The determination of the potential of Australian native plants to phytoremediate lead

Desmond Dev Menon

*Edith Cowan University*
Edith Cowan University

Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study.

The University does not authorize you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following:

• Copyright owners are entitled to take legal action against persons who infringe their copyright.

• A reproduction of material that is protected by copyright may be a copyright infringement. Where the reproduction of such material is done without attribution of authorship, with false attribution of authorship or the authorship is treated in a derogatory manner, this may be a breach of the author’s moral rights contained in Part IX of the Copyright Act 1968 (Cth).

• Courts have the power to impose a wide range of civil and criminal sanctions for infringement of copyright, infringement of moral rights and other offences under the Copyright Act 1968 (Cth). Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.
The Determination Of The Potential Of Australian Native Plants To Phytoremediate Lead

Desmond Dev Menon

Thesis submitted in partial fulfilment of the requirement for the award of B. Sc. (Biological Science) Honours

Department of Applied Science

Edith Cowan University

February 1998
USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.
ABSTRACT

Industrial activities and natural occurrences of mineral ores can both result in the presence of high concentrations of heavy metals in the soil. These toxic metals have been shown to adversely affect human health and the environment.

Currently, three main technologies are being used to solve this problem of soil contamination. These are incineration, landfill construction and physico-chemical treatments.

The economic and environmental costs of each of these technologies tend to outweigh the merits of their application, especially where contaminated areas are small. These techniques do not necessarily remove the heavy metals from the soil, and can also inhibit biological activity by affecting the physical structure of the treated medium.

Recent research has seen the development of alternative methods, which utilise living systems, for less expensive land rehabilitation. Phytoremediation is the process of utilising plants with some tolerance of soil contamination to rehabilitate polluted sites. One category of such plants comprises species that are able to accumulate high concentrations of heavy metals. Experiments carried out with herbaceous species (e.g. *Alyssum tenium* and *Brassica juncea* L.) and trees (e.g. *Populus* spp.) have presented valuable and successful reports regarding their application. The capacity of native Australian plants to fulfil these requirements is relatively unknown.

Many species of Australian native flora are well adapted to the adverse conditions of arid and semi arid landscapes. Some are currently being utilised in the reclamation of such soils (Marcar & Termaat, 1990). Research with tree species in particular have seen
the successful rehabilitation of saline and waterlogged soils. The economic values of these plants provide substantial incentive for their use in these applications.

This study was aimed at initiating the development of research into the prospects of utilising Australian plants to carry out the phytoremediation of heavy metal-contaminated soil.

Species from three families were selected for this study. These were *Eucalyptus camaldulensis*, *E. lesoufii* and *E. globulus* from the Myrtaceae family, *Acacia heteroclita*, *A. saligna* and *A. quadramarginea* from the Mimosaceae family and *Casuarina obesa* and *Allocasuarina verticillata* from the Casuarinaceae family. Experiments were carried out to determine the potential of these selected plants for the accumulation of lead.

Seedlings of these species were subjected to lead treatments in both hydroponic and soil experiments. The quantity of accumulated lead in leaf, stem and root tissue of seedlings grown in varying lead concentrations was measured. The results obtained for species subjected to hydroponics experimentation was shown to correspond to results obtained for the same species in soil experiments. The results also showed that there were variations in physiological responses and levels of lead accumulated within tissues for each individual species. *A. heteroclita* accumulated the highest levels of lead in its tissues and showed mild toxic symptoms.
DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief it does not contain any material previously published or written by any other person except where due reference is made in the text.

Desmond Dev Menon

5 February 1998
I wish to thank my supervisor Dr Ian Bennett for his patience, guidance and being an inspiration throughout this project. I am also grateful to my Co-supervisor Dr Mary Boyce for her time, effort and enthusiasm.

I would like to acknowledge all those who work in the research laboratory in the Department of Applied Science, Edith Cowan University, for their assistance and encouragement throughout the project.

Finally, to my mother, Juliana Menon, to whom I am eternally grateful for believing in me and providing me with the opportunity to do my best.
TABLE OF CONTENTS

ABSTRACT .............................................................................................. i

DECLARATION ..................................................................................... .iii

ACKNOWLEDGEMENTS ........................................................................ iv

TABLE OF CONTENTS ............................................................................ v

LISTS OF FIGURES AND TABLE ............................................................ viii

ABBREVIATIONS .................................................................................. xii

CHAPTER 1 INTRODUCTION ........................................................................... 1

1.1 GLOBAL PROBLEM ........................................................................... 1

1.2 CLEAN-UP OF HEAVY METAL CONTAMINATED SOILS ...................... 3
  1.2.1 Conventional Methods .............................................................. 3
  1.2.2 Incineration ........................................................................... 3
  1.2.3 Landfill .................................................................................. 4
  1.2.4 Physico-Chemical Treatments .................................................... 4
  1.2.5 Shortfalls Of These Methods...................................................... 4

1.3 PHYTOREMEDIATION ..................................................................... 6
  1.3.1 A Novel Stratagy For Waste Removal From The Environment .......... 6
  1.3.2 Current Application ................................................................. 10
  1.3.3 Properties Of Heavy Metal Hyperaccumulators ............................. 11
  1.3.4 Mechanisms Involved In Phytoremediation .................................. 12
    1.3.4.1 Phytochelatins ................................................................. 12
    1.3.4.2 Root Uptake Mechanism ..................................................... 15
    1.3.4.3 Metal Solubilising Mechanism ............................................ 16
    1.3.4.4 Transport Within Plants .................................................... 18
    1.3.4.5 Accumulation ................................................................. 18
    1.3.4.6 Heavy Metal Tolerance ...................................................... 19

1.4 UTILISATION OF AUSTRALIAN PLANTS FOR PHYTOREMEDIATION ....... 23
  1.4.1 Current Hyperaccumulators ...................................................... 23
  1.4.2 Australian Plants .................................................................... 23
    1.4.2.1 The Eucalypts .................................................................. 25
      1.4.2.1.1 Eucalyptus globulus .................................................. 25
      1.4.2.1.2 Eucalyptus camalidulensis ......................................... 25
      1.4.2.1.3 Eucalyptus lesouefii .................................................. 26
    1.4.2.2 The Casuarinas ................................................................. 26
1.4.2.1 Casuarina equisetifolia ........................................ 26
1.4.2.2 Casuarina obesa ............................................. 27
1.4.2.3 Allocasuarina verticillata .................................. 27
1.4.2.3.1 A. saligna, A. heteroclita and A. quadrimarginata ........ 28

1.5 AIMS ........................................................................ 28
1.6 HYPOTHESES ............................................................ 28

CHAPTER 2 HYDROPONIC EXPERIMENTS ........................................ 30

2.1 INTRODUCTION ................................................................ 30

2.2 MATERIALS AND METHODS ............................................ 32

2.2.1 Plant Material and Maintenance .................................. 32
2.2.2 Pre-Experiment Set Up ............................................. 33
2.2.3 Hydroponic Set Up .................................................. 35
2.2.4 Hydroponic Lead Experiment ...................................... 36
2.2.5 Harvesting Of Plant Material ...................................... 36
2.2.6 Fresh And Dry Weight Analysis ................................. 36
2.2.7 Lead Analysis Of Plant Material ................................. 37
2.2.8 Statistical Analysis .................................................. 37
2.2.9 Data Analysis .......................................................... 38

2.3 RESULTS ........................................................................ 39

2.3.1 Lead Solutions ............................................................. 39
2.3.2 Acacia Experiment ....................................................... 39
2.3.3 Casuarina Experiment ............................................... 43
2.3.4 Eucalyptus Experiment ............................................. 47

2.4 DISCUSSION ................................................................... 51

2.4.1 Acacia Experiment ....................................................... 53
2.4.2 Casuarina Experiment ............................................... 53
2.4.3 Eucalyptus Experiment ............................................. 54

CHAPTER 3 SOIL EXPERIMENTS .................................................. 57

3.1 INTRODUCTION .................................................................. 57

3.2 MATERIALS AND METHODS ............................................... 58

3.2.1 Soil Preparation .......................................................... 58
3.2.2 Plant Material and Maintenance .................................. 58
3.2.3 Soil Experiment Set Up .............................................. 59
3.2.4 Soil Lead Experiment ............................................... 59
3.2.5 Harvesting Of Plant Material ...................................... 60
3.2.6 Fresh And Dry Weight And Lead Analysis ........................................60
3.2.7 Lead Analysis ................................................................................60
3.2.8 Data Analysis ................................................................................60

3.3 RESULTS ........................................................................................................61
3.3.1 Acacia Experiment ................................................................................61
3.3.2 Eucalyptus Experiment ........................................................................65

3.4 DISCUSSION ....................................................................................................69
3.4.1 Acacia Experiment ................................................................................69
3.4.2 Eucalyptus Experiment ............................................................................70

CHAPTER 4 GENERAL DISCUSSION ...................................................................72

REFERENCES .....................................................................................................77
LIST OF FIGURES AND TABLES

Figure 1.1. Pb ions being taken up into the plant through the aid of the three components of phytoremediation. Namely, phytoextraction, phytostabilisation and rhizofiltration. (Adapted from Salt et al. 1995)

Figure 2.2. Chemical structure of the phytochelatin molecule isolated from higher plants (Adapted from Zenk, 1996).

Figure 3.3. Cadmium ions entering the cell and actively being taken up into the vacuole. (Adapted from Zenk, 1996)

Table 2.1. Composition of nutrient components

Figure 2.2 Hydroponic set up where nine seedlings of each species were subjected to different lead concentration treatments for a period of two weeks.
Figure 2.3. Lead content in roots and shoots of a) *A. heteroclita*, b) *A. quadrimarginea* and c) *A. saligna*, grown in 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated from 9 replicates and the vertical bars represent standard error.

Figure 2.4. Lead content in shoots of *A. heteroclita, A. quadrimarginea* and *A. saligna*, grown in 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated from 9 replicates and the vertical bars represent standard error.

Figure 2.5. Mean accumulation of Pb ions in the leaves, stems and roots of a) *Casuarina obesa* and shoots and roots of b) *Allocasuarina verticillata* under three hydroponic treatments of Pb(NO₃)₂. The means were calculated from 9 replicates and the vertical bars represent standard error.

Figure 2.6. Lead content in the stems of *C. obesa* and shoots of *Allocasuarina verticillata* grown in 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 9 replicates and the vertical bars represent standard error.
Figure 2.7. Lead content in roots, stems and leaves of a) *E. lesouefii*, b) *E. globulus* and c) *E. camaldulensis*, grown in 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated from 9 replicates and the vertical bars represent standard error.

Figure 2.8. Lead content in leaves of *E. lesouefii*, *E. globulus* and *E. camaldulensis*, grown in 0 μM, 50 μM and 100 μM lead nitrate solution. Means. The means were calculated over 9 replicates and the vertical bars represent standard errors.

Figure 2.9. Lead content in stems of *E. lesouefii*, *E. globulus* and *E. camaldulensis*, grown in 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated from 9 replicates and the vertical bars represent standard error.

Figure 3.1. Lead content in roots and shoots of a) *A. heteroclita*, b) *A. quadrimarginea* and c) *A. saligna*, grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and vertical bars represent the error bars.

Figure 3.2. Lead content in leaves of *A. heteroclita*, *A. quadrimarginea* and *A. saligna*, grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and the vertical bars represent standard error.
Figure 3.3. Lead content in stems of *A. heteroclita*, *A. quadrimarginea* and *A. saligna*, grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and the vertical bars represent standard error.

Figure 3.4. Lead content in roots, stems and leaves of a) *E. globulus* and b) *E. camaldulensis*, grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and vertical bars represent the error bars.

Figure 3.5. Lead content in leaves of *E. globulus* and *E. camaldulensis* grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and the vertical bars represent standard error.

