Potential for Phytoremediation of a Metalliferous Mine Site at Mt. Costigan, NSW

Hylton B. Hobday (B.Sc.)

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CERTIFICATE OF AUTHORSHIP / ORIGINALITY

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I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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List of Abbreviations

CERCLA	Comprehensive Environmental Response Compensation and Liability Act
CF	Concentration factor
DLWC	Department of Land and Water Conservation (NSW)
DMR	Department of Mineral Resources (NSW)
dwt	Dry weight
EC	Electrical conductivity
EPA	Environmental Protection Authority (NSW)
ER	Enrichment ratio
g	Gram
GBH	Tree girth at breast height
ha	Hectare
LOI	Loss on ignition
N.P.K.	Nitrogen:phosphorus:potassium ratio
NSW EPA	New South Wales Environmental Protection Authority
OECD	Organization for Economic Cooperation and Development
OM	Organic matter
RG	Reagent grade
SO_4	Sulphate
t	Tonne
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USBM	United States Bureau of Mines
± se:	Standard error of the mean
µS	microSiemen

Abstract

Mt. Costigan mine, on the Western Slopes of the Great Dividing Range, NSW, was worked intermittently (1887 – 1928) for copper, lead, zinc, silver and gold. The entire mine site was originally cleared and contaminated with mining wastes, but had naturally revegetated with eucalypt woodland in parts. However, a barren section remains despite recent remediation efforts by the NSW Department of Mineral Resources (DMR), and problems of metal contamination, acid saline seepage, erosion and the threat of contaminated runoff into the catchment persist.

This study utilized the triad approach of field ecological and chemical-impact assessment at the barren site, using revegetated woodland as a reference site, and glasshouse toxicity trials of soils from both the barren and reference sites. Copper, lead, zinc and cadmium levels in barren site soils all exceed NSW Environmental Protection Agency (residential) limits in soil. Remediation by the DMR of the barren site using biosolid amelioration while this project was being carried out resulted in decreased metal contamination at the site, without significant changes in salinity. Vegetation analysis of the barren site before and after remediation did not indicate significant changes, though this may have been due to seasonal variation in plant growth.

A glasshouse bioassay using neat site soils and several dilutions with river sand was designed to determine the dose-response relationships in native plants *Eucalyptus sideroxylon*, *Acacia hakeoides* and *A. salicina*, endemic to Mt. Costigan. The objective was to evaluate soil toxicity and the potential of native species for phytoremediation at Mt. Costigan. *Avena sativa* (oats) was included as a standard test species for phytoxicity studies, and was the only species to survive in all soils. *A. salicina* proved well suited to much of the barren site, but *E. sideroxylon* did not grow well, and was better adapted to woodland soils. Acacias and eucalypts both showed strong accumulator tendencies for copper, zinc and manganese in diluted site soils. The reverse was true for cadmium, however, with plant-tissue concentrations of this metal increasing in proportion to soil content. Most metals were selectively concentrated in root tissue, but acacias leaves showed high copper and manganese content.

Phytoremediation is likely to prove effective in a multifaceted program of physical, chemical and biological characterization and remediation. It is suggested that phytostabilization of the severely contaminated parts of the barren site be initiated by planting adaptable species such as *A. sativa,* and that less-contaminated areas be planted with *A. salicina*. This could be followed by amelioration with biosolid, mixed into top layers, before further planting with the less-tolerant *E. sideroxylon.* The resulting humic buildup and reduction in soil toxicity would allow other indigenous plant communities to return and restore ecological balance at Mt. Costigan site.

Chapter 1: Overview

1.1. Project aims

The primary aim of this project was to investigate the potential use of Australian native plants for phytoremediation and rehabilitation of a derelict mine site at Mt. Costigan, located on the Western Slopes of the Great Dividing Range, New South Wales (NSW) (Fig. 1.1). Specifically, the study objectives included:

- Determining the existing metal contamination levels at the mine site;
- Evaluating the toxicity of a range of site soils to selected native plant species;
- Evaluating the effectiveness of soil remediation and replanting measures carried out by the NSW Department of Mineral Resources (DMR) at the site; and
- Investigating the feasibility of using selected indigenous plant species in future remediation efforts.

An ecotoxicological, triad approach was used in this study, comprising a combination of field ecological and chemical assessment and glasshouse toxicity trials.

1.2 Project rationale: remediation of contaminated sites

The term remediation has various connotations. According to Bradshaw (1996), remediation can imply:

- Restoration, which involves returning the site to its original state;
- Rehabilitation, representing only a partial return to the original state; and
- Reclamation, where the final state differs from the original.

In this study, the term is used in a broad context (Johnson, 2000) of improving the ecosystem by reducing contaminants, reestablishing indigenous plant species, halting

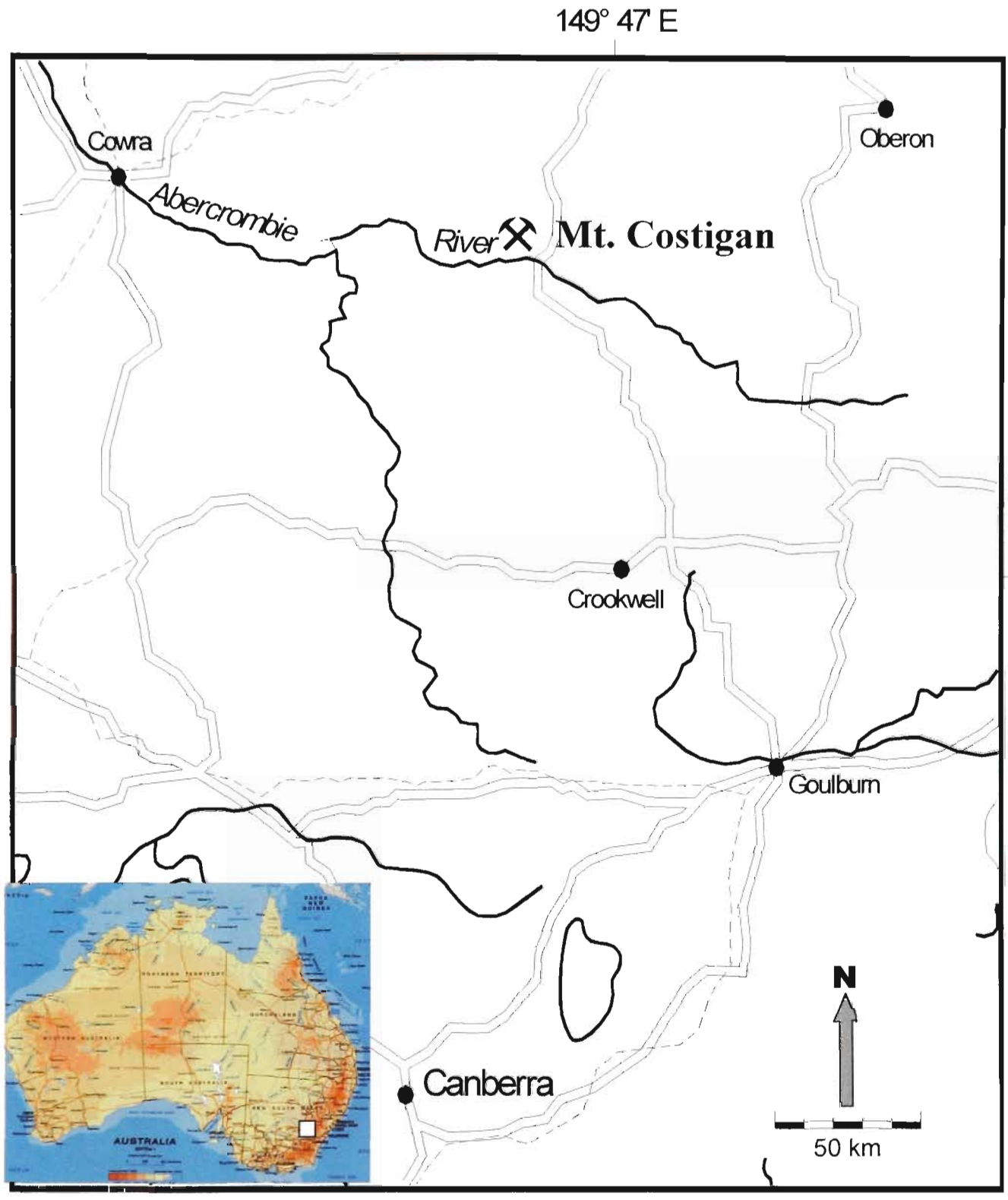


Figure 1.1 Mt. Costigan study area. Location shown by rectangle on inset map, situated on the Western Slopes of the NSW Southern Highlands. Modified from Minfo (1997) and Geoscience Australia (2002).

soil erosion, improving biological diversity, and achieving a self-sustaining condition compatible with its natural surroundings.

1.2.1 Problems presented by contaminated sites

Many anthropogenic activities result in active or abandoned sites that are left in a contaminated state. Contaminants include both organic and inorganic compounds in solid, liquid and gaseous states. Contaminated sites are a cause for concern since they degrade the environment and downgrade land that is otherwise suitable for food production, and can be hazardous to human health. Without remedial action, the problems presented by contaminated sites tend to persist. Freedman (1995) cites the example of Roman lead-mine wastes that remain a barren scar to this day, with only a few scattered pioneer plant species. Apart from the local impacts of contamination, there is a tendency for toxic metals to be redistributed by water or wind (Mailman, 1980), or to enter the food chain via terrestrial plants to arthropods, herbivores and carnivores (Freedman, 1995). Alternatively, metals from contaminated soils or site runoff may be concentrated in plant crops consumed directly by humans, leading to poisoning and disease (Francis, 1994).

The world's land surface affected by high levels of chemical contamination from all sources, including heavy metals, totals an estimated 43 million hectares (Oldeman, 1994, 1998). Apart from this direct impact, chemical contamination also contributes to severe physical degradation and erosion, which affects a further 262 million hectares of the earth (Lal, 2001).

The largest volume of data relating to contaminated sites is from the United States, where the majority of the "Superfund" sites identified by the US Environmental Protection Agency (USEPA) are contaminated with organic or organometallic compounds (Newman *et al.*, 1998). Heavy-metal contamination also poses a serious problem in the USA, especially around old mine sites (Society for Mining, Metallurgy and Exploration, 1998). This is also the case in Australia (Johnson, personal communication). In NSW, the Department of Mineral Resources (DMR), charged with the responsibility for regulating the management and rehabilitation of

contaminated sites, has recently established the Derelict Mines Rehabilitation Program (Johnson, 2000).

1.2.2 Contaminated soils

Soil is a crucial part of the biosphere. Apart from its role in providing an anchoring substratum and a medium for nutrient uptake for plants, as well as a niche for a wide range of soil organisms, soil is a geochemical sink for contaminants. It also serves as a buffer that restricts migration of toxic substances from the soil to the atmosphere, hydrosphere and biota (Lal and Bruce, 1999; Lal, 2001; Grace *et al.*, 1995).

The fate of contaminants in the soil (frequently concentrated to abnormally high levels resulting from human activities) is determined as much by physical and chemical properties of the soil as by the physico-chemical characteristics of the contaminants themselves. Soil supports life by purifying ground water and detoxifying pollutants (Lal, 1995, 2001). However, the length of time that certain contaminants remain in the soil is much longer than in other components of the biosphere. Although soil metals are redistributed by erosion, deflation, leaching and plant uptake, metals are elemental forms that can be neither degraded nor metabolized (Francis, 1994).

1.2.3 Remediation of abandoned mine sites

Mine closures arise from several causes: depletion of ore or a decline in ore grade to sub-economic levels, falling commodity prices, competing land use, and as a result of legislation or litigation. In Australia, economic factors have been paramount in the decisions to close mines (Markham, 1961).

Apart from metal-contamination problems on abandoned base-metal mine sites, other toxic elements may be present, for example arsenic compounds used as a lixiviant (solvent) for silver (D.K. Hobday, personal communication). Acid mine tailings, such as at Mt. Costigan, pose an especially serious problem since the solubility and bioavailability of most metals increases at acidic pH, thereby increasing potential toxicity (Freedman, 1995). Concentration and spreading of metal contaminants and

poor soil quality may result from these extractive processes. This in turn leads to extensive soil erosion and, in extreme cases, development of "badlands" topography, downgrading catchment water quality and habitability. Apart from aqueous transport, dust from contaminated tailings may be spread by wind (Peters, 1984; Freedman, 1995).

1.2.4 Current methods of remediation

The requirement to remediate contaminated sites, including derelict mines, is imposed by regulatory bodies such as governmental environmental protection agencies, acting under specific legislation and with well-defined guidelines (Johnson, 2000). Many of the remediation methods used today are engineering-based (Society for Mining, Metallurgy, and Exploration, 1998). These techniques tend to be expensive and may be ineffective or damaging to the soil structure, making it unfit for agriculture. However, the pace of industrial development projects generally requires the rapid results achieved by engineering methods, rather than the more benign, less disruptive, but slower procedures of bioremediation and phytoremediation.

Engineering-based remediation techniques that are currently used for metal-contaminated sites include (Markert, 1993; Society for Mining, Metallurgy, and Exploration, 1998):

i) Covering the polluted area with clean soil or impermeable media such as clay to isolate contaminants from water infiltration and oxygenation;

ii) Excavation, removal and disposal of contaminated material (*e.g.* tailings down old mine workings), without additional treatment;

iii) Excavation and treatment of soil, including sieving and jet washing, with offsite disposal of the contaminated fine fraction; also, agitation in dilute acid to remove metal hydroxide precipitates;

iv) Self-sealing and immobilization, whereby chemical reactions form an impermeable barrier;

v) Vitrification or *in-situ* encapsulation to provide permanent immobilization;

vi) Enclosure of toxic sites using near-surface barriers, such as heavy plastic, that confine the contaminants; and

vii) Electromigration and electroosmosis involving creation of an electrical field by electrodes in the soil.

1.2.5 Development of alternative remediation methodologies

Several techniques are being developed as alternatives to the existing engineering-based approaches. These can be classified as bioremediating methodologies based on the use of biota to extract and/or modify the soil contaminants of concern. The organisms used for this purpose are microorganisms, plants or a combination of both. The term "phytoremediation" refers to the use of plants for remediation.

Remediation measures for sites with organic contaminants often combine bioremediation and engineering methods specifically tailored to the organic compound and local site conditions (Newman *et al.,* 1998). In some cases, phytoremediation alone may achieve excellent results, for example a 98% reduction in trichloroethylene within two years using *Populus trichocarpa* (Newman *et al.,* 1998). Results of field trials currently underway on a range of other contaminants such as carbon tetrachloride, pentachlorophenol, formaldehyde, tricloroethane, benzenes and toluenes, suggest that this promises to be a powerful technique (Newman *et al.,* 1998).

The applications of bioremediation to metal-contaminated sites include:

i) Bioleaching, which involves the activity of soil microorganisms in releasing metals from the soil into the interstitial water. The contaminated ground water is then collected and purified, leaving the soil structure largely undisturbed. This technique has been used for some time in ore leaching (Markert, 1993); and

ii) Phytoremediation, both in the various forms outlined in Section 1.3.1 and as hybrid technologies, in combination with traditional engineering techniques, has been successfully employed to remove metals from contaminated sites (Cunningham *et al.*, 1995; Newman *et al.*, 1998).

1.3 Phytoremediation

1.3.1 Definition

The term phytoremediation is derived from the Greek prefix *phyto*, for "plant," with the Latin suffix *remedium*, "to remedy" (Cunningham *et al.*, 1997). This emerging technology draws on the natural process of genetic adaptation of metal-tolerant plant ecotypes that survive and grow in metal-contaminated environments (Freedman, 1995).

