5	3.61	448	ns	481	106	386	8.68	1.47	9.88
6	4.24	265	1060	355	35.8	330	6.24	1.80	31.0
7	4.38	70.1	748	279	31.4	289	5.49	1.84	44.4
8	5.28	22.0	169	248	29.8	242	5.86	1.90	64.1
9	4.61	11.5	273	184	27.1	217	5.31	2.11	ns
10	7.02	9.72	162	207	24.3	235	6.36	2.27	ns
11	5.49	7.65	121	159	20.7	204	5.52	2.05	ns
12	4.72	4.84	104	169	28.3	975	3.28	5.74	ns

ns = no sample

APPENDIX K. ANALYSIS OF GEOCHEMICAL REFERENCE MATERIAL (GXR-3)

The geochemical reference material (GXR-3) was submitted to the AAC, JCU for microwave acid digestion and ICP MS analysis at the beginning of 2002 and 2003. The reference material accompanied two large batches of samples for data presented in Chapters 3-4 and Chapters 5,6 and 7 respectively.

Replicate	Total Element Concentration ($\mu g\ g^{-1}$)				
	Ag	As	Cd	Pb	Zn
2002	2.4	3970	1.5	15	207
2003	2.3	3971	1.8	15	208
Mean	2.4	3971	1.7	15	208
Std. Dev.	0.1	0.7	0.2	<0.1	0.7