Figure 3.6. Lead content in stems of *E. globulus* and *E. camaldulensis* grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and the vertical bars represent standard error.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.P.A.</td>
<td>Environmental Protection Authority</td>
</tr>
<tr>
<td>EXAF</td>
<td>extended X-ray absorption fine structure</td>
</tr>
<tr>
<td>F.A.A.S.</td>
<td>flame atomic absorption spectrophotometry</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene dianinotetracetic acid</td>
</tr>
<tr>
<td>glu</td>
<td>Glutamic acid</td>
</tr>
<tr>
<td>cys</td>
<td>Cysteine</td>
</tr>
<tr>
<td>PC</td>
<td>Phytochelatin-Complex</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>NH$_3^-$</td>
<td>amino group</td>
</tr>
<tr>
<td>COO-$^-$</td>
<td>carboxyl group</td>
</tr>
<tr>
<td>Cd</td>
<td>cadmium</td>
</tr>
<tr>
<td>S</td>
<td>sulphur</td>
</tr>
<tr>
<td>Cu</td>
<td>copper</td>
</tr>
<tr>
<td>Zn</td>
<td>zinc</td>
</tr>
<tr>
<td>Ca</td>
<td>calcium</td>
</tr>
<tr>
<td>Ni</td>
<td>nickel</td>
</tr>
<tr>
<td>Pb</td>
<td>lead</td>
</tr>
<tr>
<td>Fe</td>
<td>iron</td>
</tr>
<tr>
<td>Mn</td>
<td>manganese</td>
</tr>
<tr>
<td>Mg</td>
<td>magnesium</td>
</tr>
<tr>
<td>Al</td>
<td>aluminium</td>
</tr>
<tr>
<td>Cr</td>
<td>chromium</td>
</tr>
<tr>
<td>Hg</td>
<td>mercury</td>
</tr>
<tr>
<td>Element</td>
<td>Substance</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>Mo</td>
<td>molybdenum</td>
</tr>
<tr>
<td>B</td>
<td>boron</td>
</tr>
<tr>
<td>Si</td>
<td>silica</td>
</tr>
<tr>
<td>Sn</td>
<td>tin</td>
</tr>
<tr>
<td>Au</td>
<td>gold</td>
</tr>
<tr>
<td>Ag</td>
<td>silver</td>
</tr>
<tr>
<td>As</td>
<td>arsenic</td>
</tr>
<tr>
<td>Bi</td>
<td>bismuth</td>
</tr>
<tr>
<td>Cl</td>
<td>chloride</td>
</tr>
<tr>
<td>NO$_3$</td>
<td>nitrate</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

1.1 GLOBAL PROBLEM

Contamination of soils with industrial and domestic pollutants is a major problem throughout the world. This can occur in a number of different ways, including contamination from a range of organic toxins, and inorganic wastes such as heavy metals. Natural occurrences of mineral outcrops, or ore bodies, and industrial activities, can result in high concentrations of heavy metals in the soil (Baker et al., 1994 b). Toxic metal contamination of soils, streams and groundwater is a major environmental and human health problem in need of affordable and effective solutions (Salt et al., 1995 a).

Two particularly important heavy metal contaminants are cadmium and lead. Cadmium pollution of the biosphere has accelerated dramatically since the beginning of the industrial revolution, and its accumulation in soil and water now poses a major environmental and health problem (Salt et al., 1995 b). Cadmium is potentially toxic if accumulated in the human body. Renal dysfunction and pulmonary emphysema, and possibly bone demineralisation, are diseases associated with low level exposure to cadmium from dietary intake (Salt et al., 1995 b).

Lead compounds have been used in paint manufacture, gasoline, explosives and radioactive container linings. Mining and smelting, and the disposal of municipal sewage sludge enriched in lead has contributed to contamination of the environment (Huang & Cunningham, 1996). Lead contaminated soil has caused a variety of
environmental problems including loss of vegetation, groundwater contamination and lead toxicity in plants and animals, including humans (Huang & Cunningham, 1996).

Careful management is necessary to sustain soil resources (Martin & Allenby, 1989). For example, land degradation problems have emerged in Australia in the 200 years of European settlement. These rival the problems of many developing countries such as India, Pakistan, and Bangladesh (Martin & Allenby, 1989). In 1995 the Environmental Protection Authority (E.P.A.) of Western Australia reported 1,500 sites, which have been used as dumping grounds for a range of pollutants. The majority are the result of unsatisfactory industrial procedures for the removal or storage of chemicals and for the containment and disposal of wastes (E.P.A., 1995). Of these sites, the Department of Minerals and Energy has identified 1,100 on the Swan Coastal Plain as having the potential to contaminate the groundwater (E.P.A., 1995). The state of Western Australia relies heavily on its supply of groundwater for domestic use, and for supplementing natural resources in industries such as agriculture and fisheries (E.P.A., 1995).

In addition to posing a threat to public health and the environment, site contamination has led to: delays in the development of contaminated areas, the requirement for multi-million dollar clean-ups, and legal implications concerning liabilities (E.P.A., 1995).
1.2 CLEAN-UP OF HEAVY METAL CONTAMINATED SOILS

1.2.1 Conventional Methods

Decontamination of soils polluted with heavy metals remains one of the most intractable problems of clean-up technology (Baker et al., 1994). The remediation of lead contaminated soils represents a significant expense to many industries and government agencies (Huang & Cunningham, 1996). Lead contaminated sites have been remediated through a relatively narrow range of engineering based technologies (Huang & Cunningham, 1996). Techniques currently in use are based on thermal, isolation and containment, or decontamination procedures. These methods include incineration, landfill construction, and physico-chemical technologies. They vary in their effectiveness, cost and environmental impact. More recently, bioremediation, in particular the use of plants (phytoremediation) has been used as an alternative method for heavy metal clean-up.

1.2.2 Incineration

Incineration enables the handling of many types, or combinations of wastes through the judicious selection of specific refuse treatment designs, as well as combustion operating, and gas cleaning devices (Miller, 1992). Incineration reduces the volume of waste going to landfill by almost 60% (Miller, 1992). Two classes of incinerators exist. These are the 'mass-burn' and the 'trash-to energy' types. Most incinerators belong to the former class, and burn mixed waste without separating out hazardous material (Miller, 1992). The latter type is utilised for a more selective waste management program, where wastes suitable as fuel are sorted from the rest (Miller, 1992).
1.2.3 Landfill

Landfill entails the engineering of a specific area for the disposal of both hazardous and non-hazardous wastes. Landfill sites are normally chosen on the presumptions that the area has little or no economic value, and is geographically stable (Knight, 1985). The chosen site then has waterproof sheeting wrapped round the edges before it is filled. This prevents liquids moving out of the site. The bottom is lined with a solid backing such as limestone prior to dumping the waste. The waste is then covered either by topsoil, or with concrete if there are no future intentions for the site (Health Department of Western Australia, 1993). Heavily contaminated soils are usually excavated, stabilised by containment in cement, and then placed in secured landfill (Health Department of Western Australia, 1993).

1.2.4 Physico-chemical Treatments

The third method is physico-chemical treatment. Wastes subjected to this process undergo decomposition via chemical treatments (Miller, 1992; Salt et al., 1995 a). These techniques are precise enough to target specific contaminants within the treated media. Examples include acid leaching and electro-osmosis, or immobilisation in situ via vitrification (for radionuclides) (Baker, et al., 1994 a; Cunningham et al., 1996).

1.2.5 Shortfalls Of Conventional Methods

These techniques of disposal contain many limitations. Incineration exchanges one form of pollution for two. Smoke, containing a mixture of gases, rises into the atmosphere, and a residue of metal remains to be carted away, probably to a landfill (Harris & Dolan, 1992).
Despite technological advances in air pollution control devices, incinerators emit small amounts of highly toxic particles of lead, cadmium and mercury into the atmosphere (Miller, 1992). The residue has an increased toxicity, with a higher potential to leach into the groundwater than the initial bulky material that might have been placed into landfill (Miller, 1992).

Landfills are only containment techniques, hence do not really remove the contamination from the soil, and they deteriorate with age (Cunningham et al., 1996). Contaminated leachates have been found to seep out of old or abandoned sites due to liner failures (Miller, 1992). These methods can bring about more damage to the environment through the dispersal of the pollutants.

Physico-chemical treatments utilise specific technologies to remove the unwanted pollutants from the medium. These techniques strip all biological activity from the treated medium and adversely affect its physical structure (Baker et al., 1994 b; Cunningham, et al. 1996). Accordingly, it is not cost effective to utilise such processes for the decontamination of small areas of land where the benefits of potential land use or development are outweighed by the cost of this treatment (Baker et al., 1994).

With the high cost of conventional clean up technology, it is not surprising that the clean up of contaminated sites has not proceeded at a rapid pace (Cunningham & Ow, 1996). The cost of maintaining incinerators and landfill sites are comparatively high. Physico-chemical treatments such as those in ‘super-critical water’ or ‘dechlorination’
plants require specialised equipment and operations. These systems involve the use of specific apparatus that is expensive to construct, monitor and maintain (Cunningham & Berti, 1993). As a result, this option has not played a major role in the disposal of wastes, except where they have been extremely hazardous (Mohammed et al., 1996).

The limitations of conventional methods have prompted the search for more cost-effective approaches that do not damage our surroundings in the processes. Bioremediation is one such approach. It includes strategies such as; the use of biofilters and bioscrubbers to remove inorganic materials from residual-gases and waste in industrial processes (Dixon, 1996). The term bioremediation is used to describe the manipulation of living systems to derive a desired physical and chemical change within a confined environment (Cacciatore & McNeil, 1995). According to Karl Engesser of the University of Stuttgart in Germany, biological methods are the most cost-effective approaches of cleaning waste (Dixon, 1996). In situ bioremediation of contaminated soils has been used where extraction costs would be excessive, or for sites with restricted access to current methods (Cacciatore & McNeil, 1995). One such technology, which utilises plants, is phytoremediation.

1.3 PHYTOREMEDIATION

1.3.1 A Novel Strategy For Waste Removal From The Environment

Phytoremediation is a new field of technology that has received considerable research and development over recent years. This process utilises specific plants for on-site treatment of soils contaminated with toxic heavy metals, or organic and nutrient contaminants (Salt et al., 1995).
All plants possess the ability to accumulate metals from the soil which are essential for their growth and development. Such metals include iron, manganese, zinc, copper, magnesium, molybdenum, and perhaps nickel. However, if not for the discovery of the existence of a small group of plants called hyperaccumulators, it would have been presumed unlikely that plants could contain more than 2% metal content within their tissues (Cunningham & Bertó, 1993).

This small selection of plants, many of which are endemic to metalliferous soils, have shown the capacity to hyperaccumulate essential and non-essential (even potentially phytotoxic) metals, which have no known biological function. Metals such as zinc (Zn), nickel (Ni), chromium (Cr), cadmium (Cd), lead (Pb), copper (Cu), cobalt (Co), mercury (Hg) and silver (Ag) have been shown to accumulate in the tissue of these plants (Baker, et al., 1994a).

Phytoremediation of heavy metals encompasses three components (Fig. 1). Firstly, phytoextraction is the process whereby metal-accumulating plants transport and concentrate metals from the soil into harvestable parts of roots and above ground shoots. Secondly, rhizofiltration involves the absorption, precipitation and concentration of toxic metals from polluted effluent by the roots. Finally, phytostabilisation is the reduction of heavy metal ion mobility in soils by heavy metal tolerant plants, preventing the leaching of these contaminants into groundwater or from the dispersal of such particles through the air by wind erosion (Salt et al., 1995).
A plant suitable for phytoremediation may use any combination of these three methods. In proposed phytoextraction processes, several sequential crops of hyperaccumulating plants could be used to reduce the concentration of heavy metals in the soil to environmentally acceptable levels (Cunningham & Berti, 1993). Several studies have examined the possibility of utilising these hyperaccumulators as a low cost approach to phytoremediate contaminated land. Field trials conducted in the Mariupol and Chernobyl regions of the Ukraine and the Pennine region of England have used *B. juncea* to extract Pb, Cr, Cd and Ni ions from the soil (Salt *et al.* 1995). Cropping with metal-accumulating plants could serve as a cheap, visually unobtrusive, and practical solution for the removal and recovery of metals from superficially contaminated soils. By harvesting the dry matter, the heavy metals of contaminated agricultural soil can be reduced without disabling the biological activity and physical structure of the soil (Huang & Cunningham, 1996).

Companies such as Dupont, Phytotech and Phytokinetics are currently investigating the possibility of using additional techniques to increase the efficiency of phytoremediation. In particular, soil amendments and agronomic practices can contribute to its success. Cunningham & Ow (1996) have shown that the most successful amendments include the addition of chelates such ethylene diaminetetracetic acid (EDTA) and hydroxyethylethylene diaminetriacetic acid. These chelates increase the soil solution levels of some heavy metal ions, such as Pb, by almost one thousandfold. They also alter the root/shoot partitioning in most crop plants for these metals (Cunningham & Ow, 1996). Researchers at Dupont are also looking at electrokinetics (movement of soil ions under a direct current), which speeds up the migration of relatively immobile ions.
technique combined with the chelation process has increased phytoextraction of metal ions (Cunningham & Ow, 1996).