Initially, the phytoremediation process was proposed as a means of extracting contaminants from the soil by plant physiological processes. The sequence of root uptake, translocation to shoots and harvesting is shown in Fig. 1.2. Several complementary processes outlined below from Chaudhry *et al.* (1998) may be involved:

- Phytostabilisation harnesses plant production of compounds that chemically immobilize metals at the soil and root interface, while the roots physically bind the soil and resist erosion and redistribution of toxic substances;

- Phytodegradation, or phytotransformation, involves enzyme-catalyzed metabolism of toxins, usually organic contaminants (Newman *et al.*, 1998), within plant tissues;

- Rhizofiltration, a process whereby roots of terrestrial plants extend into polluted waters and are able to concentrate selected elements (Brooks, 1997; Brooks and Robinson, 1997).

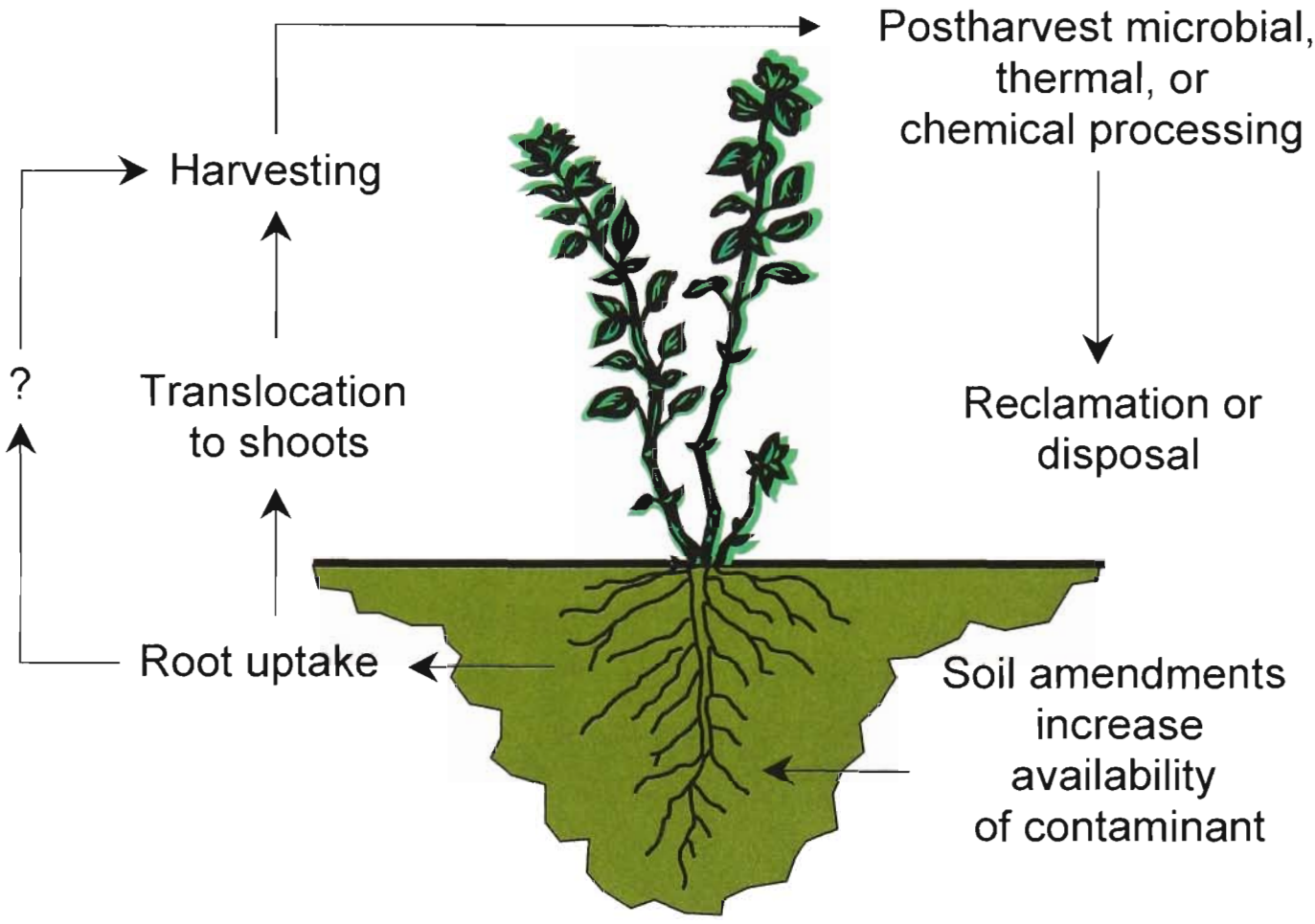


Figure 1.2 Processes involved in phytoextraction of contaminants from soils. Taken from Cunningham *et al.* (1995).

- Enhanced rhizosphere biodegradation, also known as plant-assisted bioremediation, involving release by plant roots of nutrients for bacteria and fungi, thus enhancing biological activity in the rhizosphere; and

- Absorption by plant roots of volatile organics (phytoextraction) and metabolism and release through the plant leaves by the process of phytovolatilization.

1.3.2 Origins of phytoremediation

Raskin coined the word "phytoremediation" in 1989 (Raskin *et al.*, 1994; Kumar *et al*, 1995). The association between plant communities and mineral composition of soil had been noted as early as the 16th Century, when Cesalpino identified a "serpentine flora" in Tuscany, Italy (Brooks, 1997, 1998). Raskin and coworkers expanded upon Russian research conducted in the 1930s on metal-accumulating plants in mineralized soil (Chase, 1995; Bing, 1996; Salt *et al.*, 1998). Since Raskin's pioneering work, the physiological plant uptake and bioaccumulation of toxicants have been increased substantially by selective breeding, genetic engineering, and chemical or microbial stimulants to enlarge plant biomass and accelerate growth rates (Cunningham *et al.*, 1995).

1.3.3 Application of phytoremediation to metal-contaminated sites

Examples of plant trials on metal-contaminated sites to date include the following:

- Swiss experiments showed that *Salix viminalis, Thlaspi caerulescens*, *Alyssum murale* and *Nicotiana tabacum* were capable of extracting nickel, zinc and cadmium from soil (Felix, 1997).

- Experiments in Belgium demonstrated the role of plant nutrients in metal uptake and established toxicant thresholds (Tolra *et al.*, 1996). *Thlaspi caerulescens* was shown to be capable of accumulating up to 4% zinc and high levels of cadmium (Brown *et al.*, 1994).

- On the US Pacific coast, *Streptanthus polygaloides*, which is endemic to the serpentine soils, was able to accumulate as much as 4,810 μg/g nickel in plant tissue, compared with 3,820 μg/g in the soil (Nicks and Chambers, 1997).

- Rehabilitation of mine tailings at Sudbury, Ontario by slope and drainage modification, lime, nitrogenous fertilizer and mulching provided an initial cover of grasses and legumes, followed by voluntary invasion of birch trees (Peters, 1984).

Trees are particularly well suited to phytoremediation, since they have substantial biomass and tend to be fast growing and hardy. Many species thrive in marginal land without need for soil improvement or irrigation. Tree root systems tend to be deep and pervasive, and the diverse soil microorganisms in their rhizosphere contribute to microbial detoxification (Stomp *et al.*, 1993). If trees need to be cut down to dispose of sequestered contaminants, many species regenerate freely from the stumps, thereby perpetuating the phytoremediation process.

Revegetation programs and phytoremediation trials have been conducted in several parts of Australia. Soils collected from three polluted sites in Sydney were used to test *Armeria maritima*, a known accumulator of lead and copper, *Impatiens balsamina*, an indicator plant for copper, *Alyssum saxatile* and *Brassica oleracea*, or cabbage, which provided a control. These experiments demonstrated that the soil texture and organic composition exerted a strong influence on metal uptake (Kelly and Guerin, 1995).

Research by Chaudhry *et al.* (1998) on the waste-slag heaps at the BHP Port Kembla steelworks showed that the metals were mainly present as tightly bound oxides, and therefore not in a bioavailable form. Despite this limitation to metal uptake, *Foeniculum vulgare* and *Sonchus oleraceus* accumulated cadmium, and *Ricinus communis* was effective in absorbing zinc.

1.3.4 Advantages of phytoremediation

Phytoremediation may be applied in isolation or in combination with other remediation methods within the broader fields of bioremediation and ecotoxicology. Plant-based remediation creates minimal disturbance, is environmentally friendly and aesthetically appealing (Nwosu *et al.*, 1991; Cornish *et al.*, 1995; Bing, 1996; Chaudhry *et al.*, 1998). The method has considerable cost advantages over conventional techniques (Table 1.1). Engineering-based remediation typically costs between A$20 and A$600/m^2, but phytoremediation may be as low as A$0.10/$m^2$ (Watanabe, 1997). Plants provide ecologically based solutions to mine sites (Table 1.2) plagued with physical, nutritional and toxicity problems (Bradshaw, 1996).

1.3.5 Practical limitations of phytoremediation

Apart from its slowness to take full effect, phytoremediation is not widely recognized by regulatory agencies, and there are liability concerns (Bradshaw, 1996; Chaudhry, *et al.*, 1998; Table 1.1). Phytoremediation is restricted to the depth of root penetration, ranging from a few millimetres to as much as 10 m, but maximum efficiency is achieved within the top 30 cm of the soil (Cunningham *et al.*, 1995). This may be well above the zone of maximum pollutant concentration (Cunningham and Berti, 1993). Furthermore, toxic substances may be bound in clay lattices, adsorbed in the humic fraction, occluded by oxide coatings, or chemically stable and thus extremely difficult to mobilize by plants. Even where phytoremediation is effective, contaminants may be reintroduced by ground water influx or windborne dust (Cunningham and Berti, 1993).

1.4 Metals in soils and plants

1.4.1 Factors influencing metal availability

The influence of metals on biota is determined by the chemical and physical state of the metal. This in turn will determine the ability of organisms to take up metal and transport it through their tissues. Factors influencing the rate and amount of metal

Table 1.1 Benefits and limitations of phytoremediation. Adapted from Cunningham *et al.* (1995) and Chaudhry *et al.* (1998). * Costs from Watanabe (1997).

Benefits	Limitations
Cost effective; as little as A$0.10/m^3 compared with as much as A$60-600/m^3 for conventional engineering methods*	Still mainly in developmental stage, so tends to be overlooked in favour of established methods
Aesthetically appealing, can offer natural parkland setting with recreational opportunities and minimal environmental disturbance	Slow to achieve full effect
While phytoremediation is underway, the site can be used for other purposes	Legal liability concerns
Can be applied to very large areas at the same time	Unsuited to treatment of severely contaminated sites
High level of public acceptance	Long lead time; regulatory agencies may impose limitations and constraints
Generally few toxic byproducts; low levels of waste generated	Some natural degradation products are highly toxic; potential contamination of food chain by grazing animals
Does not entail excavation, spoils dumps, dust emissions and acid drainage	Effectiveness limited by depth of root penetration, so unsuited to deeper contamination
Applicable to a wide range of contaminants, ranging from metals to organics	Ineffective in removing contaminants that are immobile or adsorbed to particle surfaces
Metal contaminants can be removed from the residues and recycled	Potential disposal problems
Potentially synergistic with other remediation technologies	Phased or parallel remediation methods not always possible.

Table 1.2 Major problems associated with mine sites, and their treatments. Adapted from Bradshaw (1996).

Limiting factor	Variable	Problem	Short-term treatment	Long-term treatment
1. Physical	Soil structure	Compacted	Rip or scarify	Revegetation
		Open	Compact or cover with fine material	Revegetation
	Stability	Unstable	Stabiliser or mulch	Regrade or vegetation
	Moisture	Wet	Drain	Drain
		Dry	Organic mulch	Regenerate with tolerant species
2. Nutritional deficiencies	Macronutrients	Nitrogen deficiency	Fertilizer	Legumes or other N-fixing species
		Other deficiency	Appropriate fertilizer	Fertilizer, or tolerant species
	Micronutrients	Deficient	Fertilizer	
3. Toxicity	pH	High levels	Organic matter or pyrite waste	Weathering, or tolerant species
		Low levels	Lime	Lime, or tolerant species
	Nutrients and metals	High levels	Organic matter or tolerant species	Inert covering, or tolerant species
	Salinity	High levels	Irrigation, gypsum, or tolerant species	Weathering, or tolerant species

taken into the plant include soil pH, organic content, cation exchange capacity, conductivity and transformations by fungal and microbial processes. Several of these factors are interdependent (Cunningham *et al.*, 1995; Azadpour and Matthews, 1996. Boyd, 1996; Brooks, 1997). A schematic portrayal of the processes involved in metal speciation in soils is shown in Fig. 1.3. These processes associated with mineral weathering range from liberation of free ions and their uptake by plants to complexing with organic matter, microbial ingestion, precipitation, adsorption, diffusion and occlusion (Azadpour and Matthews, 1996).

Soil pH. Solubility of metals is strongly influenced by pH (Kabata-Pendias and Pendias, 1984; Azadpour and Matthews, 1996). Plants normally show a negative correlation between metal uptake and increasing soil pH (Kabata-Pendias and Pendias, 1984). Conversely, lowering soil pH will allow for trace metal desorption from particulate matter and enhance metal mobility and concentration in solution. Thus, for phytoextraction, the use of acidifiers, such as ammonium-containing fertilizers, can increase metal bioavailability and hence plant uptake (Chaudhry *et al.*, 1998).

Extreme acidity is created by breakdown of pyrite (Ferm, 1974). Subsoil acidity inhibits deep root penetration and the uptake of nutrients and moisture (Marschner, 1986). Excessive manganese levels and deficiencies of calcium, magnesium and phosphorus are characteristic of plants in acidic soils of this type. Plant adaptation to very acid soils requires a high manganese tolerance and efficient use of mineral nutrients (Marschner, 1986).