Fig. 1.1 The diagram demonstrates Pb ions being taken up into the plant through the aid of the three components of phytoremediation. Namely, phytoextraction, phytostabilisation and rhizofiltration. (Adapted from Salt et al. 1995)
1.3.2 Current Application

Plants with the capacity to tolerate and accumulate heavy metals are uncommon, but have been found in a wide range of taxonomic groups (Cunningham & Ow, 1996). These plants often occur in heavily impacted regions of industrial, mining and urban activities (Cunningham & Ow, 1996).

In Europe, there are two genera of plants, *Alyssum* and *Thlaspi*, within the Brassicaceae family, which have been shown to accumulate heavy metal ions. For example, *Alyssum* species are found on serpentine soils in southern Europe, which are rich in nickel and chromium. *Thlaspi* species found in calamine soils have been shown to accumulate zinc, cadmium and lead to more than 3%, 0.1% and 0.8% dry weight respectively (Baker *et al.*, 1994 a). Hyperaccumulators of zinc, nickel, copper and cobalt have also been found in the tropics and the sub-tropics (Baker *et al.*, 1994 b). These species can accumulate nickel in excess of 2% in dry-weight.

To date, metal-accumulating plants which have been identified are either slow growing, small, and/or weedy plants that produce low biomass. They also have uncertain growth requirements and characteristics (Kumar *et al.*, 1995). Nevertheless, these ‘wild metal-accumulators’ have been useful models for studying the characteristics and mechanisms used by hyperaccumulators. Using *Brassica juncea* and *Thlaspi caerulescens*, the mechanisms involved in the accumulation and mobility of heavy metals, such as cadmium, have been determined. The suggested mechanism is a detoxification system, which transfers the toxic metal ions into the trichomes in the leaves (Salt *et al.*, 1995). This experiment, and other projects conducted with *Brassica* and *Thlaspi* species, have
provided considerable information on the mechanisms and components involved in heavy metal accumulation.

1.3.3 Properties Of Heavy Metal Hyperaccumulators

Recently, the concept of using plants to remediate heavy metal contaminated soil has received increasing attention (Cunningham & Berti 1993; Baker et al., 1994). The hyperaccumulation of heavy metals incorporates two strategies, phytostabilisation and phytoextraction (Huang & Cunningham, 1996; Cunningham & Berti 1993; Salt et al., 1995 a). This combination results in the removal of toxic heavy metal contaminants from soils. There are some essential characteristics required of hyperaccumulating plants.

Ideally, these hyperaccumulators of heavy metals should not only contain a strong metal-accumulating genotype, to sequester heavy metals and withstand high metal toxicity, but also an extensive root system and a high biomass (Kumar et al., 1995). These plants should also be fast growing and respond rapidly to nutrient supplements, thereby accomplishing their purpose more quickly (Baker et al., 1994 b).

A deep and extensive root system would provide the plant with the capability to affect a larger surface area (Baker et al., 1994). Plants with high biomass would increase the potential for accumulating and storing a higher concentration of heavy metal ions within its tissues (Salt et al., 1995). This high biomass can be located within the shoots, leaves or the roots or a combination of these. Experiments carried out by Salt et al. (1995) have shown that Indian mustard (B. juncea L.) has a high biomass and utilises it to retain
cadmium in both its root and shoot tissue. Another successful hyperaccumulator, *Alyssum tenium*, has, under experimental conditions, accumulated sequestered nickel into its aboveground biomass (Baker *et al.* 1994). As mentioned, *T. caerulescens* is capable of taking up high levels of cadmium into its shoots from soil and hydroponic solutions. However, the low biomass of this species limits the potential of the plant to be a successful heavy metal hyperaccumulator (Salt *et al.* 1995).

Other economic or productive qualities might also help to foster the choice of the plant to be utilised. These essentially provide a post-remediation use for the plant, such as wood pulp for paper, timber, or used as charcoal, after which the respective accumulated metal can be collected.

### 1.3.4 Mechanisms Involved In Phytoremediation

There are several key mechanisms pertaining to the survival of plants in heavy metal laden soil. These include the biological uptake, translocation, accumulation, and tolerance of heavy metals within the plant tissue (Salt *et al.* 1995). Phytochelatin molecules have been shown to play an important part in these processes and are the principal components required for plants to sustain themselves in such conditions.

#### 1.3.4.1 Phytochelatins

Metallothioneins are polypeptides that act as antioxidants in animals, contributing to the sequestering of excess metal ions within the body (Robinson *et al.*, 1993). Genes that encode proteins with some sequence similarity to metallothioneins have been isolated from several plants. The structure of the main peptide was elucidated as \((\text{NH}_3)^+\gamma\text{-glu-}\).
cys-γ-glu-cys-γ-glu-cys-glu-COO' by the process of Edman degradation (Fig. 1.2). Solid phase synthesis and classical solution synthesis with maximum side-chain protection strategy also verified this (Zenk, 1996). These molecules have been referred to as phytochelatins. Phytochelatins, the primary metal-binding polypeptides of plants, and phytochelatin synthase, the enzyme catalysing their biosynthesis, constitute the primary molecule involved in sequestering metal ions in plants (Steffens, 1990).

![Chemical structure of the phytochelatin molecule isolated from higher plants](Adapted from Zenk, 1996).

Phytochelatin formation in response to heavy metal ions within the plant is one of the few adaptive stress responses seen in plants (Steffens, 1990). However, unlike other plant stress responses elicited by a number of inducers, phytochelatin biosynthesis is tightly regulated by the availability of free metal ions which act as primary inducers (Steffens 1990; Robinson et al., 1993). In an experiment to show this, cell cultures inoculated onto normal tissue culture media underwent a transient induction and build up of phytochelatins that ceased when free Cu and Zn ions were depleted (Zenk, 1996).
Steffens (1990) and Zenk (1996) also showed that after the addition of EDTA or metal-free phytochelatins in vivo, the synthesis of phytochelatins ceased immediately. These experiments have led to the suggestion that metal ions activate the phytochelatin synthase enzyme in a feedback loop. The presence of sufficiently synthesised phytochelatins to complex the metal ions ceases the synthesis activity.

The synthesis of heavy metal-complexing phytochelatins has been considered an important metabolic activity in higher plants. According to Zenk (1996), and Robinson et al. (1993), the presence of toxic levels of metal ions in the biosphere seems unlikely to have exerted a selection pressure for the evolution of heavy metal detoxification. However, a phylogenetic survey on the occurrence of phytochelatins established that phytochelatins are invariably expressed as constituents of plant species exposed to heavy metals (Steffens, 1990). No other thiol-rich, heavy metal-binding constituents other than phytochelatins were detectable in the many plants assayed (Zenk, 1996).

Continued studies done on phytochelatins have elucidated the various roles played by these molecules. They play a role in various stages of essential metal ion maintenance and subsistence in the plants. Phytochelatins are involved in homeostasis of essential metals in plants, regulating the availability of metal ions in the plant cell (Zenk, 1996). Salt et al. (1995 b) showed that Cd ions in the soil led to the influx of S-ligand phytochelatin accumulation in the roots of B. juncea. The intracellular presence of Cd-phytochelatin-complex is convincing evidence of its role in transient accumulation and storage (Zenk, 1996). Phytochelatin-metal-complex formation also seems to be the
principal heavy metal tolerance mechanism in plant heavy metal accumulation (Baker et al., 1994; Zenk 1996).

1.3.4.2 Root Uptake Mechanism

Essential metal ions such as Cu\(^{2+}\) and Zn\(^{2+}\) have to enter the cell in order to become part of catalytic proteins or structural elements. The metal ions enter the roots of plants through the extracellular (apoplastic) pathway or intracellular (symplastic) pathway (Delhaize, 1996). In both cases most metal ions enter plant cells through energy dependent, saturable processes, which utilise either specialised or generic metal ion carriers. Specialised plasma membrane carriers provide specific metal-chelate complex mechanisms for transport across the plasma membrane (Salt et al. 1995 b). Generic carriers also exist, allowing both essential and non-essential metals to be transported. This effectively allows non-essential cations to compete for the same transmembrane carriers (Salt et al. 1995 b; Kumar et al. 1995). This could be a route for the transport of non-essential metal ion uptake into plants.

According to Salt et al. (1995) kinetic data has produced examples where essential heavy metals such as Cu\(^{2+}\) and Zn\(^{2+}\), and non-essential heavy metals such as Ni\(^{2+}\) and Cd\(^{2+}\) compete for the same transmembrane carriers. The lack of selectivity in the transmembrane ion transport system of the root cells also allows metals to enter against a concentration gradient (Salt, et al., 1995 b; Baker, et al., 1994 b). Huang and Cunningham (1996) conducted experiments, using voltage-gated Ca\(^{2+}\) channels isolated from the plasma membrane vesicles, in root-cell plasma membrane of wheat and corn to observe this phenomenon. In the uptake of metal ions from the soil, it was observed that
Pb$^{2+}$ was also transported into the root cells across the root cell plasma membrane. Pb$^{2+}$ significantly inhibited the voltage-gated Ca$^{2+}$ channel activity in the plasma membrane of wheat roots (Huang and Cunningham, 1996). Hence they deduced that one possible pathway was the utilisation of plasma membrane cation channels such as Ca$^{2+}$ channels. The inhibition of the Ca$^{2+}$ channels by Pb$^{2+}$ could arise from Pb$^{2+}$ ions blocking the channel, or the competitive transport of Pb$^{2+}$ ions through the Ca$^{2+}$ channels (Huang and Cunningham, 1996). Experiments using Fura-2 to monitor Pb$^{2+}$ entry into isolated bovine chromatin cells detected the permeation of Pb$^{2+}$ ion through Ca$^{2+}$ channels providing evidence for the latter action.

1.3.4.3 Metal Solubilising Mechanism

An important aspect in the uptake of such cations is the solubilisation of these metal ions. This is achieved by formation of soluble polypeptide complexes. Plants take up 'soil-bound' metal ions through the mobilisation of these cations by first forming solutions ('soil solutions') with them, which is achieved in a number of ways (Salt, et al., 1995). Phytosiderophores are metal-chelating molecules, secreted into the rhizosphere, that solubilise soil-bound metals. Some examples of phytosiderophores are mugineic and avenic acids of graminaceous plant species, which are effectively released in response to Fe and Zn ion deficiency (Salt, et al., 1995 b). Some plants also release plasma membrane-bound metal reductase from their roots. This reduces certain metal ions attached to the soil, as observed by experiments conducted with pea plants deficient in Fe or Cu ions. Here an increased ability to reduce Fe(III) and Cu(II) in the soil was demonstrated (Delhaize, 1996). The roots of some plants exude protons to acidify the soil environment, increasing the solubility of heavy metals in the soil, thus allowing a
higher release of soil-bound metal ions into soil solutions (Salt, et al., 1995 b). Fe-deficient dicotyledonous plants use similar mechanisms for Fe ion mobilisation (Delhaize, 1996). However, researchers have also observed non-specific uptake of metal ions such as Mn, Fe, Mg, Cd and Cu. Kumar, et al. (1995), showed in a hydroponics experiment that B. juncea increased uptake of Fe and Cu ions, when nutritionally stressed. This led to the increase in uptake of other metals such as Pb\textsuperscript{2+}. This suggests that non-essential metals and essential metallic elements share some uptake mechanisms.

Short term exposure to low concentrations of cadmium has revealed the use of organic acids and cell wall carriers in cadmium binding. However, higher concentrations of cadmium resulted in the influx of S-ligand polypeptides accumulating in the roots. These molecules are involved in binding a significant amount of cadmium in the roots (Salt, et al., 1995 b). Studies into this occurrence have revealed the chelating function of phytochelatin proteins to be the predominant mechanism for non-specific metal uptake into the roots (Salt, et al., 1995 b; Zenk, 1996; Steffens, 1990; Kumar, et al., 1995). B. juncea, upon exposure to Cd laden soil, secretes phytochelatins to form complexes which are then accumulated into the roots (Salt, et al., 1995 b). Baker et al. (1994 b) has established that wide variations occur in the extent to which the accumulated metal ions are transported from the root system to the shoot with respect to Al, Cr, Cu, Fe, Pb, Zn, Ni, Co, Mn, Hg, Mo and Cd.
1.3.4.4 Transport Within Plants

Metal ions that have entered the roots can either be stored, or transported to the shoots. They are exported into the shoot through the symplastic pathway into the xylem tissue. Xylem cell walls have a high cation exchange capacity which could retard the metal ion movement. Consequently, the availability of appropriate metal-chelate-complexes facilitates the movement of the metal ions through the transpiration stream (Salt, et al., 1995b). The presence of oxygen atoms supports the association of organic acids with metal ions, playing a role in the translocation in both the xylem and the phloem. One such example is the chelation of translocated Cd with oxygen atoms in B. juncea. Experiments also show the involvement of phytochelatins binding to metal ions in xylem and phloem sap. This non-specific transport of heavy metal ions through the phloem can be observed through the use of nicotianamine, which is essentially an Fe transporter, but has the ability to bind with and transport Zn, Co, Ni and Cu ions (Salt et al., 1995b).