Organic content. Organic matter complexes with metals and under certain conditions can render them temporarily innocuous. However, accumulation of metals bound to organic matter in soils can, under certain conditions, become a source of metal contamination. Organic matter in soils results from the breakdown of vegetation and animal products by predominantly bacterial processes. Decomposition of terrestrial plant material under water-saturated conditions yields humic matter, whereas breakdown of algal matter yields hydrogen- and lipid-rich

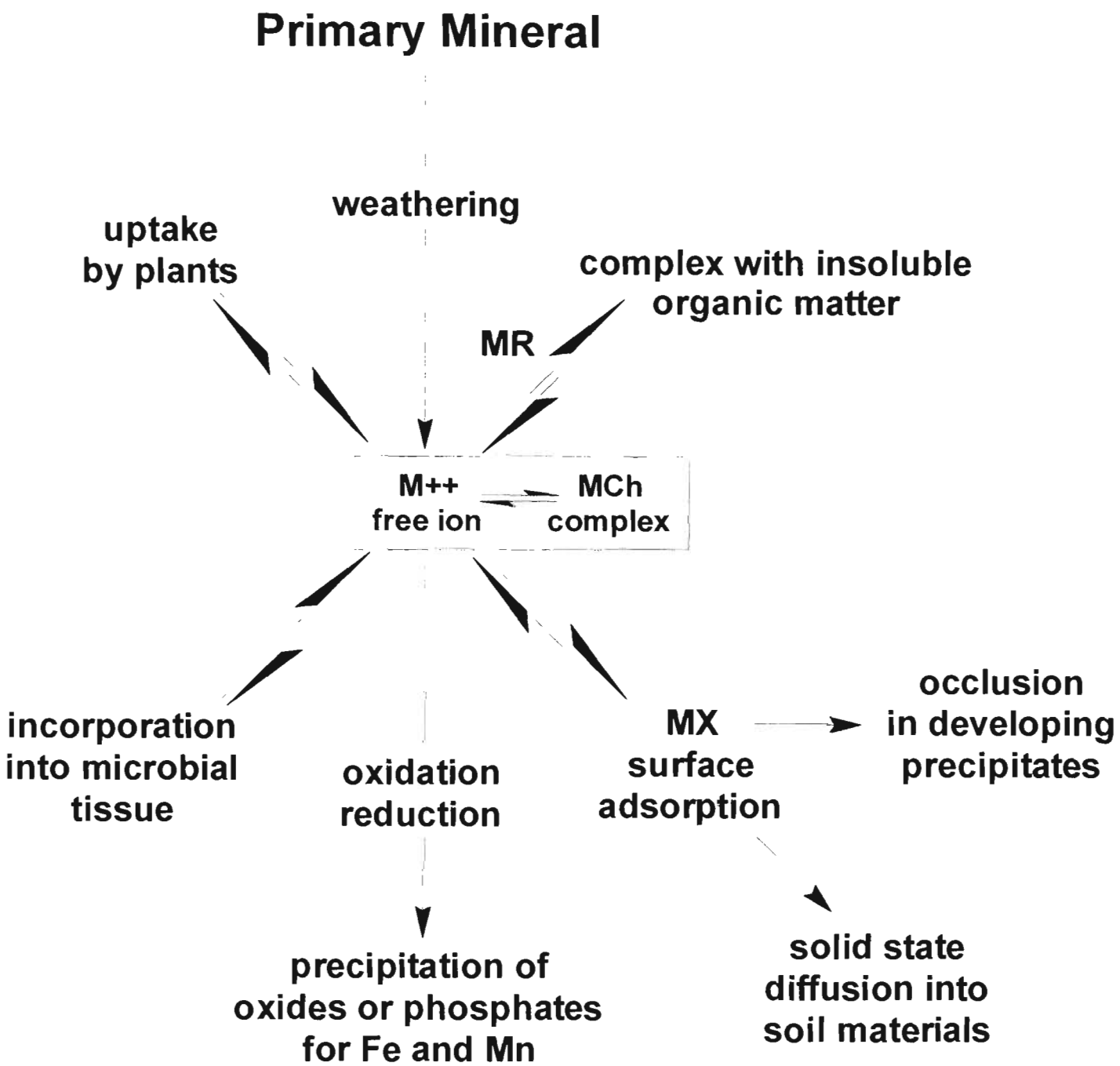


Figure 1.3. Factors affecting bioavailability of metals in soils to plants. Taken from Azadpour and Matthews (1996). M = metal; MCh = metal chelate complex. M^{++} and MCh complex are in solution.

sapropelic matter, precursors of liptinite (D.K. Hobday, personal communication). Humic substances bond to clay particles in soil and interact with metals by ion exchange, sorption and chelation. As organic matter accumulates, it reduces soil toxicity by complexing the available metals (Bradshaw, 1996).

Humic substances have a very strong affinity for cations. A hectare of soil containing 4% humus is capable of binding almost 18,000 kg of iron, 4,500 kg of lead, 1,517 kg copper, 1,015 kg zinc and 913 kg manganese (Kabata-Pendias and Pendias, 1984). These complexes are relatively insoluble, and plants grown in peatland soils may show a deficiency in copper and zinc because the metals are strongly bound by the insoluble humic acid. Conversely, addition of organic material may sometimes increase activity by microorganisms that can reduce iron and manganese, and therefore increase their bioavailability (Kabata-Pendias and Pendias, 1984). Thus, organic soils can act both as a source and as a sink for metals.

Given the crucial role of humic matter in soils and aquatic ecosystems because of their ability to sequester pollutants, recent studies are focusing on their molecular structure using *in situ* techniques. Microspectroscopic studies of the electronic and physical properties of humic substances reveal differences in biological reactivity and affinity for sorbing contaminants (Astheimer *et al.*, 2000).

Cation exchange capacity. Cation exchange capacity (CEC) reflects the ability of a cation in solution to replace another cation that is bound to silica-alumina clay mineral assemblages (Bates and Jackson, 1980). Bioavailability generally decreases with increased cation exchange capacity. The simple act of adding lime to soils increases both pH and CEC, thereby reducing bioavailability (Azadpour and Matthews, 1996).

Electrical conductivity. Electrical conductivity (EC), a measure of current flow under an applied electrical field (Bates and Jackson, 1980), is used to measure soil salinity in soil-water slurries. Conductivity tends to be high in mine tailings, where

it can limit plant growth. In agriculture, most crops are badly affected where soils with plentiful moisture have electrical conductivity values of 4,000 to 8,000 μS/cm. Only the most tolerant crops will grow within the range of 8,000 to 16,000 μS/cm. If EC readings are above 16,000 μS/cm, no crops will survive. In areas such as the Captains Flat dumps in NSW, the saturation extracts of slimes and solids have electrical conductivity readings as high as 31,000 μS/cm to 42,000 μS/cm (Craze, 1977 a and c). In some Colorado mine wastes, readings have been as high as 63,000 μS/cm (Craze, 1977 a and b).

Conductivity is commonly high in modern tailings because of pyrite decomposition (Ferm, 1974), as well as the presence of carbonates and evaporites. Chemical additives as well as natural processes may result in elevated salinity. Apart from the effect on pH, and hence bioavailability of metals, these salts inhibit establishment of a healthy vegetative cover. High electrical conductivity values, as are present in the Captains Flat, NSW dumps, may therefore provide obstacles to phytoremediation (Craze, 1977 a and b).

Effect of metal oxides. The presence of iron and manganese oxides commonly decreases bioavailability of many metals and renders them relatively immobile. However, plant roots have the ability to reduce iron from the ferric to ferrous form, which is more readily assimilated by plants. A low oxidation level of iron compounds could therefore contribute to high uptake, attaining toxic levels (Kabata-Pendias and Pendias, 1984).

1.4.2 Metals in plants

Some metals have an important physiological role in plants whereas others have no known or defined function (Table 1.3). Metals that are also trace elements (*e.g.* copper, zinc, manganese) cause deficiencies if they are present in too low concentrations in the soils, and lead to toxicity if the levels are too high. Some of the biochemical functions of metals in plants are shown in Table 1.3.

Copper. Copper is fixed in soils by a range of processes including adsorption, occlusion and coprecipitation, organic chelation and complexing, and microbial fixation (Kabata-Pendias and Pendias, 1984). Copper plays an important role in photosynthesis and is therefore essential for plant health, but optimal levels in most plants are easily exceeded. Copper tends to be concentrated in plant root tissue, with limited capacity to migrate to the shoots except during phases of intense growth (Kabata-Pendias and Pendias, 1984). Compartmentation of copper within cytoplasm and in vacuoles as soluble or insoluble complexes is the most important process of copper tolerance (Marschner, 1986).

Table 1.3 Principal functions of selected metals in plants. Adapted from Kabata-Pendias and Pendias (1984).

Metal	Biological association	Biochemical functions in plants
Copper	Various plastocyanins, and oxidases	Oxidation, photosynthesis, carbohydrate and protein metabolism, also bound into miscellaneous small molecules including antibiotics and defence mechanisms, catalyse valence changes in the substrate, protection mechanisms for frost-hardy and drought-resistant plants.
Zinc	Anhydrases, dehydrogenases, peptidases and proteinases	Carbohydrate and protein metabolism, also storage or transport functions, catalyse valence changes in the substrate, protection mechanisms for frost-hardy and drought-resistant plants
Iron	Hemoproteins and nonheme iron proteins, ferrodoxins and dehydrogenases	Photosynthesis, nitrogen fixation, valence changes, incorporation into plant structural materials, storage or transport functions, catalyse valence changes in the substrate
Lead	None known	No known function in plants
Manganese	Many enzyme systems	Photoproduction of oxygen in chloroplasts, also storage or transport functions, catalyse valence changes in the substrate, protection mechanisms for frost-hardy and drought-resistant plants
Cadmium	None known	Complexed by macro molecules and proteins. No known function in plants

Zinc. Zinc is freely mobilized and reprecipitated in the soil zone where it contacts sulphides, or is bound with organics or clays (Kabata-Pendias and Pendias, 1984). Under suitable low pH, soluble forms of zinc are readily available to plants. Uptake of zinc, mainly as a divalent cation, bears a direct relationship to its concentration in soils (Kabata-Pendias and Pendias, 1984). Other elements such as copper, iron and cadmium can occupy the same exchange sites on root surfaces, so an abundance of any of these competing elements may inhibit plant uptake of zinc.

Iron. Uptake of iron by plants is metabolically controlled. The ability of plant roots to reduce Fe^{3+} to Fe^{2+} is thought to be essential to the absorption of the cation by most plants. Iron is not readily transported in plant tissues, and the levels of iron are relatively low in tissue of plants experiencing active growth (Kabata-Pendias and Pendias, 1984). Nonetheless, most iron is stored in green leaves, where it is concentrated in chloroplasts (Marschner, 1986). Iron uptake and transport between plant organs are strongly influenced by soil pH, oxidation level, calcium and phosphorous content, and proportions of competing metals (Kabata-Pendias and Pendias, 1984).

Lead. Lead is the "most immutable of substances" (Francis, 1994), potentially highly toxic, but generally insoluble and relatively immobile in soils (Cole, 2000). Close association of lead with organic-rich soils confirms the role of carbon absorption and formation of insoluble lead chelates with organic matter (Lepp, 1981). With few exceptions such as plants belonging to the genus *Thlaspi* and several species from the family Brassicaceae, little lead tends to be taken up by plants regardless of its soil concentration (Kabata-Pendias and Pendias, 1984). The presence of calcium further decreases the ability of plants to assimilate lead (Lepp, 1981). The small quantities of lead that are taken up by roots are immobile, with only minor amounts reaching the plant shoots (Kabata-Pendias and Pendias, 1984).

Manganese. Manganese mobility is highly sensitive to redox conditions in the soil (Kabata-Pendias and Pendias, 1984), and its bioavailability is highest in acidic,

poorly drained soils (Lepp, 1981). Oxygenation decreases bioavailability of manganese, but reducing conditions increase uptake of the metal to potentially toxic levels (Kabata-Pendias and Pendias, 1984). Manganese is taken up readily and moves freely within plants (Kabata-Pendias and Pendias, 1984). Manganese is crucial to photosynthesis and its requirement as a mineral nutrient relates to its presence in metalloproteins.

Cadmium. Cadmium is more mobile than lead, especially in low pH conditions (Francis, 1994), such as exist at Mt. Costigan. Plant uptake of cadmium is rapid, with as little as 1-10 ppm affecting plant growth, although toxic thresholds vary according to plant species (Francis, 1994).

1.4.3 Metal tolerance in plants

Essential elements are those that cannot be substituted for by others in their specific biochemical role, and without which the plant cannot grow properly and complete its metabolic cycle. Either an insufficiency or excessive supply of trace elements will hinder plant development and lead to physical abnormalities. The associations and functions of trace elements in plants vary (Kabata-Pendias and Pendias, 1984).

Plants have an ability to concentrate and metabolize a range of toxicants, but they cannot metabolize metals. One adaptation to contaminated soil is that plants develop mechanisms for accumulating metal without inducing any harmful effects (Cunningham *et al.*, 1995; Boyd, 1996). Boyd (1996) cites the example of Pennycress, a member of the genus *Thlaspi*, capable of accumulating as much as 125 kg of zinc per hectare in a year, while improved varieties may be capable of four times higher concentrations.

According to Marschner (1986), plant growth response to an essential metal has three clearly defined regions, as shown in Fig. 1.4:

i) Firstly, the growth rate increases progressively with increasing nutrient supply (deficient range);

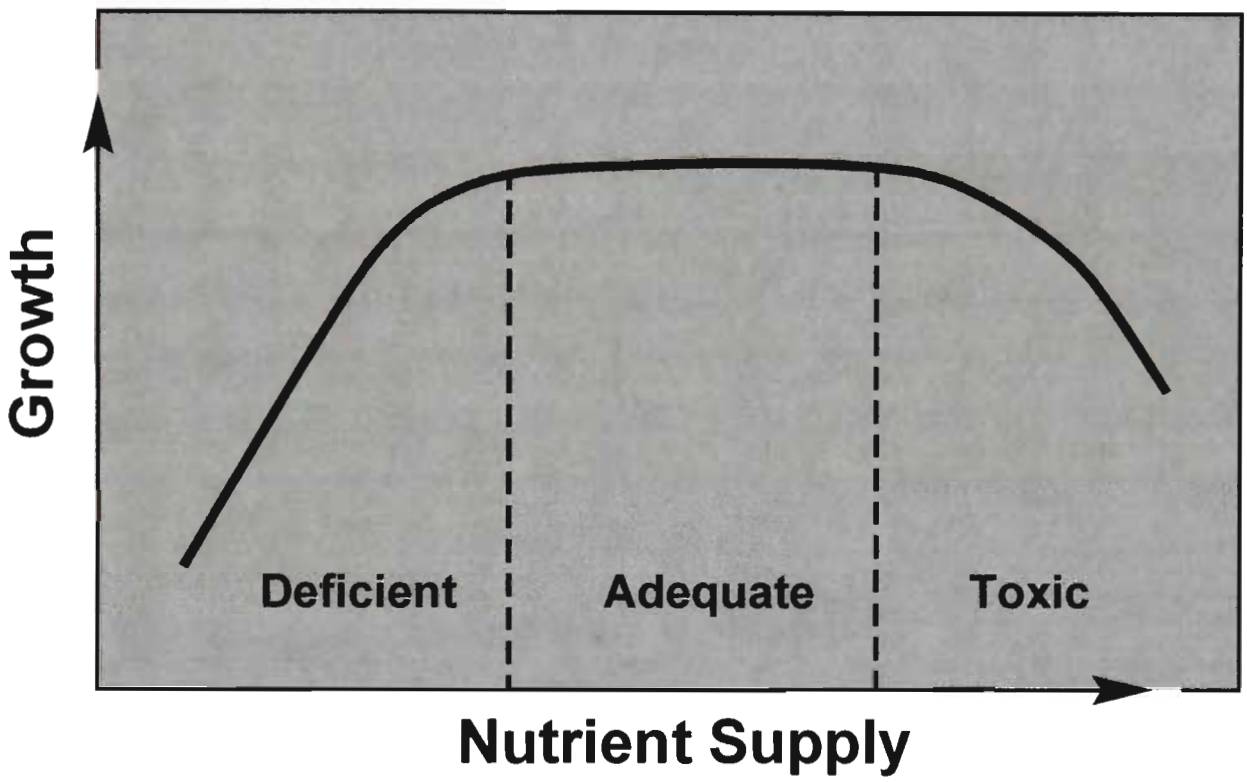


Figure 1.4 Plant response to nutrient uptake. Growth occurs within the deficient range, a plateau represents maximum uptake, with decreasing growth marking the toxic range. Taken from Marschner (1986).

ii) Secondly, the growth rate reaches a maximum, flattens out, and remains unaffected by further increase in nutrient supply (adequate or plateau range); and

iii) Finally, the growth rate falls with increasing nutrient supply (toxic range).

Plant growth is therefore maximized with optimal nutrient uptake between the critical deficiency and toxicity levels. The critical levels above and below optimal are not fixed amounts, but are defined as those levels at which growth is 5 to 10% below maximum. Critical toxicity levels can be determined in growth experiments under controlled environmental conditions, such as a glasshouse, by varying the metal levels in test soils over a wide concentration range (Marschner, 1986). The degree of toxicity depends on the plant's physiological state (*e.g.* reproductive activity) and the speciation of metal in the soil. Soil conditions also come into play in determining toxicity thresholds (Thurman, 1981).