1.3.4.5 Accumulation

The mass flow of transpiration-driven transport can result in the accumulation of several ions such as Ca, B, Si and Cl into various parts of a plant (Salt, et al., 1995b). The key molecule binding to non-essential heavy metal ions is a phytochelatin polypeptide. The presence of these ions will immediately activate the latent, fundamental phytochelatin-synthesis enzyme, synthesising phytochelatin molecules of varying lengths to chelate metal ions. The formation of stable metal-phytochelatin complexes helps to protect sensitive metabolic enzymes. Eventually these complexes are transported from the cytosol to the vacuoles where they accumulate (Zenk, 1996). The vacuole is most likely
the ultimate storage compartment for these heavy metal ions (Zenk, 1996). In cell cultures, the rapid accumulation of phytochelatins occurred as a result of the introduction of free copper and zinc. This continued until the medium became depleted of these ions (Zenk, 1996). Upon entering the cell vacuole, the acidic conditions of the vacuole cause the metals to be liberated from the phytochelatin complexes. The metal-free phytochelatin molecules are subsequently degraded and the individual amino acids re-enter the cytosol. Metal ions then bind to organic acids found within the vacuole (Zenk, 1996).

This is a likely mechanism by which the plant salvages the valuable reduced sulphur present in cysteine molecules of phytochelatins (Zenk, 1996). This system also permits plants not only accumulate to metals from high soil background concentrations typical of their native metalliferous soils, but also from low soil concentrations (Baker et al., 1994 b).

1.3.4.6 Heavy Metal Tolerance

It is unlikely that the evolution of heavy metal-resistant, biochemical, metabolic processes could occur as viable heavy metal resistance mechanisms, because once the heavy metals accumulate within the plant tissue, their presence would still require detoxification. Hence, according to Salt et al., (1995), the only strategy that phytoremediating plants can use to alleviate the effects of accumulated heavy metal ions is to detoxify them.
The detoxification of heavy metals within the plant tissue can occur through various processes, according to the heavy metal accumulated. The pathways and enzymes available to the plant also vary, depending on the plant. Isolated intact vacuoles from tobacco and barley exposed to Zn ions, have been shown to contain high levels of this metal (Zenk, 1996). Here, Zn may chelate to organic acids and accumulate within the vacuole. The roots of *T. caerulescens* have also been shown to exhibit this mechanism. This process of detoxification has also been supported by reports of Zn exposure causing increasing vacuolar volume fraction of meristematic cells, within the root tip of *Festuca rubra* (Salt *et al.* 1995). These pathways also provide plants with efficient mechanisms to obtain essential elements for metabolic functions.

The homeostatic function of phytochelatin can be seen by the induction of these peptides with the inoculation of cell cultures in fresh nutrient medium (Zenk, 1996). Phytochelatin production is the simplest natural compound in plants engaged in the homeostasis and detoxification of metals through metal-thiolate formation. This polypeptide plays a dual role, in being able to complex toxic metal ions, tightly binding them in the vacuole, and transferring the essential metal ions to newly synthesised apoenzymes that require metals such as Cu²⁺ or Zn²⁺ for catalytic activity, or to nucleic acid structures known as zinc fingers.

Phytochelatins function as the key mode of detoxification of heavy metal uptake (Zenk, 1996). Plants resist the toxic effects of heavy metals by detoxifying the accumulated metals ions. To protect themselves from heavy metal poisoning, most plant cells have developed a mechanism to complex and inactivate metal ions entering the cytosol of the
cell, preventing any catalytic or structural protein inactivation (Fig. 1.3). Using cell suspension cultures of higher plants, Zenk (1996) has provided evidence for this mechanism. The presence of the heavy metal ions shifts the reaction between active and inactive phytochelatin-synthase in the forward direction, preventing the release of toxic metals by forming stable metal-phytochelatin-complexes. Phytochelatin synthase enzymes facilitate the chelation of toxic metal ions entering the cytosol, preventing them from inhibiting the metabolic enzymes within the cell (Zenk, 1996). It is likely that the inorganic sulphide in the complexes stabilises the compound by increasing its capacity to bind to cadmium ions (Salt et al. 1995).

Fig. 1.3. Cadmium ions entering the cell activate the PC synthase that catalyses the formation of PC. Cd^{2+}-PC complex is actively taken up into the vacuole and is stored there while the PC peptide is degraded. (Adapted from Zenk, 1996)
In *B. juncea*, the family of thiol-rich phytochelatins have been found to chelate with Cd. These complexes, as well as Cd ions, have also been shown to be transported across the tonoplast and accumulated in the vacuoles (Salt, *et al.*, 1995). This information and recent extended X-ray absorption fine structure data show that Cd detoxification was achieved by the accumulation of Cd as Cd-phytochelatin complexes (Zenk, 1996).

Using non-serpentine and serpentine *Thlaspi goesingense* populations Baker, Reeves & Hajar (1994) provided evidence of a non-specific metal detoxification system in these closely related populations. Experiments conducted with *Rauvolfia serpentina* cell cultures have shown that other metals could also be sequestered by forming metal-binding complexes with the same phytochelatin molecule. However, these heavy metals demonstrated differing metal-complex binding strengths in the decreasing order of: Cd$^{2+}$ > Pb$^{2+}$ > Zn$^{2+}$ > Sb$^{3+}$ > Ag$^+$ > Hg$^{2+}$ > As$^{5+}$ > Cu$^+$ > Sn$^{2+}$ > Au$^{3+}$ > Bi$^{3+}$ (Zenk, 1996).

Such induced increases in the metal uptake can, however, result in toxicity symptoms and often death of plants not possessing inherent abilities to tolerate or detoxify the high levels of accumulated heavy metals (Salt, *et al.*, 1995). The growing knowledge on the mechanisms of heavy metal hyperaccumulation can provide a basis for genetic modification or selection of plant species for improved performances (Cunningham & Ow, 1996). Perhaps in the selection of plants adapted for remediation, another set of traits should also be considered. These characteristics would include the ability to tolerate contamination within the soil, the ability to detoxify the contaminants and accumulate the contaminants within its above ground structures (Stomp *et al.*, 1994).
1.4 UTILISATION OF AUSTRALIAN PLANTS FOR PHYTOREMEDIATION.

1.4.1 Current Hyperaccumulators

The list of plants that possess traits required for phytoremediation is limited, but growing. Currently acknowledged species have been discovered in a range of families including: Brassicaceae (e.g; *Thlaspi* sp., *Brassica juncea* and *Alyssum bertolonii*), Caryophyllaceae (e.g; *Silene cucubalus*), Plumbaginaceae (e.g; *Armeria maritima*), Asteraceae (e.g; *Viola calaminaria* and *Haumaniastrum katangense*) and several others. Most of the plants investigated so far have a herbaceous habit, and some work has been done with trees such as poplars (*Populus*). Experiments have been done with most of these plants to elucidate the mechanisms involved in metal uptake and accumulation.

1.4.2 Australian Plants

The decision to carry out phytoremediation on heavy metal contaminated soils in Australia might involve serious considerations of releasing new and foreign species into the Australian environment. If improperly managed, this might result in the increased degradation of the already fragile Australian vegetation through the invasion of these species (Scheltema & Harris, 1995). Thus, native Australian plants should be considered to prevent the impacts associated with the introduction of a foreign species.

Known hyperaccumulators of heavy metals tend to be characterised by a low biomass. This limits the amount of metal accumulated during the growing season. Trees, however, generally possess growth attributes desirable for successful remediation of contaminated soils. The root system penetrates further than most herbaceous plants and the large aboveground structures and leaf surface area creates a massive transpirational
stream (Stomp, et al., 1994). Experiments conducted with five year old hybrid poplar trees showed that these trees were capable of removing 20g of material per year, present in groundwater (Stomp, et al., 1994).

In recent years, a considerable amount of information has been collected regarding the interaction of plants with toxic compounds. Consequently, several species have been identified with desirable traits for phytoremediation. However, only field data can substantiate the application of a particular plant for phytoremediation. Therefore, similar field experiments should be conducted to ascertain the potential of other plant species to carry out similar functions.

The species chosen for this study are all trees and have been selected based on either their economic significance or because they occur naturally in areas known for their high metal content, such as serpentine soils. The experiments will help provide important information to the study of the selected families and their potential for treatment of heavy metal soils. In the process, this project hopes to extend the application of these Australian plants.

Most of these plants have also been used in land rehabilitation, and show variation in their tolerance to a range of environmental conditions. They include three species from the Myrtaceae family (Eucalyptus camaldulensis, E. lesoufii and E. globulus), three species from the Mimosaceae family (Acacia heteroclita, A. saligna and A. quadramarginea) and three species from the Casuarinaceae family (Casuarina obesa, C. equisetifolia and Allocasuarina verticillata).
1.4.2.1 The Eucalypts

There are over 500 species of eucalypts and all but three species are naturally confined to Australia (Eldridge et al., 1993). Several of these are widely propagated in plantations throughout the world. It is expected that eucalypt plantations by the year 2000, may exceed 10 million hectares. Generally they are grown for pulp production but other uses such as timber, charcoal, fuelwood and oil are also very important. Eucalypts have also been used for the revegetation of damaged landscapes and mine spoils (Farrell et al., 1996 a). This is because a number of these species have demonstrated tolerance to extreme conditions, such as variable pH, found in such regions (Chippendale, 1973). 

_Eucalyptus_ species also possess the ability to act as water pumps, restabilising waterlogged lands (Blake, 1981; Farrell et al. 1996 a).

1.4.2.1.1 _Eucalyptus globulus_

This mesophytic species was one of the first eucalypts to be planted outside Australia. _E. globulus_ can produce very high growth rates with excellent pulping qualities (De Little et al., 1992). These economic attributes mark _E. globulus_ as the species of choice for plantations both in Australia, and temperate regions overseas.

1.4.2.1.2 _Eucalyptus camaldulensis_

This species is also widely grown outside Australia. It produces quality wood for timber and coppicing (Eldridge et al., 1993). Its selection is based on its ability to tolerate the adverse conditions of semi-arid and arid lands, and drought or infertile conditions, as opposed to a rapid growth (Farrel, et al, 1996). Its leaves also produce high levels of cineole which might have important economic value (Eldridge, et al., 1993).
1.4.2.1.3 *Eucalyptus lesouefii*

*E. lesouefii* has a low coppicing quality, and is only used as firewood in localised areas in Australia (Chippendale, 1973). This species has been noted to grow on serpentine soils such as those of the Kambalda-Widgiemooltha goldfield area and mining sites where saltbushes (*Atriplex*) are also found (Brooks 1987; Chippendale 1973; Van Der Moezel *et al.*, 1989a). They also occur in sandy loam, showing resilience to saline and arid conditions, though they are adaptable to most other soils (Chippendale, 1973).

1.4.2.2 The *Casuarinas*

*Casuarina* species are naturally distributed throughout the equatorial and sub-equatorial regions of the world. Trees from this genus are used for fuel wood, land reclamation, dune stabilisation, and as shelter belts (Landquist & Torrey, 1984). Casuarinas are ideal candidates for the rehabilitation of marginal lands. The main qualities that favour the use of these species are their adaptation to adverse environments, including saline and nutrient-poor sites. (Sougoufara *et al.*, 1987).

1.4.2.2.1 *Casuarina equisetifolia*

This species occurs along seacoasts or brackish water streams. Despite slight provenance variations, this species is generally noted for its high propagation value, and has been used in the study of land rehabilitation (van der Moezel *et al.*, 1988). It has also been used for dune stabilisation and salt tolerance in subtropical and tropical regions (Sougoufara *et al.* 1987).
1.4.2.2 Casuarina obesa

*C. obesa* has a high survival rate in saline soils (Bell *et al.*, 1994 b). This is probably because the mechanisms involved in the tolerance of adverse soil conditions by *C. obesa* may be less energy-requiring than that of Australian trees such as eucalypts (van der Moezel, *et al.*, 1988).

1.4.2.3 Allocasuarina verticilatta

There is little information for this species as a potential rehabilitation plant. Experiments conducted have, however, suggested it may be useful for rehabilitation of highly saline soil (Dawson & Gibson, 1987).

1.4.3 The Acacias

*Acacia* species occur in semi-arid and arid regions of subtropical and tropical areas of Africa, Egypt, Australia and Asia, and are well adapted to such conditions (Nabil & Coudret, 1995). According to Alexander (1989), some *Acacia* species naturally colonise spoils of mounds created from open cast tin mining. Trials conducted with this species in Jos Plateau in Africa have shown that land rehabilitation with *Acacia* plantations clearly result in beneficial chemical and physical alterations in the soil composition (Alexander, 1989).
1.4.2.3.1  *A. saligna, A. heteroclita* and *A. quadrimarginea*

These plants play an important role in many revegetation programs throughout Western Australia. *A. quadrimarginea* occurs naturally on serpentine soils, and therefore might be heavy metal tolerant. *A. saligna* has been shown to be moderately salt tolerant and it is therefore considered useful for re-planting of saline soil (Brooks, 1987).

1.5  AIMS

This project aims to determine the potential of a range of Australian native plants that have economic and environmental uses, for phytoremediation. If these plants show a positive result to heavy metal hyperaccumulation, this project also aims to initiate research in utilising Australian native flora in the economical rehabilitation of heavy metal contaminated sites.

In the process, the experiments are designed to measure variation of the responses to growing in high lead concentrated media and ascertain the location of the accumulated heavy metals.

1.6  HYPOTHESES

1. Australian native plants have the potential to accumulate significantly high amounts of lead from lead contaminated media.

2. The selected plants can withstand the amount of lead accumulated into their tissues.
3. Hydroponic experiments are capable of providing information in the determination of potential phytoremediating plants.
2. HYDROPONIC EXPERIMENTS

2.1 INTRODUCTION

Several experimental models have been designed in an attempt to determine the potential of particular plants for phytoremediation. No particular model has shown the capacity to cover all the essential parameters to measure a plant’s potential in this area (Cunningham et al., 1996 b). Hence, to date, consistent efforts have provided several models emulating the processes of phytoremediation. Ideally, a soil medium should be chosen which would allow plants to be placed in conditions closest to those which potential phytoremediating plants might be used. However, the heterogenous nature of a soil medium prevents the comparison of plants under equal parameters such as contaminant concentrations and bioavailability, which are uncontrollable variables. Often such experiments only produce significant results after long periods of experimentation and extended periods of study (Cunningham et al. 1996 b).

An alternative experimental approach is to use hydroponics. Hydroponic experiments allow the process of phytoextraction to be more easily isolated from the other two processes within phytoremediation, namely, phytostabilisation and rhizofiltration. These studies help increase our knowledge on physiological characteristics and biological processes, of intracellular uptake, vacuolar deposition and translocation to the shoots involved in translocation and accumulation of heavy metals within plants, without any external interference (Huang & Cunningham, 1996). This in turn provides information on which part of the plant to harvest (Kumar et al., 1995).
Hydroponic experiments also enable external interferences that occur in the form of algae and fungi to be confronted and dealt with promptly and effectively. According to Huang and Cunningham (1996) this approach also acts as a protocol for rapid screening of potential phytoextracting plants. Hydroponic experiments have also been used by Salt et al. (1995) and Kumar et al. (1995).

Plants for these experiments were trees selected from three Australian families. They include three species from the Myrtaceae family (*Eucalyptus camaldulensis*, *E. lesouefii* and *E. globulus*), three species from the Mimosaceae family (*Acacia heteroclita*, *A. saligna* and *A. quadramarginea*) and three species from the Casuarinaceae family (*Casuarina obesa* and *Allocasuarina verticillata*). The chosen species from each family possess economic significance or naturally occur on soils with high heavy metal content. These plants have also been used in land rehabilitation projects.

In the following experiments, the lead phytoextraction efficiency of seedlings of these species was examined. Seedlings were subjected to different lead concentration treatments to compare the accumulated lead. Categorising these plants into three sections, namely leaves stems and roots, lead accumulated in these locations was determined. Observational signs of physiological stress were used as a measure of tolerance to the heavy metal accumulated.
2.2 MATERIALS AND METHODS

2.2.1 Plant Material and Maintenance

Seeds of *Eucalyptus camaldulensis*, *E. lesouefii*, *E. globulus*, *Acacia heteroclita*, *A. saligna*, *A. quadramarginea*, *Casuarina obesa*, *C. equisetifolia* and *Allocasuarina verticillata* were purchased from Kim Seed Co. These seeds were germinated in 50 mm diameter plastic (T4) pots of perlite. In each pot 4 seeds were planted in perlite. For each species, one tray of forty pots of seeds was germinated. This procedure was conducted once a month for a period of five months to obtain a series of staggered height growth for each species. The trays were lined and sprayed with Mancozeb fungicide to deter fungal infection.

Prior to planting the *Acacia* seeds, unscarified seeds from these species were soaked in boiling water. Seeds of the *C. equisetifolia* species were aerated in water, until the first signs of germination, before planting in the T4 pots. Plants were watered once daily and fertilised twice a week (1 g L$^{-1}$) with Thrive® (Yates and Co. Pty Ltd, NSW, Australia). An iron supplement, Librel Fe.Lo®, (Atlas Interlates, Lancashire, England) was also given to plants fortnightly.

Once the seedlings were approximately 5 cm in height, the pots were thinned to one plant per pot. From each species, healthy individuals of approximately 8 cm in height were selected for the hydroponics experiments.
2.2.2 Pre-Experiment Set Up

Prior to subjecting the plants to hydroponic experiments, seedlings of each species underwent a pre-experiment phase (Figure 2.1). During this stage, seedlings were adapted to a hydroponic medium. Seedlings for each species were immersed in aerated nutrient solution throughout this stage, to generate sufficient roots from each seedling.

![Figure 2.1 Pre-Hydroponic System](image)

Figure 2.1 Pre-Hydroponic System. Seedlings grown in T4 pots of perlite are encouraged to adapt to hydroponic conditions. The bases of the pots are immersed in aerated nutrient solution for a period of two weeks.

Three different nutrient solutions were trialed to determine a suitable medium to supply sufficient nutrients and that would not precipitate the added lead. The nutrient solutions chosen were taken from experiments conducted by Salt et al., (1995), Huang & Cunningham (1996) and Hoagland and Arnon (1950) (Table 2.1). Trials conducted with the modified Hoaglands solution, where the levels of phosphate and sulphate were
<table>
<thead>
<tr>
<th>Nutrient Solution Source</th>
<th>Components</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rorison’s Solution</td>
<td>In ppm:</td>
<td></td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Mg^{2+}</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>K^{+}</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>NO_{3}^{-}</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>PO_{4}^{3-}</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>SO_{4}^{2-}</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Fe-EDTA</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Huang &amp; Cunningham, 1996</td>
<td>in mM;</td>
<td></td>
</tr>
<tr>
<td>K (as KNO_{3}, KH_{2}PO_{4} &amp; KCl)</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Ca (as Ca(NO_{3})_{2}</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>MgSO_{4}</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>NH_{4} (as NH_{4}NO_{3})</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>NO_{3} (as NH_{4}NO_{3}, Ca(NO_{3})<em>{2} &amp; KNO</em>{3}</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>P (as KH_{2}PO_{4})</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>in μM;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl (as KCl)</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>B (as H_{3}BO_{3})</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Mn (as MnSO_{4})</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Zn (as ZnSO_{4})</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Cu (as CuSO_{4})</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Mo (as Na_{2}MoO_{4})</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Ni (as NiSO_{4})</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Fe-EDTA</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Hoagland &amp; Amon, 1950 (Modified)</td>
<td>in mg/L;</td>
<td></td>
</tr>
<tr>
<td>H_{3}BO_{3}</td>
<td>2.86</td>
<td></td>
</tr>
<tr>
<td>Ca(NO_{3})_{2}</td>
<td>656.4</td>
<td></td>
</tr>
<tr>
<td>CuSO_{4}.5H_{2}O</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Fe-EDTA</td>
<td>5.32</td>
<td></td>
</tr>
<tr>
<td>KNO_{3}</td>
<td>606.6</td>
<td></td>
</tr>
<tr>
<td>ZnSO_{4}.7H_{2}O</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Mg(NO_{3})_{2}</td>
<td>120.35</td>
<td></td>
</tr>
<tr>
<td>MnCl_{2}.4H_{2}O</td>
<td>1.81</td>
<td></td>
</tr>
<tr>
<td>MoO_{3}</td>
<td>0.016</td>
<td></td>
</tr>
</tbody>
</table>
minimised, allowed the lead ions to remain in solution. Once seedlings achieved an approximate root length of 8 cm, they were subjected to the hydroponic tests.

2.2.3 Hydroponic Set Up

The hydroponics design emulated that constructed by Huang & Cunningham (1996). The set up provided sufficient apparatus to conduct experiments on three species of one family simultaneously. This allowed observable differences to be compared between species throughout the experimental period. For each species, nine 4 L polyethylene buckets with lids were set up as in figure 2.2. In each lid, three equi-distant holes were bored to house three T4 pots containing seedlings. The modified Hoaglands nutrients solution (3 L) was added to the 4 L buckets, immersing the bases of the T4 pots. The nutrient solution was aerated by aeration stones connected to tubes fed through holes drilled through the lids. An air pump evenly distributed air into the buckets.

Fig. 2.2 Hydroponic set up. Nine seedlings of each species were subjected to different lead concentration treatments for a period of two weeks.
2.2.4 Hydroponic Lead Experiment

From the pre-experiment tub, 27 healthy, and comparatively even sized seedlings for each species, were immersed in the lead treatments. For each species, three buckets were used for each lead treatment, providing a total of 9 replicates of one species, in one lead concentration. The lead treatment solutions used were the modified Hoaglands solutions spiked with the appropriate amount of \( \text{Pb(NO}_3\text{)}_2 \) [0 \( \mu \text{M (control), 50 } \mu \text{M and 100 } \mu \text{M} \)]. During the two week experimentation period, the hydroponics nutrient medium was maintained at pH 5.5 - 6, using 2 M HCl (aq) and 2 M NaOH (aq). The level of nutrient solution was maintained by the addition of distilled water.

2.2.5 Harvesting Of Plant Material

After growing for two weeks in the hydroponic set up, the plants were harvested. Individual T4 pots were removed and the plants washed and stored in separate sections. Each plant was first washed in water to remove most of the perlite. The plants were then separated into roots, stems and leaves, and again washed in distilled water separately removing the remaining perlite. Two more successive washes in distilled water were performed to remove external lead, which might be contained in the rhizosphere. Using a sieve, detached roots belonging to the respective plants were also collected and washed. The individual plant sections were then stored in labelled paper bags.

2.2.6 Fresh And Dry Weight Analysis

The fresh weights of plant sections were recorded and replaced into their bags and stored in an oven for three days at 80 °C. Dry weights were then recorded.
2.2.7 Lead Analysis Of Plant Material

Dried plant material (200 – 400 mg) was frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. With larger sample sizes (> 0.4 g), an electric wiley mill was used. Accurately weighed dried plant material (200 mg) was transferred to digestion tubes and 5 ml of a nitric perchloric acid mixture (9:1) was added. The digestion tubes containing the plant material were left to stand for approximately 8 hours. The samples were subsequently heated in a block digester set at 160 °C in a fumehood until the volume fell to 0.5 ml or white fumes were emitted. On cooling, the digested samples were diluted to 10.0 ml with milli-Q (double deionised) water. The samples were analysed for lead using a Varian 20 flame atomic absorption spectrometer (F.A.A.S.). All apparatus used in the experiments was immersed in a 2 M HCl (aq) acid bath and then washed with Pyroneg™ to ensure the removal of any residual lead.

2.2.8 Statistical Analysis

The experimental designed allowed both one way and two way analysis of variance (ANOVA) to be conducted. Equal number of replicates for each species and treatment were utilised at the start of the experiment. Two way ANOVA were conducted to test for differences between species and effects of treatments. Where there was a difference due to either species or treatment, a one way ANOVA was performed to determine the differences between means.
2.2.9 Data Analysis

The concentrations of lead present in the 1 g dry weight samples were calculated from the absorption values obtained from the F.A.A.S. The mean values were then used to compare lead accumulation for each species.
2.3 RESULTS

2.3.1 Lead Solutions

The nutrient solutions were tested to ensure a nutrient composition that would satisfy two main objectives. Firstly, that the components provided sufficient nutrients throughout the experiment and secondly, that the added lead would not be precipitated from the solution.