1.4.4 Metal uptake and tolerance mechanisms

As a rule, plants tend to concentrate elements initially in their roots before transport to the plant shoots occurs. The amount transported to the plant tops depends on the chemistry of metal (Thurman, 1981). For example, zinc is quite mobile, but cadmium and iron are much less so, and lead is immobile. In addition, the photosynthetic tissue of plant tops has a limited tolerance and storage capacity for metals, further restricting transport. Three different patterns of uptake of metals into the shoots have been identified (Fig. 1.5). Accumulator species thrive despite absorbing abnormally high amounts of metals in their tissues. Indicator species show a more or less linear relationship between metal levels in the plant and soil, while excluder species tend to have lower metal levels than in the soil (Ross, 1994; Chaudhry *et al.,* 1998). The physiological and biochemical potential of a plant to accumulate, store and/or immobilize excess metal that has entered its tissues depends largely on the genetic tolerance that classify it as an accumulator, indicator or excluder.

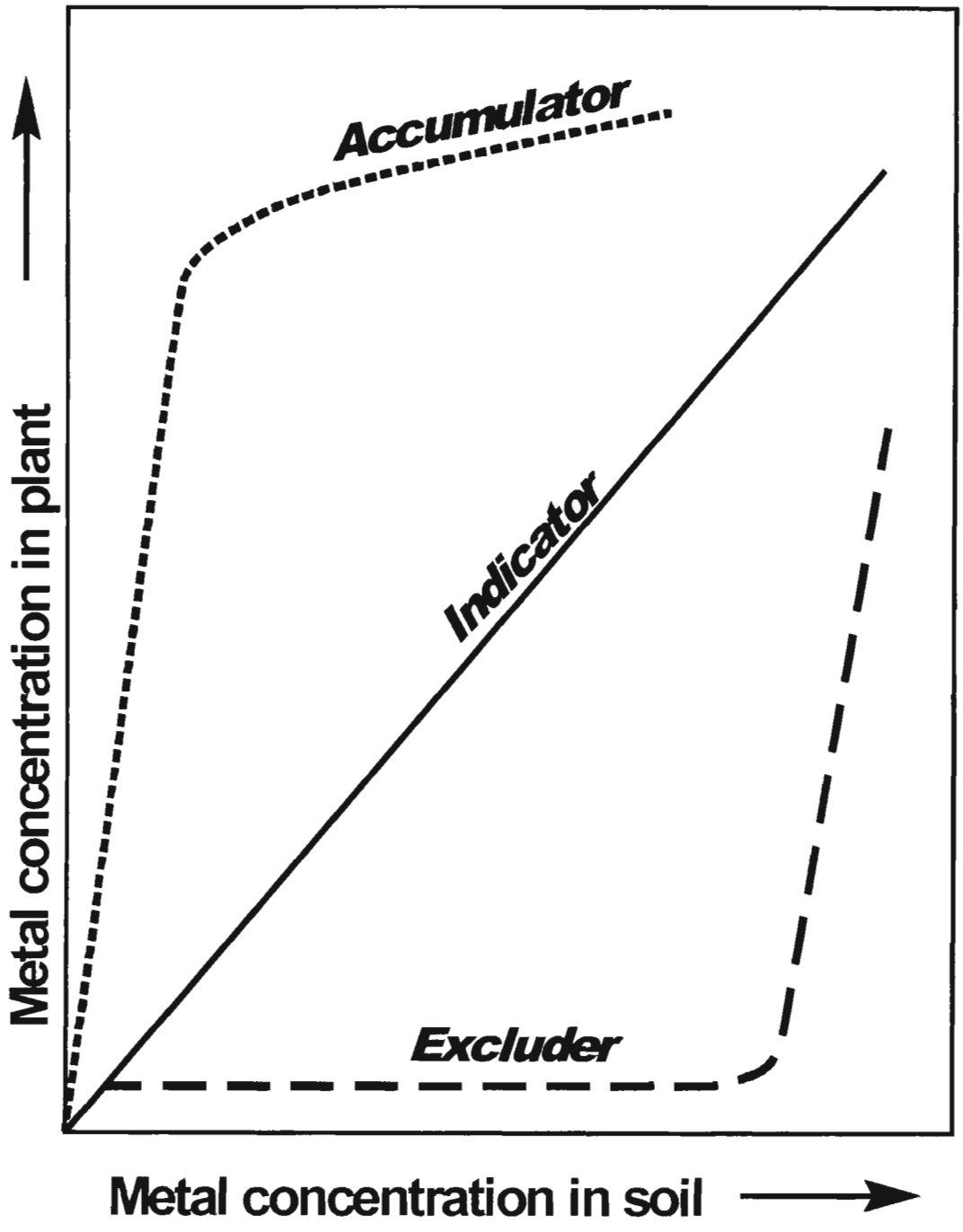


Figure 1.5 Different patterns of metal uptake by plants. From Ross (1994) and Chaudhry *et al.* (1998).

Root uptake. Metal absorption by plant roots can be either active (metabolic) or passive (nonmetabolic). The rate of trace element uptake tends to correlate positively with the available metal pool at the root surface, although varying markedly in absolute concentrations depending on plant tendencies, whether accumulator, indicator or excluder. Passive uptake is the diffusion of ions from the soil into the root endodermis, whilst active transport (also occurring in the roots) requires metabolic energy and occurs against a chemical gradient (Kabata-Pendias and Pendias, 1984).

The metals most readily available to plants are those that are in solution and adsorbed on clay minerals, whereas those fixed by oxides and bound onto microbes are less readily available. Ions and other materials released into the soil influence nutrient absorption by roots. States of cation oxidation around roots are of great importance in these processes. As pointed out by Kabata-Pendias and Pendias (1984), changes in the pH of the root ambient solution may play a significant role in the rate of availability of some trace elements. Acidification of soil can create a large increase in bioavailable soil metal (Freedman, 1995).

Some plants are more effective than others in taking up and tolerating elements from the soil, as discussed in detail below in Section 1.4.5. Further, the ability of plants to absorb different trace elements varies. Under the right conditions such as low pH, metals such as copper and zinc are easily absorbed, while lead remains less readily available to plants. Although elements such as lead tend to be extremely immobile in soil, a combination of factors such as soil chemistry, fungal processes and the presence of chelating agents can increase uptake (Cunningham *et al.*, 1995; Azadpour and Matthews, 1996; Boyd, 1996; and Brooks, 1997). Fungi have a specific affinity for some trace elements and are known to accumulate high levels of copper, zinc and cadmium (Kabata-Pendias and Pendias, 1984).

Foliar uptake. Dust can spread heavy-metal particles through the air and deposit them onto plant leaves. Foliar uptake consists of two phases: nonmetabolic

cuticular penetration, which is the major route of entry, and a primary metabolic mechanism. The latter allows element accumulation against a concentration gradient, and is responsible for ion transport across the plasma membrane into the cell protoplast (Kabata-Pendias and Pendias, 1984).

Trace elements absorbed by leaves can be sent to other plant tissues, including the plant roots. Rates of trace-element movement among tissues vary depending on the plant age, the plant organ and the element involved. Cadmium, lead and zinc absorbed by leaves do not move readily to the roots, whereas copper is comparatively mobile (Kabata-Pendias and Pendias, 1984).

Transport of metals within the plant. According to Kabata-Pendias and Pendias (1984), transport of ions within plants involves several processes:

i) Movement in the xylem;

ii) Movement in the phloem; and

iii) Storage, accumulation and immobilization.

Chelating ligands are very important in the control of cation movement in plants. Numerous other factors such as pH, redox state, hydrolysis, competing cations, polymerization, and formation of insoluble salts also govern metal mobility within plants (Kabata-Pendias and Pendias, 1984).

Transport of trace elements through vascular tissues (xylem and phloem) is partly related to root pressure and the intensity of transpiration (Marschner, 1986; Society for Mining, Metallurgy and Exploration, 1998). The rate of water flux, and hence absorbed metal transport across the root (short-distance transport) and in the xylem vessels (longer distance transport), is affected by soil moisture content, plant age and nutritional state, and the length of daylight. According to (Marschner, 1986), high soil metal concentrations enhance transpiration rate and metal transport, with uncharged molecules more mobile than ions. Maximum storage of most metals is observed in root tissue. Accumulation varies with the type of plant and growth season, and differs from one element to another. For example, zinc is bound to

organic compounds, whilst manganese is only partially complexed (Kabata-Pendias and Pendias, 1984).

Tolerance strategies. Plant tolerance strategies include exclusion or isolation in the rhizosphere, both of which take place before entry into the plant biomass. Once the metal has entered the plant cell, mediated tolerance comes into play. The following actions can then occur: absorption, restricted influx through plasma membrane, active efflux, production of phytochelatins, loading to vacuoles, transport to leaves, and adsorption to cell walls (Markert, 1993). These mechanisms allow plants to become more tolerant of metals taken up from the soil (Verkleij, 1991). Furthermore, tolerance may derive from development of metal-resistant enzymes, transport of metals to less-sensitive parts of the plant, and alteration of metabolic process (Azadpour and Matthews, 1996).

An unusual example of tolerance is found in a group of plants called hyperaccumulators, a term coined by Brooks (1977) for plants accumulating greater than 1,000 μg/g nickel on a dwt basis. Brooks (1997) noted that this value represents concentrations about 100 times higher than the maximum encountered in non-accumulating plants growing on the same serpentinite soils. Some hyperaccumulator species can exceed conventional plant metal levels by a factor of more than a thousand (Cornish *et al.*, 1995). Hyperaccumulating plants can take up and tolerate shoot concentrations of heavy metals in excess of 0.01% by dry weight of Cd, 0.1% Ni, Co, Pb, Cu and Cr, and 1-3% Zn and Mn (Cunningham *et al.*, 1997; Chaudhry *et al.* 1998). These plants are taxonomically widespread; however, the hyperaccumulating trait is quite rare, thus indicating a late appearance in the evolution of modern species (Cunningham *et al.*, 1997).

Tolerance within a particular plant species tends to be metal-specific, but most metalliferous soils contain a suite of metals (Azadpour and Matthews, 1996). For example, silver, lead, zinc, copper and cadmium commonly coexist. Further, tolerance varies within particular plants. In the case of the ryegrass *Lolium perenne*, cadmium levels were higher than mercury in the shoots, whereas mercury levels were greater than cadmium in the roots (Azadpour and Matthews, 1996).

Mechanisms of manganese tolerance are located in the shoots of some plant species. In mature leaves of tolerant species, the metal is distributed evenly but in non-tolerant species with the same manganese content, local spot-like accumulations of manganese occur (Marschner, 1986). Although many plant species show an affinity for a particular metal, Freedman (1995) noted that plants develop "cotolerance" mechanisms, whereby an ability to take up significant amounts of one metal will allow them to increase their uptake of other metals present in only small amounts, *e.g.* copper and zinc.

According to Kabata-Pendias and Pendias (1984), there are different mechanisms of resistance to metal uptake in plants, involving several internal metabolic processes. These are:

- Selective uptake of ions;
- Reduction of permeability of membranes or other changes in the structure and function of the membranes;
- Immobilization of metal ions in roots, shoots and seeds;
- Removal of ions from metabolism by storage in insoluble and/or fixed forms in organs and organelles;
- Changes in metabolic patterns;
- Adaptation to toxic metal replacement of a physiological metal in an enzyme; and
- Release of ions from plants by leaf shedding, leaching from foliage and root secretion.

Therefore, metals enter a plant by a transport process that bypasses defense mechanisms until the plant has absorbed an excessive amount. Non-essential metals can get in via a piggyback effect, for example where elements with a chemical similarity such as zinc and cadmium can be taken up together (Kabata-

Pendias and Pendias, 1984). Examples of metal concentrations in the shoots of plants grown on contaminated sites are shown in Table 1.4, but these high levels of metal uptake in plants are not a normal response.

1.4.5 Characteristics of toxic response

All metals are toxic to organisms if present in bioavailable form in excessive amount, with lead, cadmium, mercury, selenium and tin most severely affecting ecological health (Francis, 1994). According to Kabata-Pendias and Pendias (1984), metals most toxic to higher plants are copper, lead, cadmium, cobalt, nickel and mercury. Effects on plants of excessive metal concentrations in soil are shown in Table 1.5. Metal poisoning disrupts chlorophyll synthesis, reduces resistance to drought, limits nutrient uptake, and decreases photosynthesis because of damage to plant leaves. Azadpour and Matthews (1996) noted that cross-linking or binding of metals to pectin molecules makes cell walls rigid, but this reduces cellular elasticity and results in damage to the membrane, causing potassium leakage and restricting plant growth. Visible symptoms vary among species, and even among individual plants. However, necrosis, stunted growth, chlorotic or brown spots on leaves, and deformed coralloid roots are typical of metal excess (Kabata-Pendias and Pendias, 1984).

Freedman (1995) noted that soil acidification, such as is associated with sulphide-rich mine waste at Mt. Costigan, has a negative impact on plants, including symptoms such as root, leaf and cell damage, impaired metabolism, and susceptibility to metal toxicity and disease. Because lowered pH increases uptake of several metals to toxic levels (Section 1.4.3), acidification may serve to reinforce the toxic response of plants to metals.

There is a wide range of metal-toxicity symptoms among the various plant species. Although varying from one plant species to another, the critical toxicity level of copper is between 20 to 30 μg/g dwt in leaf tissue, above which there is chlorosis and inhibited root growth (Marschner, 1986). Kabata-Pendias and Pendias (1984) noted darkening of leaves and thick, short, or barbed-wire root development and

Table 1.4 Metal concentrations in shoots of hyperaccumulating plants. All values are expressed as tissue dwt basis. Modified after Cunningham *et al.* (1995) and Chaudhry *et al.* (1998).

Metal	Plant Species	Concentration %
Copper	*Aeollanthus biformifolius*	>0.1
	Ipomoea alpina	1.22
Zinc	***Thlaspi caerulescens***	2.1-5.1
	Thlaspi calaminare	>10
Lead	*Armeria maritima*	>1
	Brassica juncea	>10
	Helianthus annuus	>10
	Thlaspi caerulescens	0.1
	Thlaspi rotundifolium	0.08
Manganese	*Macadamia neurophylla*	5.2
	Psychotria douarrei	4.7
Cadmium	*Alyssum murale*	0.13
	Thlaspi caerulescens	0.02-0.11

depressed tillering as symptomatic of copper toxicity. Furthermore, excessive copper creates deficiencies in other elements, especially iron, causing induced iron chlorosis. Zinc toxicity is reflected in inhibited root elongation (Marschner, 1986), inter-veinal chlorosis, chlorotic and necrotic leaf tips, retarded growth and deformed roots. Toxic symptoms of excessive iron include dark-green foliage with purple-brown discoloration and stunted growth, while excessive lead uptake creates short, brown shoots and dark, wilted leaves (Kabata-Pendias and Pendias, 1984).

Table 1.5 Metal levels in plants and their effects on plant function. Adapted from Kabata-Pendias and Pendias (1984) and Marschner (1986).

Metal	Normal range (ppm dwt)	Excessive range (ppm dwt)	Effect on plants of excessive amounts of metal in soil
Copper	5-30	20-100	Changes in permeability of the cell membrane; accumulation in reproductive tissue
Zinc	27-150	100-400	Disrupted chlorophyll synthesis
Iron	variable	unknown	Excessive iron is rare because of restrictions to plant uptake
Lead	5-10	30-300	Changes in permeability of the cell membrane, and reactions of thiol groups with cations; minor potassium leakage
Manganese	20-300	300-500	Restricted calcium and manganese uptake
Cadmium	0.05-0.2	5-30	Changes in permeability of the cell membrane; markedly reduced cell elasticity

According to Marschner (1986), evidence of manganese toxicity is common, with crinkled leaves and brown spots representing precipitation of $Mn0_2$ surrounded by chlorotic zones. Stunted shoots and dried leaf tips are also symptomatic (Kabata-Pendias and Pendias, 1984). Excess manganese may induce deficiencies in other elements such as iron, magnesium and calcium, or reduce nitrogen uptake in plants such as acacias (Marschner, 1986).

1.5 Ecotoxicological approach

Ecotoxicology deals with the response of organisms to an impacted environment. It seeks to identify those factors, primarily chemical, which have a negative effect on the dynamics of organic populations and ecosystems, to quantify the effects of these relationships in ecosystems, to evaluate physical and functional changes in organisms exposed to natural or anthropogenic compounds, and to predict future impacts (USEPA, 2002 a and b). Phytoremediation may be applied in isolation, or in combination with other remediation methods within the broader fields of bioremediation and ecotoxicology.

1.5.1 Toxicity testing

The ecotoxicological approach includes the application of toxicity tests and bioassays to determine the composition, concentration, distribution and bioavailability of contaminants. The USEPA defines bioassay as "a test to determine the relative strength of a substance by comparing its effect on a test organism with that of a standard preparation". Procedures and standards are provided by the USEPA (2002 b) for chemical fields (the contaminants), species fields (the organisms), test-condition fields, exposure fields and impacts. Critical phytotoxicity levels can be determined in controlled glasshouse trials, but the physical and chemical state of the soil, metal diversity and speciation, and the plants' physiological state will affect toxic response thresholds (Marschner, 1986).

A limitation of many bioassays and toxicity tests is that they ignore the effects of combining more than one toxicant or the impact of "multiple stressors" (USEPA, 2002 a). For example, uptake of some metals is strongly affected by the presence of other metal elements (Tolra *et al.*, 1996), so single stressors may be misleading. Furthermore, microbial factors are not considered, and these may have a pronounced effect on biouptake (Kabata-Pendias and Pendias, 1984). According to the USEPA (2002), "investigation procedures are needed that can successfully

identify the stressors and lead to appropriate corrective measures through habitat restoration..."

1.5.2 The triad approach

The triad approach was developed by the USEPA as an integral component of ecological risk assessment for contaminated sites (Pascoe and DalSoglio, 1994). It involves:

- Chemical analyses at the site to establish the presence, distribution and concentration of contaminants;
- Ecological field assessment of the impacts; and
- Laboratory toxicity studies to establish dose-response relationships between the ecological effects and chemical contamination.

The USEPA guide for conducting ecological assessments at hazardous waste sites describes this triad of information as essential to establish a solid relationship between toxic wastes and ecological effects, and sets out the procedures employed (Pascoe and DalSoglio, 1994). An example of the triad approach was the Milltown Reservoir-Clark Fork River Superfund Site, Montana, USA. The area had been mined since 1864, with mine tailings surrounding the historical mining areas containing elevated levels of arsenic, cadmium, copper, lead and zinc, an association generally similar to Mt. Costigan. In 1989, the USEPA initiated a baseline risk assessment to characterize potential human health and ecological risks associated with soil contaminants. The foundation for this document was provided by the 1980 Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and the 1990 National Contingency Plan (Pascoe and DalSoglio, 1994).

1.6 This Study

1.6.1 The problem

Mining activities are a prolific source of metal contaminants, especially from ore stockpiles and tailings waste dumps, which require stabilization, rehabilitation, detoxification, or removal (Gardea-Torresdey *et al.*, 1996). Derelict mine sites number several hundred in NSW alone, and present environmental or safety problems (Johnson, 2000). Johnson (2000) notes that the rural communities in which these old mines are located generally lack the resources to conduct rehabilitation. The DMR therefore assumed the task of administering these problem sites (Section 2.3.2). Problems associated with mine sites typically involve poor soil structure, lack of nutrients, and toxic conditions (Tables 1.2 and 1.4).

The focus of this study is one such site, an abandoned mine site at Mt. Costigan, that has been subjected to unsuccessful remediation efforts in the past (Johnson, 2000). These remediation efforts were based mainly on soil amelioration and engineering modifications. No investigations were made concerning the suitability of plant species for replanting, though an attempt was made at a later stage to improve soil quality by surface addition of organic fertilizer, biosolid. Mt. Costigan was therefore recommended by the DMR as a suitable site for further investigations (Johnson, personal communication).

1.6.2 The approach

The investigations at the study site were based on the triad approach and therefore involved:

i) Chemical analysis of site soils to investigate soil quality and distribution and concentration levels of toxic metals;

ii) Ecological field assessment of plant distribution, speciation, and plant abundance relative to soil metal contamination, to determine potentially negative ecological impacts; and

iii) Glasshouse toxicity studies of soils from various parts of the site, and the adjacent woodland as a "reference" site, using selected native plant species to identify and document possible links between contaminant distribution and ecological impact.

1.6.3 Experimental objectives

Given the focus of this study on a biological assessment of site, and selection and testing of species that may prove useful in phytoremediation, the following objectives were addressed:

1. To assess the extent of metal contamination and soil degradation at Mt. Costigan mine site by analyzing soil chemistry, metal loadings and vegetation cover.

2. To compare the characteristics of the barren site with the adjacent woodland, which had revegetated naturally.

3. To assess, by means of laboratory/glasshouse toxicity tests, the degree of toxicity to plants of the barren-site soils, and to compare the results with those for the woodland soils.

4. To investigate, by means of toxicity tests, the effectiveness of biosolid amelioration used in prior site remediation.

5. To assess the tolerance of native tree species to degraded soil conditions, with the aim of identifying suitable species for phytoremediation.

6. Based on the findings of this study, to evaluate the methods previously used to remediate the site, and suggest future research and field trials that might be useful for future site remediation.

Section A: Field Studies

Chapter 2. Field studies: Introduction

2.1 Background

Metalliferous mining was established in the latter half of the 19th century in the Western Slopes region of NSW (Markham, 1961; Speer, 1991a; Minfact, 1997). Only a few of the many small mines proved to be economically viable, and by 1930s almost all had been abandoned (Felton, 1977). This resulted in a legacy of environmental problems such as soil degradation, loss of vegetation and wildlife habitat, subsidence, contamination of ground water and impacts on catchment health (DMR, 1998; Johnson, 2000).

Over the years, attempts by NSW government instrumentalities to remediate the damage met with only limited success. Most of the remediation efforts were based on engineering solutions of containment and reduction in soil erosion, although several attempts were made to revegetate the derelict, abandoned metalliferous mines (Johnson, 2000).

The field assessment component of this study was designed to investigate the existing vegetation and soil characteristics, including metal-contamination levels, at Mt. Costigan, one of the larger abandoned mines in the area between Goulburn and Bathurst. As with many other former mine sites, some of the disturbed area had revegetated naturally, while a section of the site remains largely barren. According to Johnson (2000), restoration attempts at revegetating the barren site had been largely unsuccessful.

The major aim of the field studies in this project was therefore:

- To investigate site factors such as soil characteristics and contaminant metal content that might have contributed to the patchy revegetation in the area; and

- To carry out complementary studies of the distribution and diversity of the vegetation at the site.

These field studies, and the subsequent soil analyses, therefore contributed two of the three elements of the triad approach to this investigation: field assessment and determination of the degree of chemical contamination of the site.

2.2 Study-site region

2.2.1 Geology

The palaeogeography of NSW (Fig. 2.1) during Mid to Late Silurian, approximately 380-435 million years ago, shows descending oceanic crust moving westward beneath the continental lithosphere (Scheibner, 1999). Landward of the accretionary prism, comprising sediments scraped off the subducting plate, were volcanic ridges and back-arc rift basins. These rift basins accumulated parallel belts of sediments, with volcanics in the south (Markham and Basden, 1975). Mt. Costigan is located in Late Silurian rocks within one of these rift basins termed the Hill End Trough, east of the dominantly volcanic Molong Rise (Fig. 2.1). Hill End Trough was tectonically unstable, accumulating marine shale and turbidites interspersed with lava and volcanic ash, or tuff (Felton, 1977). The region was subsequently uplifted and folded by Devonian earth movements (Scheibner, 1999). The present-day geological zonation, as illustrated in Fig. 2.1, extends approximately north-south and reflects several stages of deformation. Silurian Kangaloolah Volcanics east of the Mt. Costigan project study site are separated by a major fault from intercalated fine-grained sediments and acid volcanics to the west. The sedimentary rocks are described by Felton (1977) as tuffaceous shales and greywackes with minor calcareous bands, reflecting sporadic turbidite deposition in a dominantly pelagic forearc basin. The area remained volcanically active, with venting and exhalations interspersed with eruptive events. A geological sketch map from the Mt. Costigan area (Fig. 2.2) demonstrates the structural complexity.

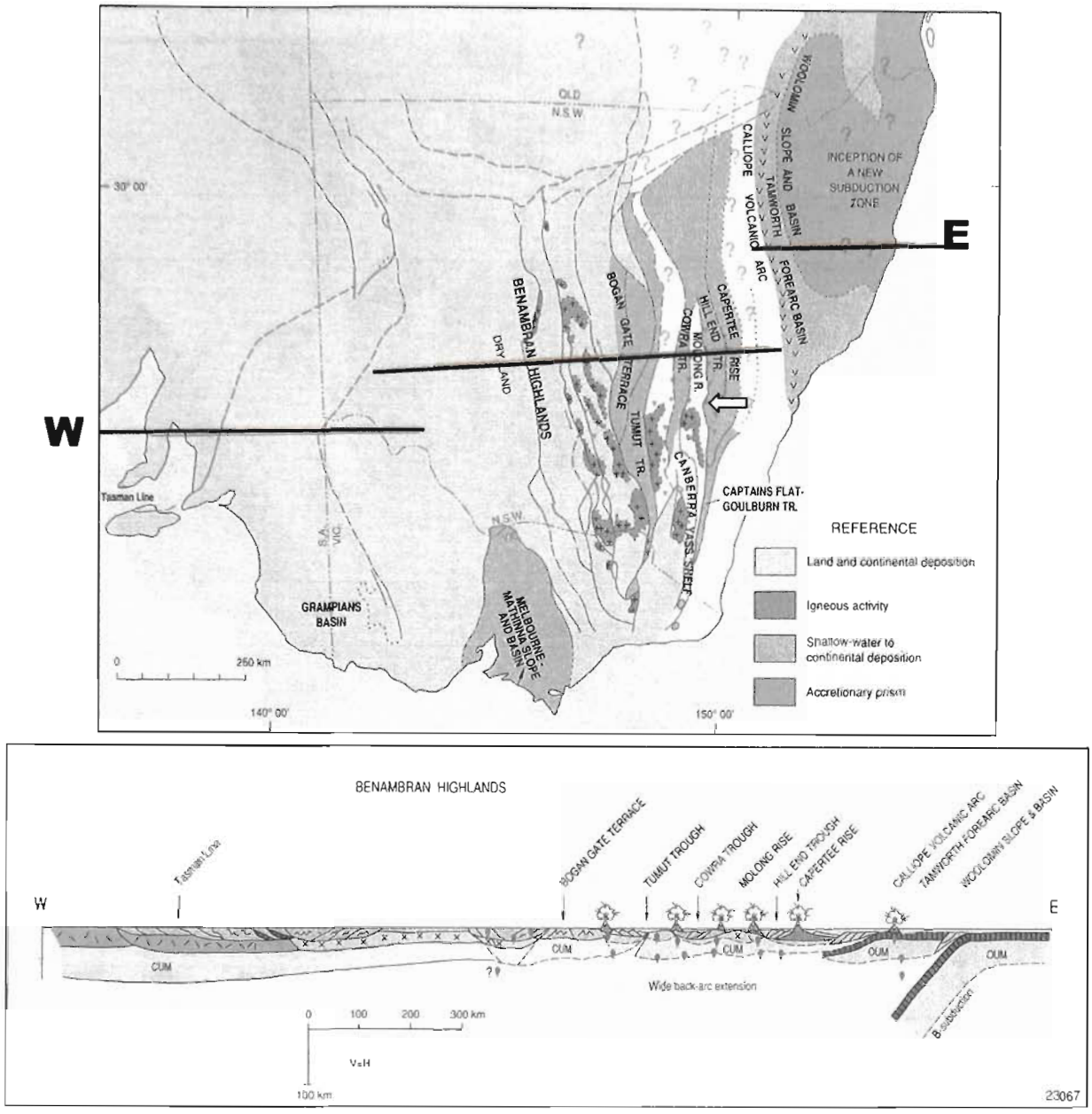
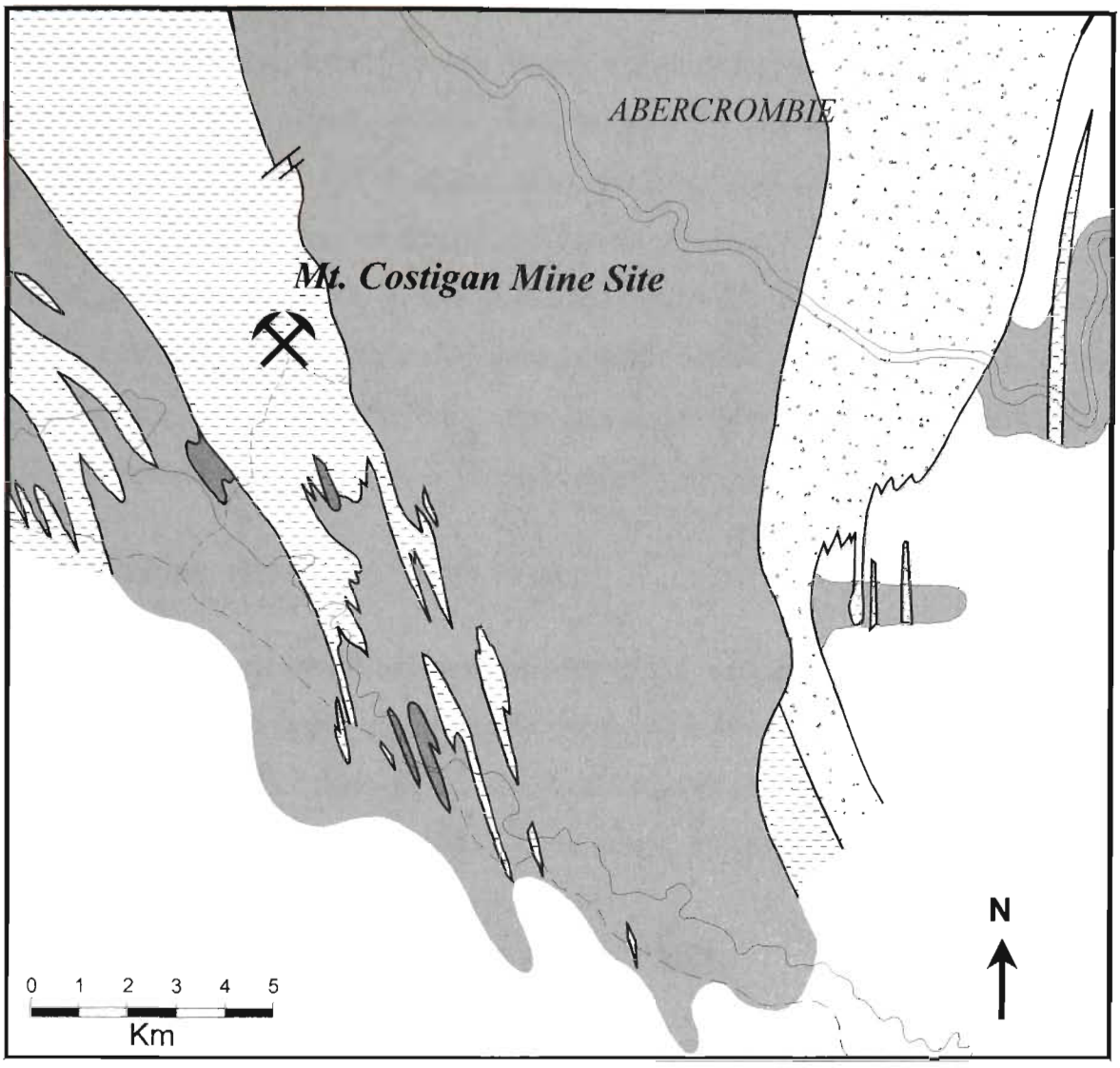


Figure 2.1. Palaeographic map (top) and reconstructed E-W cross section (bottom) for the NSW area during the Middle-Late Silurian. Mt. Costigan is located in the Hill End Trough (arrowed), west of the descending oceanic plate and volcanic arc. Taken from Scheibner (1999).