Three nutrient compositions were considered. They were a 10% Rorison solution, from Ye, Baker, Wong and Willis, (1997), a nutrient composition by Huang and Cunningham, (1996), and Hoagland’s solution by Hoagland and Arnon (1950), obtained from the Sigma Plant Culture catalogue, (1995).

Both nutrient composition obtained from Huang & Cunningham, (1996), and the 10% Rorison solution, were ascertained to be unsatisfactory candidates. Hoagland’s solution was modified by the removal of monobasic ammonium phosphate, and the replacement of magnesium sulphate with magnesium nitrate. This provided ample nutrients and allowed the required quantities of Pb(NO₃)₂ to be added without precipitation of the lead.

2.3.2 Acacia Experiment

A two-way ANOVA performed on dry weights revealed no significant difference in growth between species across the range of treatments (P=0.857). This indicates that seedlings used in the experiments were the same size.
Acacia species showed a significant increase in the lead content present in the roots with increasing lead treatment concentrations (Fig.2.3) \( (P=0.000) \). Lead accumulation in the roots varied between species, with a mean of 98.2 mg of lead per gram of dry weight for *A. heteroclita* in the 50 µM lead solution while *A. saligna* and *A. quadrimarginata* took up significantly lower quantities \( (P=0.010) \). Mean lead levels in *A. heteroclita* roots was 114 mg of lead per gram of dry weight in the 100 µM lead solution, which was again higher than the quantity in the roots of *A. saligna* and *A. quadrimarginata* in the corresponding lead treatments.

The levels of lead in the shoots increased for all species with increasing lead treatment concentrations (Fig.2.4; \( P=0.001 \)), although mean lead values were approximately 200 times lower than corresponding levels in the roots. There was no difference in mean lead levels in the shoots between species in the 50 µM lead treatment \( (P=0.4375) \), but *A. heteroclita* accumulated significantly higher levels in the 100 µM lead treatment compared to the other species \( (P=0.001) \).

*A. quadrimarginata* showed the greatest effects of lead ion toxicity, indicated by progressive browning of leaf edges, chlorosis, and eventual leaf necrosis. *A. heteroclita* was affected to a lesser extent, and *A. saligna* was least affected. Toxic effects were greatest in the 100 µM lead treatment for all species. Callus was observed on some leaves.

Transpiration remained high throughout the experiment for all treatments, and was determined from water level measurements every 3 days.
Figure 2.3. Lead content in roots and shoots of a) *A. heteroclita*, b) *A. quadrimarginea* and c) *A. saligna*, grown in 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated from 9 replicates and the vertical bars represent standard error.
Figure 2.4. Lead content in shoots of *A. heteroclita*, *A. quadrimarginea* and *A. saligna*, grown in 0 μM 50 μM and 100 μM lead nitrate solution. The means were calculated from 9 replicates and the vertical bars represent standard error.
2.3.3 *Casuarina* Experiment

All plants used for this experiment were the same size. (P=0.616). Lead accumulation in the roots of both species increased as the seedlings were subjected to higher lead concentrations (fig.2.5; P=0.015). *A. verticilatta* accumulated significantly higher levels of lead in the roots when immersed in a 50 μM lead solution when compared to *A. obesa*, with the former taking up 62.5 mg of lead per gram of dry weight, and the latter 40.0 mg of lead per gram of dry weight respectively (P=0.0026). *A. verticilatta* also accumulated more lead than *C. obesa* for the 100 μM treatment, 71.1 mg of lead per gram of dry weight and 62.8 mg of lead per gram of dry weight respectively. However, the differences between these mean values were not statistically significant.

*A. verticilatta* shoots could not be divided easily into stem and phyllodes. *C. obesa* shoots could be divided into main stem and peripheral phyllode components. However, between the stems and the phyllodes of this species, the amounts of lead accumulated for the given concentrations of lead treatments were not significantly different (P=0.7614). Hence, main stems were selected from this species to be compared with *A. verticilatta*. Lead accumulation in the shoots increased significantly for both species as the concentrations of lead in the nutrient solution increased (Fig.2.6; P=0.002), though mean levels were between 200-800 times lower than corresponding levels in the roots. *C. obesa* accumulated significantly higher lead levels in the 50 μM and 100 μM lead treatments compared to *A. verticilatta* (P=0.002).
Lead ion toxicity, indicated by phyllode-tip yellowing progressive browning of phyllodes and eventual necrosis, was most severe in *C. obesa*, though both species were increasingly affected with increasing lead concentrations in the nutrient solutions. Roots of both species showed growth retardation and browning in the 50 µM lead treatment, and more severely in the 100 µM lead treatment. Transpiration decreased with the increase in the lead concentrations in the treatment solutions.
Figure 2.5. Mean accumulation of Pb ions in the leaves, stems and roots of a) *Casuarina obesa* and shoots and roots of b) *Allocasuarina verticilatta* under three hydroponic treatments of Pb(NO₃)₂. The means were calculated from 9 replicates and the vertical bars represent standard error.
Figure 2.6. Lead content in the stems of *C. obesa* and shoots of *Allocasuarina verticilatta* grown in 0 µM 50 µM and 100 µM lead nitrate solution. The means were calculated over 9 replicates and the vertical bars represent standard error.
2.3.4 *Eucalyptus* Experiment

A two-way ANOVA performed on dry weights of all three species revealed no significant difference between species in any treatment (P=0.983). This indicated that all the plants were the same size.

A significant increase in the levels of lead (P=0.000) was recorded in the roots of all *Eucalyptus* species subjected to 50 µM and 100 µM lead solutions. The accumulation of lead within all three species increased with increasing concentration of lead in the nutrient solutions (fig.2.7). The quantity of lead detected in the roots of *E. lesoeufii* were approximately 52.6 mg of lead per gram of dry weight for the 50 µM lead treatment, and approximately 60.0 mg of lead per gram of dry weight lead for the 100 µM lead treatment. This trend was similar in both *E. globulus* and *E. camaldulensis*. *E. globulus* took up approximately 34.0 mg of lead per gram of dry weight when subjected to the 50 µM lead solution and approximately 53.0 mg of lead per gram of dry weight when subjected to the 100 µM lead solution. *E. camaldulensis* accumulated approximately 29.0 mg of lead per gram of dry weight for the 50 µM lead treatment and 33.0 mg of lead per gram of dry weight for the 100 µM lead nutrient solution.

The accumulation of lead in leaves for all eucalypt species showed no differences (fig. 2.8; P= 0.525) between species or treatments. However there was a significant difference in lead content recorded in the stems between species and treatments.
Analysing lead accumulation in stems within each species indicated that both *E. lesouefii* and *E. globulus* showed significant increase \((P=0.0009)\) in the quantities of lead in the stems of plants immersed in both the 50 \(\mu\)M and the 100 \(\mu\)M lead nutrient solutions. *E. camaldulensis* however, did not show any differences \((P=0.2854)\) for lead in the stems (fig.2.9). For *E. lesouefii*, mean value of lead intake was approximately 230 \(\mu\)g of lead per gram of dry weight in the 50 \(\mu\)M treatment and 320 \(\mu\)g /g at the 100 \(\mu\)M lead treatment. Mean values for the accumulated lead in *E. globulus* were approximately 150 \(\mu\)g /g dry weight and 170 \(\mu\)g /g dry weight for the 50 \(\mu\)M and the 100 \(\mu\)M lead treatment respectively. Mean values for lead taken into the stems of both these eucalypts were approximately 200 times lower than those found in the roots.

Visible signs of stress related to lead toxicity were observed in the plant roots. Browning and shrinking of the roots increased progressively with time and lead concentration from the 50 \(\mu\)M to the 100 \(\mu\)M lead treatments. An observation every three days indicated, plants in the 50 \(\mu\)M and 100 \(\mu\)M lead treatments did not require much distilled water replenished compared to plants the control treatments. Observable signs of lead toxicity included structural distortions in developing leaves and leaf curl on existing leaves. The simultaneous wilting of the leaves and stems followed this. Subsequently the yellowing of the leaves and browning of leaf margins occurred. Soon after necrosis and abscission resulted. All observational effects increased in severity from the 50 \(\mu\)M to the 100 \(\mu\)M lead treatments. The greatest severity of lead stress symptoms was observed in *E. camaldulensis*. *E. globulus* showed considerably less symptoms, and *E. lesouefii* even fewer symptoms.
Figure 2.7. Lead content in roots, stems and leaves of a) *E. lesouefii*, b) *E. globulus* and c) *E. camaldulensis*, grown in 0 μM 50 μM and 100 μM lead nitrate solution. The means were calculated from 9 replicates and the vertical bars represent standard error.
Figure 2.8. Lead content in leaves of *E. lesouefii*, *E. globulus* and *E. camaldulensis*, grown in 0 μM 50 μM and 100 μM lead nitrate solution. Means. The means were calculated over 9 replicates and the vertical bars represent standard errors.

Figure 2.9. Lead content in stems of *E. lesouefii*, *E. globulus* and *E. camaldulensis*, grown in 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated from 9 replicates and the vertical bars represent standard error.
2.4 DISCUSSION

Many researchers have utilised hydroponic experiments to determine the potential of plants to accumulate metals under laboratory conditions. In many instances the components present in the nutrient solution will depend on the heavy metal ions present. For example, lead solution chemistry is complex, and often most of the lead added to the solution precipitates as lead phosphate (Huang and Cunningham, 1996). Hence, phosphate and sulphate levels must be minimised in the presence of lead.

Lead precipitation, due to the presence of phosphate and sulphate occurred for the 10% Rorison solution and the nutrient solution utilised by Huang and Cunningham (1996). This is due to the low solubility of PbSO₄ and Pb₃(PO₄)₂. The low solubility products, \( K_{sp} \) for \( \text{PbSO}_4 \) and \( \text{Pb}_3(\text{PO}_4)_2 \), 1.6 x 10⁻⁸ and 5 x 10⁻³ respectively, indicate that only small amounts of \( \text{SO}_4^{2-} \) and \( \text{PO}_4^{3-} \) ions can be tolerated in a lead solution before lead will precipitate out. In Rorison’s solution lead precipitates out as \( \text{Pb}_3(\text{PO}_4)_2 \) due to the presence of \( \text{PO}_4^{3-} \). Trials conducted with the nutrient solution from Huang & Cunningham, (1996), also showed that lead salts precipitated. The modified Hoagland’s solution was the most suitable medium to carry the required amounts of lead ions without precipitation.

In Huang and Cunningham (1996), the prevention of lead precipitation was attempted by adjusting the pH to values of 4.5 - 5.0 and only adding small concentrations of phosphorus. Using a 10% Rorison solution (Ye, Baker, Wong & Willis, 1997), an
attempt was made by utilising a solution containing lower concentrations of all required
nutrients. In both cases, trials and calculations indicated that using these solutions would
encourage a certain amount of lead precipitation.

The modified Hoaglands solution used for these experiments provided ample nutrients
for the plants and did not promote lead precipitation during the experiments. Replacing
magnesium sulphate with magnesium nitrate and the removal of ammonium phosphate
reduced the concentration of sulphates and phosphates sufficiently in the solution.
However, this did not seem to reduce the required amount of nutrients throughout the
experiment. The presence of nitrates provided ample nitrogen molecules, while the
presence of zinc sulphate in small quantities provided ample sulphates without
precipitating lead.

Water moves into the roots following a negative pressure gradient, processing
considerable amounts of water soluble contaminants (Stomp et al. 1994) . Once in the
root tissue, most metal transport takes place through the xylem (Salt et al. 1995). The
rates of movement of metal ions into the shoots are limited by several factors. These are
movement across the casparian strip and the high cation exchange occurring in the
xylem (Salt et al. 1995). Movement across the casparian strips allow metal ions to cross
over to the shoot only through the symplastic pathway, and the high cation exchange
capacity severely retards the movement of cations (Salt et al. 1995). This relative rate of
contaminant accumulation has been shown to correspond to the level of tolerance of the
plant. Hence a plant more tolerant of a specific contaminant would be more tolerant to
higher accumulations of the contaminant and might carry this out at a faster rate than would non-tolerant plants (Huang & Cunningham, 1996).

2.4.1 *Acacia* Experiment

All three species of acacias showed significant accumulation of lead in the 50 µM and 100 µM lead solution treatments. However, based on the results, it is likely that *A. heteroclitia* was the best phytoextractor of lead from the hydroponic medium. Compared to *A. quadrimarginea* and *A. saligna*, *A. heteroclitia* had the highest lead efficiency and only exhibited mild symptoms of physiological stress indicating a high tolerance to the lead content within both its roots and shoots. Although *A. saligna* had the least signs of physiological stress, this could be a result of this species accumulating the lowest amount of lead in its tissues.