Geological Legend

- Acid Volcanics
- Intermediate to Basic Volcanics
- Tuffaceous Sandstone and Greywacke, Tuffs, Quartzite
- Shales

Figure 2.2. Geological sketch map of the Mt. Costigan area. Modified from undated New Consolidated Goldfields report, unpublished file at the NSW Department of Mineral Resources.

2.2.2 Landscape of the area

The natural ecology of the Western Slopes region comprises temperate, perennial pastureland (Lamp *et al.*, 1990), with stands of eucalypt woodland and an understory of acacias. Mt. Costigan mine site (Fig. 1.1) lies in the centre of this geographic region, 95 km north-northwest of Goulburn, 149^0 17' E, 34^0 29' S, at an approximate altitude of 900 metres above sea level. The landscape in the region is gently undulating in the south, with greater relief towards the north (Fig. 2.3), and includes rich pastureland (Fig. 2.4). The area is drained by the Abercrombie River and its tributaries, Tuena Creek in the west and Cooks Vale Creek in the east.

2.2.3 History of mining in the area

The Western Slopes of the Southern Highlands supported a large number of metalliferous mines that were set up in the latter half of 19th Century (Markham, 1961; Speer, 1991a). The mines attracted large numbers of workers from the overcrowded diggings in Victoria. These early prospectors identified stepwise offsets of the ore bodies. The structures provided a guide to exploring and small-scale mining of the "line of lode" (Speer, 1991 a and b).

Many of the mines were short-lived; some were closed, and then re-opened as new technologies became available or metal prices increased. Only three mines, Mt. Costigan, Cordillera and Peelwood, produced any worthwhile economic yield (Markham, 1961; Jododex Relinquishment Report, 1972). All three sites are now derelict, and like many other abandoned metalliferous mine sites in NSW, they contain high metal levels in the soil (Minfact, 1997; Johnson, 2000).

2.3 Mt. Costigan Site

2.3.1 Site Description

The Mt. Costigan site consists of a barren, west-facing slope where mining activity took place (Fig. 2.5), with open woodland and grassy undergrowth covering the

Figure 2.3. Topography and vegetation in the Mt. Costigan region. (Taken in March 1999.)

Figure 2.4. Rich pastureland in the Crookwell/Mt. Costigan district. (Taken in March 1999.)

adjacent areas (Fig. 2.6). Originally, the entire site had been cleared for mining, so that the eucalypt-dominated woodland is a result of regeneration (Johnson, 2000). The remaining barren area is sparsely vegetated by clumps of low, herbaceous plants (Fig. 2.7). A focus of the field assessment in this study was, therefore, to identify site-specific factors that contributed to only partial regeneration of the site. Thus, the revegetated woodland surrounding the barren slope was used as the reference site for this study.

2.3.2 Metal ores at Mt. Costigan

In 1953, the Electrolytic Zinc Company of Australasia Ltd. carried out a detailed mapping program which confirmed that Mt. Costigan mineralization is concentrated in fine-grained phyllites rich in quartz, sericite, and chlorite. Higher ore grades are located near the contact with coarse, volcanic tuffs. The ore bodies reflect a combination of stratigraphic and structural controls. They follow a sheared boundary between pelagic shale of the Peelwood Formation and coarse volcanic tuff of the Kangaloolah volcanics, pitching northward and dipping to the west at 60 degrees (Markham, 1961). The ore occurs both as massive replacement bodies and as small, crosscutting quartz-sulphide veins. Wall-rock alteration effects include a local bleaching in the area of the mineralization and the development of zones of silicification.

The dominant bedrock minerals at Mt. Costigan are pyrite, sphalerite, chalcopyrite and galena. Table 2.1 illustrates the typical range of metal ores, together with their chemical composition and relative abundance, at Mt. Costigan and nearby Peelwood and Cordillera mine sites. At Mt. Costigan, pyrite-rich bands with minor sphalerite and galena alternate with sphalerite-rich bands. Sphalerite commonly contains other elements such as arsenic and cadmium, both of which are present in Mt. Costigan soils (Markham, 1961). Chalcocite and pyrrhotite (analogous to pyrite but with a deficiency in ferrous ions), together with minor quantities of tetrahedrite, bournonite and jamesonite (Markham, 1961) are common metal sulphide ores (Bates and Jackson, 1980). Secondary minerals created by surface exposure and infiltration of

Figure 2.5. View of the barren site with surrounding woodland at Mt. Costigan. Terraced bands (berms) are remnants of previous remediation attempts. The northern woodland is visible as a fringe on the top right. (Taken in August, 1998.)

Figure 2.6. View of the eastern woodland at Mt. Costigan. Grassy undergrowth is mainly tussock grass; the bare foreground is the top of the barren site. (Taken in August, 1998.)

Table 2.1 Composition and relative abundance of minerals in the Mt. Costigan-Peelwood district, NSW. Mt. Costigan, Peelwood and Cordillera mines are geologically similar and in close proximity. **X** = trace; **XX** = minor amount; **XXX** = moderate amount; **XXXX** = abundant; - = absent; **?** = not known. (Modified from Markham, 1961).

Metal	Mineral	Composition	Mt. Costigan	Peelwood	Cordillera
Copper	Chalcocite	Cu_2S	X	XXXX	X
	Chalcopyrite	$(CuFe)S_2$	XXX	XXXX	XXX
	Bornite	Cu_5FeS_4	-	XXXX	-
	Pyrrhotite	Cu_2S	X	X	X
	Covellite	CuS	X	XXXX	X
	Cuprite	Cu_2O	X	?	?
	Malachite	$Cu_2Co_3(OH)_2$	X	?	?
	Azurite	$Cu_3(Co_3)_2(OH)_2$	X	?	?
	Tetrahedrite	$(CuFe)_{12}Sb_4S_{13}$	XX	XX	XXX
Zinc	Sphalerite	(Zn Fe)S	XXXX	XXXX	XXXX
	Franklinite	$(ZnFeMn)_2O_3$	?	?	?
Iron	Pyrite	FeS_2	XXXX	XXXX	XXXX
	Arsenopyrite	(AsFe)S	XX	XX	XXX
	Jamesonite	$FePb_6S_{14}$	X	-	X
	Marcasite	FeS_2	X	X	X
	Hematite	Fe_2O_3	XXXX	XXXX	XXXX
	Limonite	$Fe_2O_3(OH)$	XXX	?	?
	Magnetite	Fe_3O_4	X	?	?
Manganese	Pyrolusite	MnO_2	XX	XX	XX
Lead	Galena	PbS	XXX	XXX	XXX
	Bournonite	$PbCuSbS_3$	X	-	X
Other metals	Bauxite	Al_2O_3, $2H_2O$	XX	XX	XX
	Scheelite	$CaWO_4$	?	?	X

oxidizing ground water include hematite in its deep reddish-brown earthy form, and minor amounts of the alteration products of copper sulphides: secondary covellite, cuprite, malachite and azurite (Fig. 2.8).

2.3.3 Mining at Mt Costigan

Principal mineral resources at Mt. Costigan were copper, lead, zinc and silver, with minor amounts of gold and antimony (Markham, 1961). Records in the Geological Survey of NSW indicate that the area was worked commercially during the periods 1887-1888, 1892-1893, and 1911-1915, but only very sporadically thereafter. The first notification of gold in the Mt. Costigan region was in 1851 by Edward Hargreaves (Speer, 1991b). According to Speer (1991b) the *Goulburn Herald* reported at that time that gold prospecting in the Tuena district had been highly successful, with the more successful prospectors finding as much as 6 to 8 ounces of gold per day.

Peelwood Hill Silver Mining Company initially mined copper, with byproducts of silver and gold, later adding zinc and lead. Mining difficulties and low economic yields associated with the complex ore caused the mine to close in 1878 (Minfo, 1997). Mt. Costigan was reactivated in 1880, closing once more in 1889 because of the difficulty and expense of treating the ore. In addition to the open-cut excavations, the old workings at Mt. Costigan also comprised four shafts plus underground drives and stopes. In 1901, copper and lead were recovered from the old furnace dumps at Mt. Costigan and adjacent mine sites. The slag was sent to Sydney for extraction of the metals (Peelwood Mine Record, 1995). The *Goulburn Evening Penny Post* (cited in Speer, 1991b) mentioned a rich copper find in July 1903, when a man clearing timber found lumps of shiny metal around his fires, which had apparently smelted the ore. This renewed interest in the area, but a lack of capital soon led to abandonment of the claims (Speer, 1991a). From 1916 until 1928 there was small-scale extraction of copper from shallow, oxidized ore, and the deposits have been reworked sporadically since then, but with no worthwhile commercial returns.

Figure 2.7. Sparse vegetation on the barren slope. Grass tussocks and stunted herbaceous plants amid the rocky substratum. (Taken November, 1998.)

Figure 2.8. Secondary minerals on the surface of the barren slope. Alteration products of copper and iron minerals on the weathered surface. (Taken November, 1998.)

As of 1971, the historical values for Mt. Costigan of extracted minerals (Table 2.2) were recorded on Geological Survey of New South Wales data sheets (Markham, 1961). According to these records, the original ore body was in the modest size category of 10,000-100,000 t.

Table 2.2 Ore extracted from Mt. Costigan. The figures are an estimate of average historical grades and total tonnage of metal extracted since mining began. Data derived from Felton (1975).

Metal recovered	Copper	Lead	Silver	Gold
Average grades (%)	12	8	003	0.00015
Metal extracted (tonnes)	67	440	4	6×10^{-4}

2.4 The need for remediation of abandoned mine sites

2.4.1 Ecological and environmental concerns

The Western Slopes of the Southern Highlands (coincident with the Great Dividing Range) are in the rain shadow, and are therefore relatively arid. Consequently, water is a scarce and valuable commodity. The abandoned mines in the region are frequently poorly revegetated, with acidic soils of low organic content and high metal concentrations. In addition to this, the sites tend to be on steep slopes subject to erosion. The possible consequences of soil erosion could impact the health of the whole catchment and pose a health risk as contaminated runoff reaches streams and rivers of the drainage basins.

2.4.2 Remediation objectives

Derelict mine sites such as Peelwood and Mt. Costigan are remnants of an earlier, less-regulated approach to environmental management. New environmental regulations ensure that responsibility for contamination, site safety or offsite

impacts now lies with the landowner or controller of the site (Johnson, 2000). Since 1974, the NSW Department of Mineral Resources (DMR) has administered the Derelict Mined Lands Rehabilitation Program. The primary goals of this Program are amelioration of site-contamination sources and prevention of offsite contaminant migration, while also improving biological diversity. To complement these objectives, the DMR has formed an Environment Unit to advocate and enforce the adoption of environmentally responsible management practices throughout the NSW mining industry. The Unit's main purpose is to coordinate the management of issues related to the environment across the DMR's operational branches. The DMR is also the primary contact point for other Government agencies such as the Department of Land and Water Conservation (DLWC) and the NSW Environmental Protection Authority (NSW EPA), as well as for industry and the public, so that environmental management standards across NSW can be regulated and improved (DMR, 1998).

The DMR is particularly concerned about a number of despoiled, abandoned mines in the NSW Southern Highlands, where mining was active towards the turn of the century. Many of these abandoned sites are now experiencing problems with soil erosion and transport of contaminated water and sediment into adjoining catchments (DMR, 1998; Johnson, 2000). The Derelict Mined Lands Rehabilitation Program ensures that abandoned mines are remediated to the standards that meet expectations of the public. This Program includes input from the DMR, DLWC and EPA, who review proposed rehabilitation projects and provide advice on suitable remediation techniques (DMR, 1998).

The DMR requires that once rehabilitation is complete, the restored sites should be capable of sustained, beneficial land use compatible with activities typical of the region (DMR, 1998; Johnson, 2000). The mechanisms used by the DMR (1998) to encourage and enforce the completion of satisfactory rehabilitation on sites in NSW are:

i) Environmental audits;

ii) Environmental management plans, which monitor the progress of remediation and environmental management at mining operations. These are review and forecast documents that monitor changes in process, performance and management strategies; and

iii) Security deposits, which are lodged by mining companies to ensure compliance with the conditions of titles, mainly in relation to remediation. These are refunded after completion of an acceptable standard of rehabilitation is achieved, in compliance with DMR standards.

2.4.3 Regional remediation case studies

Over the past 50 years there have been a number of attempts to restore several derelict mine sites in the Western Slopes region of NSW. These efforts were mainly based on engineering methods, with revegetation programs being a minor component. In general, phytoremediation strategies are virtually nonexistent in the restoration of old mine sites in Australia. There have also been few attempts to assess the site-specific parameters and address these by using suitable revegetation strategies, in conjunction with other amelioration methods. Some relevant examples are provided below.

Captains Flat. One of the largest and most expensive operations to remediate a metalliferous mine site in Australia was at Captains Flat, NSW (Craze, 1977a, b, c). The mine was operated during the period 1874-1962, leaving 2.5 million tonnes of stockpiled waste covering 15 hectares. Runoff from the site, exacerbated by erosion and collapse of the waste dumps, contaminated the nearby Molonglo River, which enters the waterways of Canberra, with heavy metals. The Soil Conservation Unit of the NSW Department of Mineral Resources assumed responsibility for the revegetation of the mines. Large amounts of lime were applied to the denuded surface to reduce acidity, but vegetation was slow to return. Other remedial measures included:

- Reshaping the mine dumps and diverting surface discharge;

- Covering reshaped dumps with layers of clay, rock and soil; and

- Revegetating with a mixture of grasses and clover seeds.

Sunny Corner. There have been no restoration programs at this historic mine site, although it has been used for research projects to investigate the metal tolerance of the existing vegetation (Chaudhry *et al.*, 1998). These projects have demonstrated the capabilities of plants already growing in the area to remove metals from the contaminated site, which contained high levels of cadmium (18-34% w/w soil), copper (2-18%) and zinc (22-33%). Although the diversity of plant species was low, abundant *Baeckea utilis*, *Pinus radiata* and *Poa labillardieri* demonstrated their exceptional tolerance to high metal concentrations. Furthermore, these plants showed evidence of high metal uptake. *Baeckea utilis* contained elevated levels of copper, lead and zinc in the roots. *Poa labillardieri* roots accumulated lead to >0.1% by weight, while *Pinus radiata* accumulated lead in its roots and zinc in the leaves. Other plant species were also capable of taking up metals to varying degrees. *Lomandra longifolia* accumulated copper in its roots, and lead and zinc in both roots and leaves, while *Acacia melanoxylon* also contained elevated zinc in root and shoot tissue (Chaudhry *et al.*, 1998).

Peelwood. Peelwood mine site bears considerable similarity to the site at Mt Costigan. Like Mt Costigan, Peelwood site is privately owned. Both sites have been subjected to extensive restoration efforts by the DMR over the last 3 to 4 years, with particular emphasis on Peelwood. At Peelwood, a 7 hectare barren scar adjacent to the mine was subject to erosion, causing siltation and metal contamination of nearby creeks. Mine adits and shafts were subject to caving, adding to the hazardous nature of the site (Johnson, 2000). Soil and runoff analyses by the DMR identified copper, lead and zinc as the principal contaminants. In 1988, the DMR, funded by the Derelict Mined Lands Rehabilitation Program, contracted its Soil Conservation Unit to conduct remediation work. The abandoned mine shafts were filled with mine waste and sealed with clay to prevent leaching, the unstable upper slopes were reshaped, and berms constructed to prevent release of

silt and toxic leachate into nearby watercourses. In 1994, concrete-lined flumes and sediment dams were built, but attempts at revegetation using a range of grass, acacias and eucalypt species were thwarted by drought and acid soils (Minfo, 1997). Increased funding of the Derelict Mined Lands Rehabilitation Program led to renewed efforts to rehabilitate the Peelwood site in late 1995. Addition of Nitrohumus (a sewage sludge and compost product) augmented with lime served to reduce acidity, helping develop a soil cover for plants to become established. Post-remediation tests of water flowing from the site showed improved pH and lower metal levels. However, the revegetation attempt has not been successful, and a recent investigation has demonstrated that the combined effects of metal contamination, salinity and poor soil texture were the most likely cause for poor vegetation cover (Rowe, 2001).

Mt. Costigan. As mentioned above, a large proportion of the mountain top here had been originally cleared for mining, but most of it had revegetated naturally. There remained approximately two hectares of barren land on a steep slope with mine shafts and extensive erosion. During the early 1990s, attempts at restoration included terracing the slope, stabilising it with straw and planting with mainly exotic fodder grasses, just as had been done at Peelwood (Johnson, 2000). Neither the revegetation nor earthworks had been successful in reducing the erosion and runoff from the barren site (Figs. 2.9 and 2.10).

The field studies described in this project as pre-remediation assessment examined the site characteristics at that stage, while site soils were collected for the glasshouse toxicity studies. Subsequently, further restoration works carried out by the DMR at the site in 1999 included earthworks, erection of concrete flumes to channel surface runoff, construction of a runoff dam, surface fertilizing the site with Nitrohumus, and additional, more extensive revegetation. Post-remediation assessment of the site soils and vegetation was then carried out, and the results are presented here.

Figure 2.9. Barren slope viewed towards the edge of the eastern woodland. After heavy rains, the surface is eroded by sheetwash and small gullies. The gullies deepen over time and grow headward. (Taken November, 1998.)

Figure 2.10. Erosion at Mt. Costigan mine site. Severe erosional gullying of the upslope margin of the barren slope area bounding the eastern woodland. (Taken March, 1999.)

2.5 Outline of field studies at Mt. Costigan

2.5.1 Rationale

Because the entire site had previously been covered with tailings, a focus of these field investigations was to compare the barren site with the self-revegetated surrounding woodland with regard to vegetation, soil metal content, and other soil characteristics. The conceptual approach used was similar to that adopted at the Milltown Reservoir-Clark Fork River site, Montana (Pascoe and DalSoglio, 1994), where metal-contaminant problems were also related to historical mining activities. To complete the triad approach to the problem of metal contamination at the derelict mine site, the toxicity of the site soil and its effects on plants was examined in glasshouse experiments described in Section B of this thesis.

2.5.2 Experimental design

The comparison of soils and vegetation of the woodland and barren site was carried out in order to identify the factors governing successful revegetation. The woodland was therefore used as a reference site for what could be considered a successful natural regeneration process on a once-impacted site. The experimental design thus involved a comparison of the metal status of this predominantly eucalypt woodland, as well as the soil and vegetation characteristics. This scheme, together with the glasshouse growth trials that followed (see Chapters 5-7), provided for the first time an assessment of the site where ecological concerns and potential environmental risks were identified.

2.5.3 Experimental objectives

The primary aims of the field studies segment of this project were to investigate factors affecting the distribution of vegetation on the site in order to obtain scientific basis for further management strategies at the site.

The specific objectives of these field studies were, therefore, to:

- Identify the major differences in soil parameters, including metal content, between the naturally revegetated area and the barren site;

- Document the distribution and diversity of vegetation cover at the barren site and compare it with the naturally revegetated surrounding woodland; and

- Compare the results of post-remediation assessment, carried out during the course of this project, especially the effects on vegetation and soil, with those of pre-remediation initial assessment.

As previous restoration attempts by the DMR to revegetate the barren site have been largely unsuccessful, the results of these field studies should assist in the future remediation efforts on this site and similar abandoned metalliferous sites across southeastern Australia.

Chapter 3. Field studies: materials & methods

3.1 Field measurements

The aim of the field assessment was to investigate site characteristics likely to control spontaneous revegetation of the site, and thus identify future management strategies. Since it is known that the entire hillside was once contaminated with mining wastes, the naturally revegetated woodland was used as a reference site for the examination of the barren site. A distant, unimpacted reference site was not included because of the difficulty in matching aspect, latitude, soils and, therefore, vegetation type.

Field assessment of the soil and vegetation on the barren slope and in the adjacent woodland was carried out to establish soil conditions, metal contaminant levels and vegetation distribution patterns. A pre-remediation assessment was conducted during February 1999, when the site soil and vegetation were sampled according to the design described below. As mentioned in Chapter 2, during the second half of 1999 the DMR carried out a remediation program that included engineering modifications to the site and soil amelioration with Nitrohumus. Subsequently, a post-remediation field assessment was carried out by the candidate in April 2000, on the barren site only, to investigate the effects of remediation, using the same methodology as for pre-remediation assessment.

3.1.1 Sampling design

Woodland. Six sampling stations (M1 to M6) were selected in the woodland: two sites in the eastern woodland (M1, M2) above the mine site, two in the northern woodland (M3, M4), and two to the south (M5, M6) (Fig. 3.1). Two additional woodland locations were sampled for soil quality metal analysis by the DMR, one in the eastern woodland and one in the northern woodland. Nested quadrats (10 m^2) were laid out at each station to be used for vegetation structure and soil sampling and analyses (see Section 3.1.3). The quadrats were defined by means of four tape

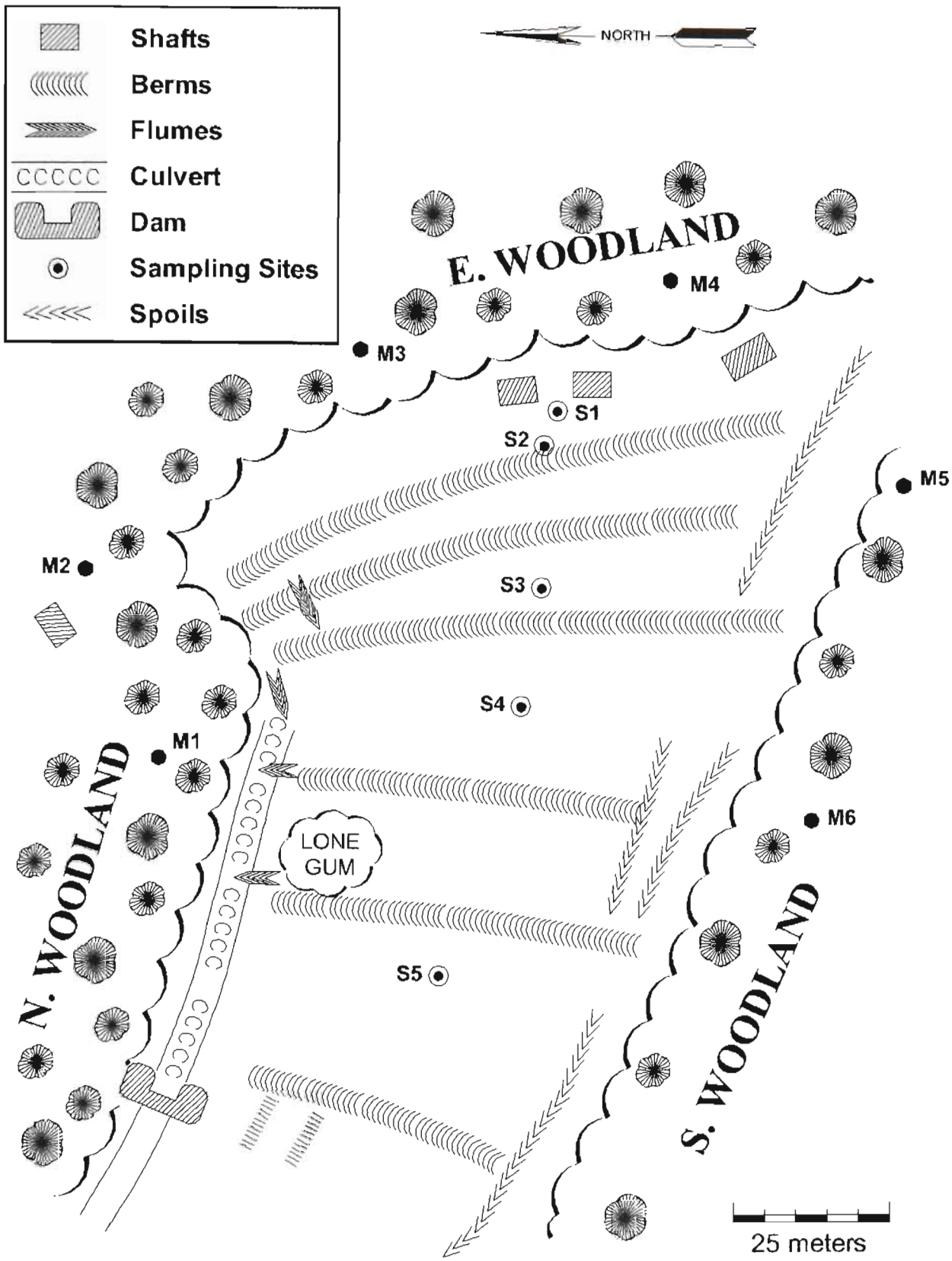


Fig. 3.1 Location of sampling sites at Mt. Costigan. Included are locations of mine shafts and DMR construction works, and sampling sites in the north (N), east (E) and south (S) woodlands (M1 to M6) and on the barren slope (S1 to S5).

measures at right angles to one another, marked unambiguously with set distances attached to a central loop. Each pair of tape measures created the diagonals of the largest quadrat, while the markers indicated the smaller quadrats nested within it (Table 3.1). The nested quadrat was set up by fastening the central loop of the tapes to the centre peg, and stretching out the tapes along the points of the compass.

Table 3.1 Nested quadrat dimensions, in metres. Based on Outhred and Buckney (1983).

Area (m^2)	Diagonal (m)	Half-diagonal (m)	Incremental area of subquadrant (m^2)
0.1	0.44	0.22	0
0.2	0.64	0.32	0.1
0.5	1.0	0.50	0.3
1.0	1.42	0.71	0.5
2.0	2.0	1.0	1.0
5.0	3.16	1.58	3.0
10	4.48	2.24	5.0

Barren site. A single transect was laid out down the centre of the barren site (Fig. 3.1). Five sampling stations (S1 to S5) were selected, starting from the top of the site and then down the transect onto each terrace. At each sampling station, a nested quadrat was laid out as described for the woodland vegetation assessment above, except that a maximum area of 10 m^2 was used for ground-cover vegetation assessment and soil sampling (Fig. 3.2).

3.1.2 Soil sampling

Soil samples were collected for later analysis of characteristics and metal content. At all sampling stations, both woodland and barren site, soil samples (1 kg) were

collected at all stations (M1-M6 and S1-S5) approximately 1-2 m on either side of the centre peg of the sampling quadrat (Section 3.1.1). The first sample was taken to the north of the peg, and the second to the south, in each case after gravel had been cleared from the surface, and soil sampled from the top 10 cm. The two samples from each station were thoroughly blended in the laboratory, and three subsamples collected from each soil mixture. Additional samples (North-1 and East-1) were taken from the woodlands for DMR soil-quality and metal analysis. Post-remediation sampling involved a repetition of the above method. Chapter 6, Section 6.2.3 outlines the preparations of these post-remediation soils.

3.1.3 Vegetation sampling and analysis

Woodland: trees. Starting from the centre, smallest quadrat of the nested sampling quadrat, each tree encountered was identified as belonging to a particular species and height class. It was then scored for tree height, crown diameter, projective foliage cover (PFC), and girth at breast height (GBH). Scoring continued until 20 individuals of same species and height class were scored. The quadrat where this occurred was noted, and this area was used for calculations of density and number. Photographs were taken to document vegetation cover, and plant specimens were collected for later identification.

Mean values were calculated for each parameter for each height class in every species. Density per hectare was also calculated, as was the biomass of trees according to the following allometric relationship (Whittaker and Woodwell, 1968):

$$\log_{10} y = A + B \log_{10} x$$

Where A = 2.2968; B = 2.1357;

y is biomass in g, and

x is diameter at breast height (DBH) in cm, derived from GBH (GBH = π. DBH).

Woodland: ground cover. Ground cover was estimated using 0.25m² quadrats (Fig. 3.3) placed in the north, central and southern points of the 10 m² nested

Figure 3.2 Nested quadrats (10 m^2) used to estimate vegetation cover on the barren slope. Mine shafts surrounded by wire fences are visible in the background.

Figure 3.3 Sampling quadrat (0.5 x 0.5 m) used to estimate ground cover.

quadrat at each station. In each of the 0.25 m^2 quadrat, each species was scored as percentage cover of the quadrat. Percent Foliage Cover (PFC) for the tree canopy was also determined for the area directly above each 0.25 m^2 quadrat. Distribution and height of ground cover were recorded. Average height was determined from three randomly selected plants of the same species within the small quadrats.

Barren site. The nested quadrats were set up as described above. The estimate of biomass here was, however, carried out in a different way from what was done in the woodland. Beginning in the innermost quadrat, the plants were recorded by name or description, the frequency counted, and percentage ground cover estimated. This was done for each of the four segments of each quadrat. Each quadrat was studied in sequence and continued until the total area of 10 m^2 was covered. The values obtained were combined to derive an average for the quadrat. This method yielded species diversity and distribution and density of each species.

Plant identification. Plant specimens were taken for identification at the UTS Herbarium, Gore Hill; identifications of the eucalypt samples from Mt. Costigan were confirmed by staff at the Royal Botanical Gardens, Sydney. Photographic records were compiled for all specimens.

3.2 Soil analysis

3.2.1 Procedures for analyses

Soils collected during the first field assessment, i.e. pre-remediation, in 1999 were analysed by the staff at geochemical laboratories of the Department of Mineral Resources (DMR), Lidcombe, NSW. This is an accredited laboratory that carries out all the soil analyses for the DMR. Barren-site soils collected after remediation, as well as southern woodland soil samples, were prepared for glasshouse experiments (See Chapter 6). Soils collected post-remediation were analysed by the candidate at UTS environmental science laboratories, at Gore Hill, NSW.

Pre-remediation soil analysis at the DMR laboratories. After sieving to <2 mm, samples were analyzed in a water leach solution (18 hr, 1:5 w/w) for pH, electrical

conductivity and sulphate content. The following analyses were then carried out on the < 150 μm and the < 2 mm soil fractions:

- Organic matter, as determined by loss of ignition at 550° C
- Sulphate content and conductivity (EC)
- Elemental analysis for copper, zinc, iron, manganese, lead, arsenic and cadmium, using AAS.

Additional pre-remediation and post-remediation soil analyses at UTS laboratories. The soil samples collected in the field were air dried and sieved using a 2 mm sieve. The <2 mm fraction was used to determine pH, conductivity and organic matter content as described below, as well as metal content as detailed in Section 3.3.

3.2.2 Determination of soil pH

The pH of air-dried soils was determined using soil (5 g) suspension series in water and in 1M KCl in ratios of 1:3 soil:liquid. Samples were shaken vigorously every 10 minutes for one hour. The series in the H_2O and KCl were measured consecutively to ensure equal settling times using a 4710 WTW pH Meter, Weilheim, Germany. The instruments were standardized according to the manufacturer's instructions.