2.4.2 *Casuarina* Experiment

Both *C. obesa* and *A. verticillata* displayed increasing lead content in their root tissues with increasing lead concentrations in treatment. *C. obesa* however, accumulated a significantly higher quantity of lead in its shoots for both the 50 µM and the 100 µM lead solution treatments even though *A. verticillata* had a higher efficiency for lead in its roots. It is likely that while *A. verticillata* possessed greater tolerance of lead in its roots, *C. obesa* possessed a greater tolerance of lead content in its shoots. Both species revealed lead levels in shoots lower than those found in their roots, indicating the translocation of lead into the shoots was being retarded to a certain extent. The higher lead content in *C. obesa* shoots could indicate that this species restricted lead
translocation to the shoots less. Since contaminant accumulation has been shown to correspond to the level of tolerance of the plant it is likely that *C. obesa* shoot tissues had a higher tolerance to systemically induced stress by lead in its shoot tissues. The observation that *C. obesa* required less distilled water to be replenished into the treatments supports this speculation.

Insufficient replicates for *C. equisettifolia* prevented this experiment to be carried out on this species.

2.4.3 *Eucalyptus* Experiment

In all three species of eucalypts, lead accumulation in root tissue increased with increasing concentration of the hydroponic treatments. *E. lesouefii* roots showed the highest efficiency for lead uptake followed by *E. globulus* and finally *E. camaldulensis*. All plants undergoing experimentation exhibited signs of physiological stress in their roots. Signs of physiological stress increased as lead content in root tissue increased, indicating a positive correlation between these factors. Thus there is a possibility that although *E. lesouefii* exhibited the strongest negative root pressure followed by *E. globulus* and then *E. camaldulensis*, there was a lack of tolerance to the accumulated lead ions. Results from this research also demonstrated that lead content in the stem tissue of *E. lesouefii* was highest followed by *E. globulus*. *E. camaldulensis* showed no significant amounts of lead in its stems. However, physiological stress symptoms were greatest in *E. camaldulensis*, followed by *E. globulus* and then *E. lesouefii*. This could suggest that varying maximum levels of tolerance to lead content existed within the
tissues of all three species of eucalypts. It is possible that surpassing this threshold could trigger these species to respond by restricting the overall intake of lead and/or essential nutrients through their roots. Wilting increased progressively as the lead concentration increased in the treatments. The amount of distilled water required to be replenished also decreased from the control treatment to the 100 μM treatment. Both these observations support the above speculation. Although no significant amounts of lead were accumulated in the leaves of all three species, varying signs of stress symptoms were observed between them.

These stress symptoms corresponded to the relative tolerance of the species to accumulated lead in its tissues. It is likely that the toxicity symptoms observed in the leaves are causes of insufficient nutrients being provided for their development. Perhaps a study to compare the relative restrictions of distilled water and hence nutrients between species should have been carried out.

The results from the hydroponic research demonstrate that plant species vary significantly in lead uptake and translocation, with roots possessing a higher efficiency for lead content than shoots. The phytoextraction coefficients for metals such as lead correlates with solubility of the respective phosphates and sulphates formed (Kumar et al. 1995). Perhaps the formation of insoluble inorganic complexes in the solution and inside the plant can significantly decrease the phytoextraction efficiency. Between all the plants used in the hydroponic experiment, A. heteroclita accumulated the most lead.
both within its shoot and root tissue, with an exceptionally higher facility for lead in its shoots than the other plants.

Insufficient sample sizes prevented the experiment from indicating whether, for this species of *Acacia*, lead translocation occurred in the stems, leaves or both. Despite the higher quantities of lead accumulation within its shoots, physiological stress symptoms expressed by these plants were mild. It is likely that little or no restriction of nutrients from the nutrient solutions occurred. This is supported by evidence that no signs of wilting were perceived.

Shoot lead quantity is the most important physiological parameter for evaluating lead phytoextraction (Huang & Cunningham, 1996). This aspect aids in the option of deciding possibilities of harvesting the accumulated lead. Thus, from the hydroponic experiment, *A. heteroclita* has shown the highest potential for accumulating lead and harvesting this accumulated lead.

Further experimentation is desired as results obtained from these hydroponic experiments are limited. Lead concentrations utilised for the solution culture do not represent an upper limit for lead uptake. The limited duration of the experiment also may not allow the full expression of this capacity. Perhaps time-dependent experiments could be carried out to aid in the determination of maximum limits for experimental plants.
3 SOIL EXPERIMENTS

3.1 INTRODUCTION

The soil experiments were conducted to evaluate lead phytoextraction efficiency in conditions that more closely emulate those under which these plants might be utilised. The difference in medium can affect the resulting accumulation of lead in the experiments, by varying the availability of lead ions present in the soil solution (Huang & Cunningham, 1996).

The efficiency of plants to phytoextract ions in soil is mainly affected by free metal ions and soluble metal complexes (Salt et al., 1995). These factors vary within the rhizosphere for each species. Conducting experiments in soil helps determine whether the proposed species release metal chelating molecules, or reductants and organic acids from their roots to obtain insoluble metals from the soil. In some cases plants have been know to acidify the soil environment by extruding protons from their roots. The lower pH releases soil bound metal ions into the soil solution (Salt et al., 1995). Mycorrhizal fungi or root-colonising bacteria can also perform these processes (Salt et al., 1995).

The selected species from the Myrtaceae family (Eucalyptus globulus and E. camaldulensis) and from the Mimosaceae family (Acacia heteroclita, A. saligna and A. quadramarginea) were grown in soil fertilised with the same nutrient solution employed for the hydroponic experiment. The same lead treatments were then supplemented and experiments were carried out for a period of three weeks.
3.2. MATERIALS AND METHODS

3.2.1 Soil Preparation

All soil utilised for the seed germination comprised pasteurised, one to one portions of coarse sand and fine white sand. The composition was pasteurised for a period of approximately fifty minutes in heshen bags and then allowed to dry.

3.2.2 Plant Material and Maintenance

Seeds of *Eucalyptus camaldulensis*, *E. lesouifii*, *E. globulus*, *Acacia heteroclitia*, *A. saligna*, *A. quadramarginea*, *Casuarina obesa*, *C. equisetifolia* and *Allocasuarina verticillata* were purchased from Kim Seed Co.. These seeds were germinated in 50 mm diameter plastic (T4) pots of pasteurised sand. In each pot, 4 seeds were planted in the sand mixture. For each species, one tray of forty pots of seeds was germinated each month. This procedure was conducted over a period of five months to obtain a series of staggered height growths for each species. The trays were lined and then sprayed with Mancozeb\textsuperscript{TM} fungicide to deter fungal infection.

Unscarified *Acacia* seeds were soaked in hot water to induce germination. *C. equisetifolia* seeds were soaked in aerated distilled water until the first signs of germination before planting in the T4 pots.

Once the seedlings were approximately 5 cm in height, the pots were thinned to one plant per pot. From each species, the healthiest looking individuals of approximately 8 cm in height were selected for the soil experiments.
3.2.3 Soil Experiment Set Up

For each species, fifteen 4 L buckets were used. A 1 cm hole was drilled at the base of each bucket for drainage. A piece of gauze was secured over the hole from the inside to prevent the loss of sand. The buckets were then filled with 4 kg of pasteurised sand mixture. Seedlings were transplanted into the buckets and left to grow for approximately two weeks prior to experimentation. In each bucket, three selected seedlings of each species were planted approximately at equal distance from each other. During this period, buckets of seedlings were watered with distilled water daily and modified hoaglands solution once a week. These buckets were also randomly shifted everyday to reduce any variation in growth from varying glasshouse conditions.

3.2.4 Soil Lead Experiment

For each species, five buckets of seedlings were used for one treatment. Three concentrations of lead were used for this experiment. They were 0, 50, and 100 μM. These lead treatments were supplied as Pb(NO₃)₂ mixed into the modified hoaglands solution. Treatments were flushed through the buckets three times over a two hour period during which a conductivity meter was used to ensure equal conductivity between the treatment solutions added, and drained. The buckets were subsequently left to drain and then maintained at field capacity. The plants were harvested after three weeks in the different lead treatments.
3.2.5 Harvesting Of Plant Material

Plants harvested from the soil experiment were removed, washed and stored in a method similar to replicates in the hydroponic experiment (section 2.2.5). In this case, however, the plants in each bucket were separated from each other by submerging the tangled roots of the plants in water and gently shaking to dislodge the sand. Once the sand had been washed off, roots were also checked for nodule signs as indications of mycorrhizal growth. The plants were again washed twice in distilled water to remove any external lead, that might have been present in the rhizosphere. The individual plant was then sectioned and stored in labelled paper bags.

3.2.6 Fresh And Dry Weight And Lead Analysis

The fresh weights of plant sections were recorded and replaced into their bags and stored in an oven for three days at 60 °C. Their dry weights were then recorded.

3.2.7 Lead Analysis

The lead analysis carried out for plant sections in this experimental design followed the procedure described in section 2.2.7.

3.2.8 Data Analysis

The experimental design allowed both one way and two way analysis of variance (ANOVA) to be conducted. Equal number of replicates for each species and treatment was utilised at the start of the experiment. Two way ANOVA was conducted to test for differences within species, between species and effects of treatments. Where there was a
difference due to either species or treatment, a one way ANOVA was performed to
determine the differences between means. The concentration of lead present in the 200
mg dry weight samples was then calculated from the absorption values derived from the
F.A.A.S as in section 2.2.9.

3.3 Results

3.3.1 Acacia Experiment

In the soil experiment, *A. quadrimarginea* was significantly smaller (*P*=0.037) in size
compared to the other species of *Acacia*, although there was no significant difference
(*P*=0.983) between the size of *A. saligna* and *A. heteroclita*. When subjected to 50 μM
and the 100 μM lead treatments, all three species of *Acacia* showed lead the
accumulation in its tissues (fig. 3.1). In both *A. heteroclita* and *A. quadrimarginea* the
amount of lead taken up in the root tissue was significantly different between the 50 μM
and the 100 μM lead treatments. *A. heteroclita* roots accumulated approximately 4.4 mg
of lead per gram of dry weight for the 50 μM lead treatment and approximately 6.5 mg
of lead per gram of dry weight for the 100 μM lead treatment. *A. quadrimarginea* roots
took up approximately 3.2 mg of lead per gram of dry weight in the 50 μM lead
treatment and approximately 4.1 mg of lead per gram of dry weight in the 100 μM lead
treatment. However, there was no significant difference (*P*=0.421) between the amount
of lead accumulated between the 50 μM and the 100 μM lead treatments for *A. saligna*.
The amount of lead accumulated at both the 50 μM and 100 μM lead treatments were
approximately 4.4 mg of lead per gram of dry weight (fig. 3.1).
All three species of *Acacia* showed a ten times lower lead accumulation in their leaf and stem tissue compared to the roots (fig. 3.2 & 3.3). For both *A. heteroclita* and *A. quadrimarginea*, the lead taken up in the stem and leaf tissue varied significantly between the 50 μM and 100 μM lead treatments. However, *A. saligna* showed no significant difference in the lead accumulated in stem and leaf tissue between the 50 μM and 100 μM lead solution treatments.

Observations made daily revealed that the plants in the soil experiments exhibited little physiological stress with most of the toxicity symptoms observed in the leaves of *A. quadrimarginea*. The leaves developed slight structural distortions and leaf curl. Leaves of all three species in the lead treatment solutions also developed callus. The browning of leaf margins and chlorosis was observed in *A. quadrimarginea* leaves. In a small percentage of the leaves this eventually led to abscission. Upon harvesting the plants after the experiment, roots showed the presence of nodule growth in all three species of acacia. These nodules appeared to be less prevalent on roots of plants undergoing lead treatments. A small percentage of the roots also showed the browning of tissue which increased with increasing lead treatment concentrations.
Figure 3.1. Lead content in roots and shoots of a) *A. heteroclita*, b) *A. quadrimarginea* and c) *A. saligna*, grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and vertical bars represent the error bars.
Figure 3.2. Lead content in leaves of *A. heteroclita*, *A. quadrimarginea* and *A. saligna*, grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and the vertical bars represent standard error.

![Graph showing lead content in leaves of *A. heteroclita*, *A. quadrimarginea* and *A. saligna*](image1)

Figure 3.3. Lead content in stems of *A. heteroclita*, *A. quadrimarginea* and *A. saligna*, grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and the vertical bars represent standard error.

![Graph showing lead content in stems of *A. heteroclita*, *A. quadrimarginea* and *A. saligna*](image2)
3.3.2 Eucalyptus Experiment

There was no significant difference (P>0.616) in the size of the plants between species and treatments for this experiment. Both species of eucalypts showed significant difference (P<0.015) in the quantity of lead accumulated in the roots between lead solution treatments. These mean accumulated lead levels increased with the increasing concentration of the lead in the treatments. A significant difference (P=0.0026) was recorded in the lead taken up in the 100 μM lead treatment between both species. The root tissue of *E. globulus* contained approximately 47.6 mg of lead per gram of dry weight, while the root tissue of *E. camaldulensis* took up approximately 43.0 mg of lead per gram of dry weight (fig. 3.4). However, this variation was not observed for the 50 μM lead treatment. Both species accumulated approximately 25.0 mg of lead per gram of dry weight for the 50 μM lead solution treatment.

The accumulation of lead in leaves of both eucalyptus species revealed no significant difference (P=0.894) between species and lead treatments (fig. 3.5). However, a significant difference occurred with lead content in the stems between species and lead treatments for this experiment. Although quantities of lead accumulated were lower by a factor of approximately 15, both eucalypts showed significant (P=0.837) amounts of lead accumulation in their stems (fig. 3.6). With *E. globulus*, the lead accumulated into the stem increased with the increasing concentrations of lead in the treatment solutions. *E. camaldulensis* did not show any significant difference in lead accumulated for both the 50 μM and the 100 μM soil lead treatments. At the 50 μM lead treatments, both *E.*
*globulus* and *E. camaldulensis* took up approximately 2.5 mg of lead per gram of dry weight of material.

Visible signs of stress related to lead toxicity were observed in the plants. Browning of the roots increased from the 50 \(\mu\)M to the 100 \(\mu\)M lead treatments.

Observable signs of lead toxicity included firstly, slight structural distortions in some developing leaves and leaf curl on existing leaves. Slight wilting of the leaves and stems followed. Some yellowing of the leaves and browning of the edges occurred. Necrosis and abscission eventually followed for some leaves. All observational effects increased from the 50 \(\mu\)M to the 100 \(\mu\)M lead treatments. *E. camaldulensis* appeared to sustain greater stress symptoms than *E. globulus*. 
Figure 3.4. Lead content in roots, stems and leaves of a) *E. globulus* and b) *E. camaldulensis*, grown in soil treated with 0 μM, 50 μM and 100 μM lead nitrate solution. The means were calculated over 15 replicates and vertical bars represent the error bars.
Figure 3.5. Lead content in leaves of *E. globulus* and *E. camaldulensis* grown in soil treated with 0 µM, 50 µM and 100 µM lead nitrate solution. The means were calculated over 15 replicates and the vertical bars represent standard error.

![Graph showing lead content in leaves of E. globulus and E. camaldulensis.](image)

Figure 3.6. Lead content in stems of *E. globulus* and *E. camaldulensis* grown in soil treated with 0 µM, 50 µM and 100 µM lead nitrate solution. The means were calculated over 15 replicates and the vertical bars represent standard error.

![Graph showing lead content in stems of E. globulus and E. camaldulensis.](image)
3.4 DISCUSSION

3.4.1 *Acacia* Experiment

The results from the experiment showed, there was significant variation in the response to the lead concentrations between the species. It also showed that signs of stress symptoms corresponded to the amount of lead accumulated by the respective species. Based on these results, it is likely that *A. heteroclita* was the best short-term phytoextractor of lead from the soil lead treatments. No physiological stress symptoms were observed although it accumulated the highest amount of lead in its tissues.

*A. saligna* also showed no sign of physiological stress, however no significant increase in lead taken into its tissues was observed with increased lead concentrations. It is possible that a mechanism exists to restrict the amount of lead entering the tissues past a threshold level preventing the toxification of its tissues.

*A. quadrimarginea* also showed an increase in the accumulation of lead in its tissues with increasing lead concentration. However, the observable signs of physiological stress indicate this species as possessing a low tolerance to the lead content in its tissues. The decrease in the nodule growth correlated with the increasing lead concentration in the treatments. No further investigations were conducted in this area.
3.4.2 *Eucalyptus* Experiment

For both *Eucalyptus* species, although no significant difference in the amount of the lead taken into the roots was indicated in the 50 μM lead soil treatments, *E. globulus* accumulated significantly higher amounts of lead, compared to *E. camaldulensis*, at the 100 μM lead soil treatment. While it is likely that both species possessed mechanisms that accumulated lead, the root tissue of *E. globulus* exhibited a higher efficiency for lead.

The increasing lead levels in *E. globulus* tissue could indicate that the process of lead translocation for this species is greater than *E. camaldulensis*. Although there were little signs of physiological stress exhibited by both species, symptoms exhibited by *E. camaldulensis* displayed were greater than those observed on *E. globulus*. It is possible that the wilting observed in both species of eucalypts is a generic response to reduce systemic toxicity from toxic substances taken in. Hence, more pronounced wilting seen in *E. camaldulensis* is an indication that *E. globulus* possessed a higher tolerance to greater amounts of lead residing within its tissues.

From this soil experiment, the results show a significant variation in the potential of the species to accumulate lead and translocate this heavy metal into its stems and leaves. This measure of lead efficiency contributes to the research to determine a suitable plant for lead phytoextraction from heavy metal contaminated soil. *A. heteroclita* took up the most amount of lead into its roots, stems and leaves indicating a higher facility for lead in its tissues compared to other species experimented with. Insufficient samples
prevented experiments to be conducted with a wider range of soil lead treatment concentrations.

Insufficient replicates also prevented this experiment to be carried out on *E. lesouefii* and *C. obesa*, *C. equisetifolia* and *C. verticillata*. Nevertheless, from the results it is likely that *A. heteroclita* possessed the highest tolerance to high lead content within its tissues. This speculation can be supported by the mild toxicity levels expressed and no signs of wilting observed by the species, providing evidence that no translocation mechanisms were restricted in response to high lead levels within it tissues.
In this study, the accumulation of lead and physiological responses to lead content within the tissues of three species of eucalyptus, acacias and casuarinas were investigated. Measurement of lead uptake and the observation of toxicity symptoms were used to assess the potential of these species to phytoextract lead from a medium. Plant species can differ in lead uptake and translocation due to different physiological responses to the concentrations used in the experiments (Huang and Cunningham 1996). This was observed for the species tested in these experiments.

Hydroponic and soil experiments provide information required to determine the potential of the selected plants to carry out phytoextraction of lead (Salt et al. 1995). In the hydroponic experiment, six of the eight species tested, indicated an increase in lead accumulation into their tissues as the lead concentration increased from 50 $\mu$M to 100 $\mu$M. These were *A. heteroclita*, *A. quadrimarginea*, *C. obesa*, *C. verticillata*, *E. globulus* and *E. lesouefii*. Results from the soil experiment showed that three of the five species tested also showed this response. These were *A. heteroclita*, *A. quadrimarginea* and *E. globulus*.

A comparison of the metal concentration in the plants sampled from the hydroponic treatment and from the soil treatment showed that lead concentration in seedlings grown in hydroponic solution were higher than in the soil experiment. The reasons for these differences might be due to differences in the environments and conditions for growth. Hydroponic solutions directly expose the plants to soluble metals and compare the
accumulation ability of plants with fewer experimental complications (Salt et al., 1995). However, edaphic conditions affecting the availability of heavy metals in soil experiments are complex, and differ from laboratory hydroponic experiments (Baker et al. 1994). In soil, the tight binding of the lead ions to the soil and plant material result in relatively lower mobility of this metal from the soil to the plant (Kumar et al. 1995). As a result, values derived from soil experiments are often lower than hydroponic experiments, although trends are similar for the same species under both experiments.

There are two basic strategies of metal uptake related to tolerance. They are the 'accumulator' and the 'excluder' strategies (Ye et al., 1997). The 'accumulator' strategy involves the active concentration of contaminants by the plant tissues over a full range of soil concentrations, implying highly specialised physiology. The 'excluder' strategy involves the maintenance of a constant level of the heavy metal contamination in the shoots by the plant until a critical soil concentration is reached, after which unrestricted metal transport results (Ye et al. 1997).

*A. heteroclita* accumulated the highest amount of lead into its tissues, both under hydroponic and soil experimentation, and showed mild or no toxicity symptoms after the experiments. These results indicate that *A. heteroclita* was possibly the most suitable species for the phytoextraction of lead from lead contaminated soil. Although these experiments have indicated that this species is suitable for the hyperaccumulation of lead from lead contaminated soil, further experiments should be carried out to gain a greater understanding of this process.
Experiments should be carried out to determine the mechanisms and molecules responsible for the accumulation of lead into its tissues. Heavy metal contaminated soil can differ in variety and concentration of heavy metals. Other factors such as pH levels and nutrients available in the contaminated area also vary. Hence, trial applications of potential heavy metal hyperaccumulators to actual contaminated sites or the utilisation of soil from heavy metal contaminated sites, are the next step in determining the suitability of the species.

*E. camaldulensis* and *A. saligna* from both the hydroponic and soil experiments did not show significant differences in the amount of lead accumulated under the 50 μM and 100 μM lead solution treatments. However, while *E. camaldulensis* showed the greatest signs of physiological stress compared to *E. globulus* and *E. iesouesfii*, under the lead treatments, *A. saligna* showed the least amount of physiological stress under the lead treatment. It is possible that although both the *E. camaldulensis* and *A. saligna* species regulated the amount of lead ions in their tissue by maintaining a low level of lead ions in their shoots. However, *A. saligna* was able to execute an 'excluder' strategy without also limiting the amount of essential nutrients and water into its system in the process. Further studies can also be conducted on these species to determine the specific mechanism responsible for the observed results.

Other studies indicate that there is no proportionality between the degree of constitutional lead tolerance and the lead content of a plant (*Ye* *et al.* 1997). Tolerant species do not always take up more metals in roots than non-tolerant ones as tolerance
and accumulation ability of a plant might be independently inherited characteristics (Ye et al. 1997). *A. heteroclita* and *A. saligna* both showed tolerance to the lead concentrations in the treatments. However, *A. heteroclita* showed a higher capacity for lead accumulation in its tissues. Perhaps this is an example of such a case where metal tolerance and accumulation are observed to be independently inherited traits.

*T. caerulescens*, a known hyperaccumulator of lead ions, recorded lead ion accumulation of 2.74 mg per gram of dry plant weight under hydroponics (Baker et al. 1994). In this project, *A. heteroclita* shoot tissue was indicated to accumulate approximately 7 mg of lead per gram of dry weight under hydroponics. Comparing the accumulated amounts between these two plants, *A. heteroclita* accumulated relatively higher amounts of lead within its tissues. However, direct comparisons are invidious in view of certain factors that exist (Baker et al. 1994). The composition of the components within a medium can play a role in the selection of plants. Some of these factors affect the growth of the plants during the experiment. These are, different background nutrient solutions used, and variations in the pH and temperature during the experiment. Other factors such as the duration of the experiments, existing differences in plant biomass and the different techniques used in harvesting the plants also give different results (Baker et al. 1994).

The hyperaccumulation of heavy metals by plants is an area of research that, not only possesses commercial application, but will also provide answers to some of the fundamental questions of plant biochemistry, nutrition, and stress physiology (Salt et al.)
The unique 'metal accumulation' genes of metal accumulating plants may directly benefit the world agriculture and environment. Phytoremediation, although in its infancy, may one day become an established environmental clean up technology (Salt et al. 1995). It is possible that plants that accumulate toxic metals may be grown and harvested economically, leaving the soil or water with a greatly reduced level of toxic metal contamination. However, further development of phytoremediation probably requires an integrated multidisciplinary research effort that combines plant biology, soil chemistry, soil microbiology, and agricultural and environmental engineering.


78


DIXON B., (1996), Realism Is The Order Of The Day For Bioremediation. *Biotechnology, 14*.


HEALTH DEPARTMENT OF WESTERN AUSTRALIA, (1993), Disposal by shaft entombment or trench burial of a range of intractable waste at the intractable waste disposal facility, Mt Walton East: Report and recommendations of the environment Protection Authority, *Bulletin 726*.


MILLER G.T., (1992), *Living In The Environment 7th edn.*, Wadsworth Publishing Co., Belmont, California


STEFFENS J.C., (1990), The heavy metal-binding peptides of plants. Annual Review of Plant Physiology and Plant Molecular Biology. 41, 553-575.