3.2.3 Determination of soil salinity

Salinity was measured via electrical conductivity (EC) of soil suspensions. Conductivity of the soil suspension series prepared in water for pH determinations was measured using LF 95 WTW Conductivity Meter, Weilheim, Germany. The conductivity meter was standardized according to the manufacturer's instructions with 0.01M KCl.

3.2.4 Determination of soil organic content

Organic content of soils was determined by Loss of Ignition (LOI) method. Initial soil mass (approximately 5 g air-dried soil) was dried in a pre-weighed Pyrex beaker at 108° C, for 24 hours, its weight recorded. It was then heated in a muffle furnace (550° C, 2 hours). After cooling, final mass was determined. The loss of weight on ignition, calculated as % of the oven-dried weight, was used as an estimate of organic content of the soil.

3.2.5 Determination of soil metal content

Air-dried soils were oven-dried (108° C, 2 days) and then finely ground using a mortar and pestle. Soil samples (0.5 g) were then digested using the nitric acid/hydrogen peroxide method of Krishnamurty *et al.* (1976). Samples were placed in 50 mL glass beakers covered with a watch glass and digested with refluxing on a sand-bath (100° C). Blank samples (HNO_3 only) and international reference standards (calcareous loam, citrus leaves) were used routinely.

The digestion was conducted over a period of several hours until the digests appeared clear or light straw-coloured. Where sufficient digestion was not occurring, HNO_3 was added. The beakers were allowed to cool to room temperature, and 2-8 ml hydrogen peroxide (H_2O_2) was added with shaking, to remove any residual organic matrix.

The digests were cooled and filtered into volumetric flasks (10, 25, or 50 ml) using Advantec 5A filter paper, with rinsing. The solutions were made up to the volume with reagent grade water. Acid-washed glassware was used throughout.

The prepared digests, including international standards and blanks, were then used to determine cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), and zinc (Zn), using the Varian SpectrAA 800, Atomic Absorption Spectrophotometer (Varian Pty. Ltd., Melbourne, Australia), with settings recommended by the manufacturer. Reference standards were made for each of the metals being measured, using analytical reagent-grade solutions.

3.3 Materials

All chemicals, including metal standards for AAS, were analytical reagent grade. Reagent-grade water was prepared and purified by reverse osmosis (RO). International standards for AAS were issued by the National Bureau of Standards (NBS), Washington, D.C. and purchased from Graham B. Jackson Pty. Ltd., Dandenong, Victoria.

3.4 Data Analysis

Statistical analyses of data were preformed using the Microsoft Excel program. Means were derived for each group, and standard error (± se) was determined and tabulated. Arithmetic means were calculated from assumed normal distributions. Student's t-Test was used to determine whether or not the means of two sample populations were significantly different.

Chapter 4. Field studies: results & discussion

4.1 Soil characteristics prior to site remediation

The investigation of soil characteristics and metal contamination was of major importance in this study because soils were expected to have a determining role in the revegetation success at the site.

4.1.1 Soil quality

Results of woodland and barren-site soil analyses are shown in Table 4.1. The pH of the woodland soils was mildly acidic, averaging 5.1, slightly lower than on the barren slope. These values are towards the middle of the pH range of 3.7-6.2 recorded from old base-metal tailings at Sudbury, Ontario (Peters, 1984).

Table 4.1 Soil characteristics of the study sites. Woodland soils were collected from north and east subsites by the candidate and analysed by DMR; soils from barren sites prior to remediation were collected from top, middle and base subsites and analysed by DMR. Post remediation analyses were carried out on top, middle and base subsite soils at UTS. Soil parameter values are shown as means ± se with n = 2 for woodland site, n = 5 for barren site prior to remediation, and n = 3 for barren site after remediation. Salinity levels were determined in 1:5 soil extracts of woodland and pre-remediation soils at DMR laboratories, while 1:3 extracts were used for post-remediation soil analyses at UTS. Trace values for SO_4 are < 5 mg/kg; - = not analysed.

Sampling location	pH	Salinity (µS/cm)	SO_4 (mg/kg)	OM (Loss On ignition %)
Woodland	5.1 ± 0.1	91 ± 94	<5	9.6 ± 5.4
Barren site (Pre-remediation)	6.5 ± 0.7	354 ± 138	130 ± 84	5.6 ± 1
Barren site (Post-remediation)	4.9 ± 0.7	497 ± 118	-	5.7 ± 1

Pre-remediation pH of the barren-site soils was higher than the woodlands, averaging 6.5. The more neutral pH of the barren-slope soils may have been the results of ground-water concentration of lime that was originally added over the entire barren site by DMR during an earlier remediation program. Values for individual locations on the barren slope will be discussed in chapter 7.

Salinities of the woodland soils, measured in terms of electrical conductivities (EC) and sulphate content, were low overall, whereas those on the barren site were several-fold higher (Table 4.1). On the other hand, levels of organic matter (OM) in the woodland soils were higher than on the barren slope.

Thus, the main difference between woodland and barren-site soils was in their salinity levels (EC and SO_4 in Table 4.1). The high salinity values recorded on the barren site are consistent with reports on derelict mine soils in literature (Kabata-Pendias and Pendias, 1984; Freedman, 1995). This is consistent with DMR observations of the presence of "acid seeps" (Johnson, personal communication) at the site. After remediation it was noted that white encrustations were present on the rocks and soil surface, presumably representing salt precipitation by evaporation of ground-water discharge.

4.1.2 Soil-metal content

Metal levels varied greatly throughout the Mt. Costigan site, both among the woodland and barren-site soils (Table 4.2). Metal levels in woodland soils were within the range for normal soils; only lead marginally exceeded international limits but was below NSW EPA residential limits (Table 4.3).

Barren-slope pre-remediation metal levels were high. Concentrations of copper, zinc and lead were substantially higher on the barren site than in the surrounding woodland, while those for iron and manganese were at least double. These values fall within the general range for mine-site soils reported by Kabata-Pendias and Pendias (1984) for copper and lead (Table 4.2), and are similar to metal levels in the old Copper Cliff tailings, Sudbury, Ontario, reported by Peters (1984). But metal loadings are an order of magnitude lower than copper and cadmium in refinery soils

near Liverpool, England (Freedman, 1995), and are also below the reported ranges for zinc and cadmium in mine-site soils elsewhere (Table 4.3).

Table 4.2 Metal levels in woodland and pre- and post-remediation barren-site soils. Metal concentrations are shown in mg/kg, except for Fe which is in g/kg. Soil parameter values are shown as means ± se with n = 8 for woodland site, n = 5 for barren site prior to remediation, and n = 3 for barren site after remediation. Woodland soils were collected by the candidate and analysed by DMR and at UTS; soils from barren sites prior to remediation were collected from top, middle and base subsites and analysed by DMR. Post remediation analyses were carried out on top, middle and base subsite soils at UTS. Pre- and post-remediation means were compared and the difference expressed as percentage change. Cd was below the method detection limit of 5 mg/kg in all soils.

Metal	Cu	Zn	Fe	Pb	Mn	Cd
	Woodland Site					
	36 ± 8	78± 22	14 ± 18	203 ± 55	86 ± 12	<5
	Barren Site (Pre-remediation)					
	800 ± 370	1,230 ± 600	40 ± 8	4,200 ± 2,400	166 ± 21	<5
	Barren Site (Post-remediation)					
	390 ± 80	530 ± 16	34 ± 5	1,260 ± 640	65 ± 13	<5
	Change after remediation (Percent)					
	- 51	- 47	- 16	- 70	- 61	-

Comparison of these metal levels with the published values for normal and contaminated soils (Table 4.3) suggests that the barren-site soils had elevated contents of copper, zinc and lead. All exceeded both international and NSW EPA limits by up to twenty-fold.

Metal enrichment may reflect ground-water processes as schematically illustrated in Fig. 4.1, although this has not been confirmed by analysis. It would be expected that soluble metals such as copper and zinc would be highest around the mid-slope where ground-water discharge had been noted. Relatively insoluble metals such as lead would show no such remobilization. These trends were present at the barren site,

though not shown in the results above because of limited sampling of soil. A single sample obtained for the mid-section of the barren slope during the pre-remediation

Table 4.3 A comparison of metal levels in natural and impacted soils. Shown are the mean international limits of soil metals, the NSW EPA investigation levels for residential areas in NSW, and pre-remediation results obtained in this study. The NSW EPA investigation levels are prescribed in Site Auditor documents for residential land. All values are shown in mg/kg (dwt). * From Kabata-Pendias and Pendias (1984) and Kabata-Pendias (1995); ** From Lacatusu (2002), calculated means of the maximum permissible metal content in soils in the United Kingdom, Germany, Poland, Austria, Japan and Canada; *** From NSW EPA (1998).

Metal	Soil levels		Investigation levels		This study	
	Typical soils* (loamy & clay soils)	Mine-site soils* (Non-ferric metals)	Inter-national limits**	NSW EPA limits*** (residential)	Barren Site	Woodland
Cu	4-70	415-2,020	96	100	125-1,990	8-76
Zn	9-362	185-53,000	310	200	150-3,560	28-215
Pb	1.5-52	15-13,000	230	600	145-11,400	38-485
Mn	45-1,500	-	-	-	101-203	45-134
Cd	0.08-1.61	1.5-144	4.2	3	<5.0-7.3	<5

sampling yielded values that were much higher than anywhere else on the barren site: 900 µS /cm salinity, 460 mg/kg SO_4, 2.0 g/kg Cu, 3.6 g/kg Zn, 8.3 g/kg Pb. Further, more intensive sampling should be carried out to establish their significance. The more mobile metals would be transported in solution (Fig. 4.1), until they are precipitated by sulphides (Galloway *et al.*, 1979). Barren-site permeability and water chemistry could change downward towards bedrock and downslope towards more clay-rich sediments. This supergene enrichment (Bates and Jackson, 1980) adds to the sulphide minerals already present near the surface. Ground-water discharge thus may have contributed to supergene enrichment (such as the "seep zone" in Fig. 4.1),

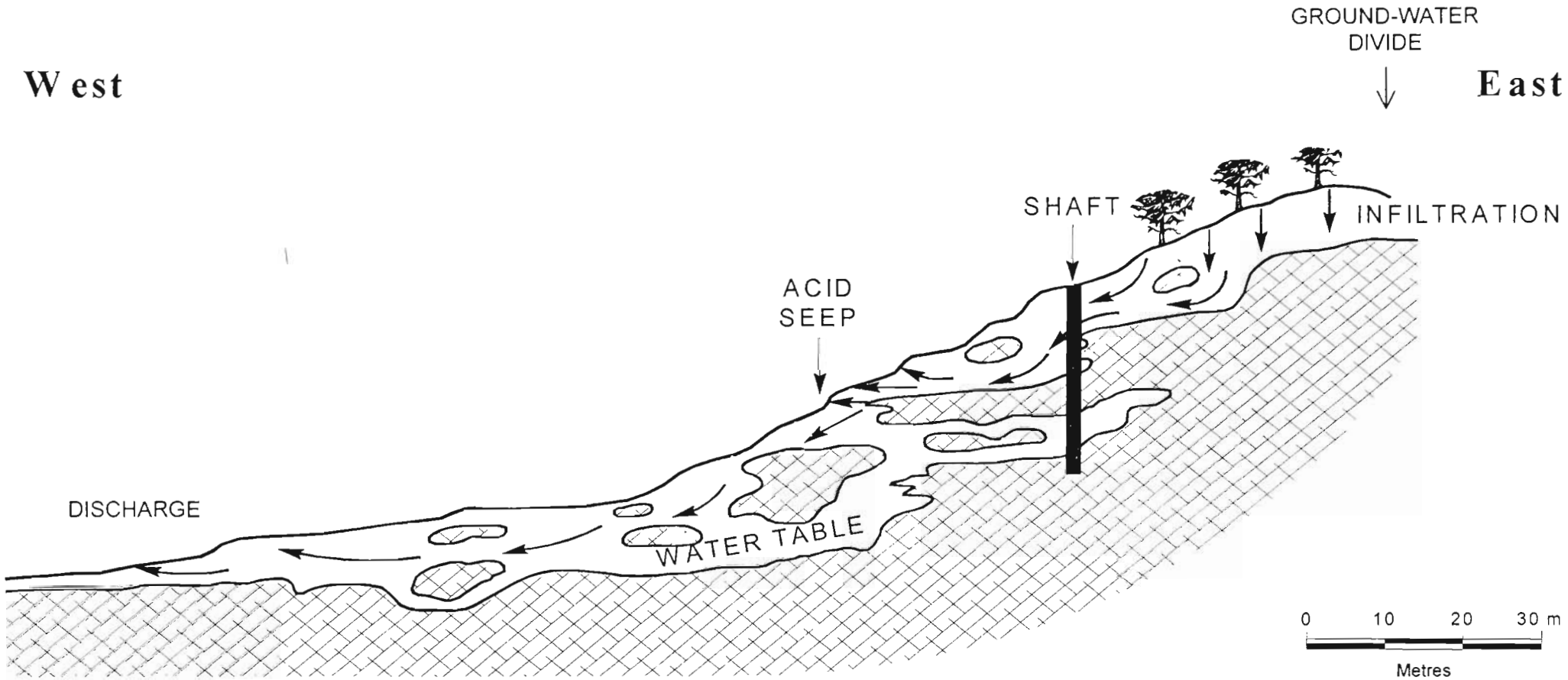


Fig. 4.1 Cross section of the barren site showing probable ground-water migration path. From the recharge area at the top of the slope, ground water flows downslope above the impermeable bedrock and discharges at the mid-slope "Acid Seep" and also at the base of slope. Interpreted by D.K. Hobday (personal communication).

as described elsewhere by Galloway *et al.* (1979). These metals, being highly conductive, may also account for the elevated EC values in this section.

This ground-water process acting on weathered surface minerals would also be expected to result in vertical redistribution of metals. Visual observations of the soil profile in the course of field investigations indicated a reddish-brown layer (Fig. 4.2), suggestive of downward changes in metal content in the woodland soils. This zonation is interpreted as resulting from common soil-forming processes described by Gilluly *et al.* (1958). According to this model, hydrous ferric oxide, formed by chemical decomposition of ferrous silicates, is leached from the surface A horizon and accumulates in the B horizon (Fig. 4.3). Over some parts of the barren site too, the deepest red colour was observed to be 10 to 35 centimetres below the surface, presumably because of iron precipitation by downward-percolating rainwater.

4.2 Vegetation at the site

4.2.1 Woodland vegetation

Three dominant tree species were present at Mt. Costigan, *Eucalyptus blakelyi* Maiden, *E. polyanthemos* Schauer and *E. macrorhyncha* F. Muell. Ex Benth (Table 4.4). *E. sideroxylon* ssp. *Sideroxylon* Cunn. Ex Woolls (Cronin, 1998) is present in the vicinity, but was not identified in the Mt. Costigan woodland. Brooker and Kleinig (1990, 1994) describe *E. blakelyi* as a widespread, medium-sized woodland tree, which is known as a "smooth-barked" eucalypt. It was the most common species at Mt. Costigan, being present on five of the six sub-sites, with an average of 9,000 trees per hectare (Table 4.4). *E. polyanthemos*, described by Brooker and Kleinig (1990, 1994) as a widespread, small to medium-sized, "half bark" woodland tree, was the smallest species, with the least height and girth at breast height (GBH); it was absent from the northern portion of the woodland. It averaged 5,500 trees/ha. *E. macrorhyncha*, known as a "full-bark", was the largest species growing on the site, with the greatest height and GBH; it was also the least numerous species, with an average of only 2,200 trees/ha (Table 4.4). Mt. Costigan woodland biomass was

Figure 4.2 Incipient soil zonation exposed in an eroded section in the woodland. This immature profile shows the pale, leached surface soil or "A" horizon, underlain by the reddish-brown, metal-enriched "B" horizon, which lies above the paler "C" horizon.

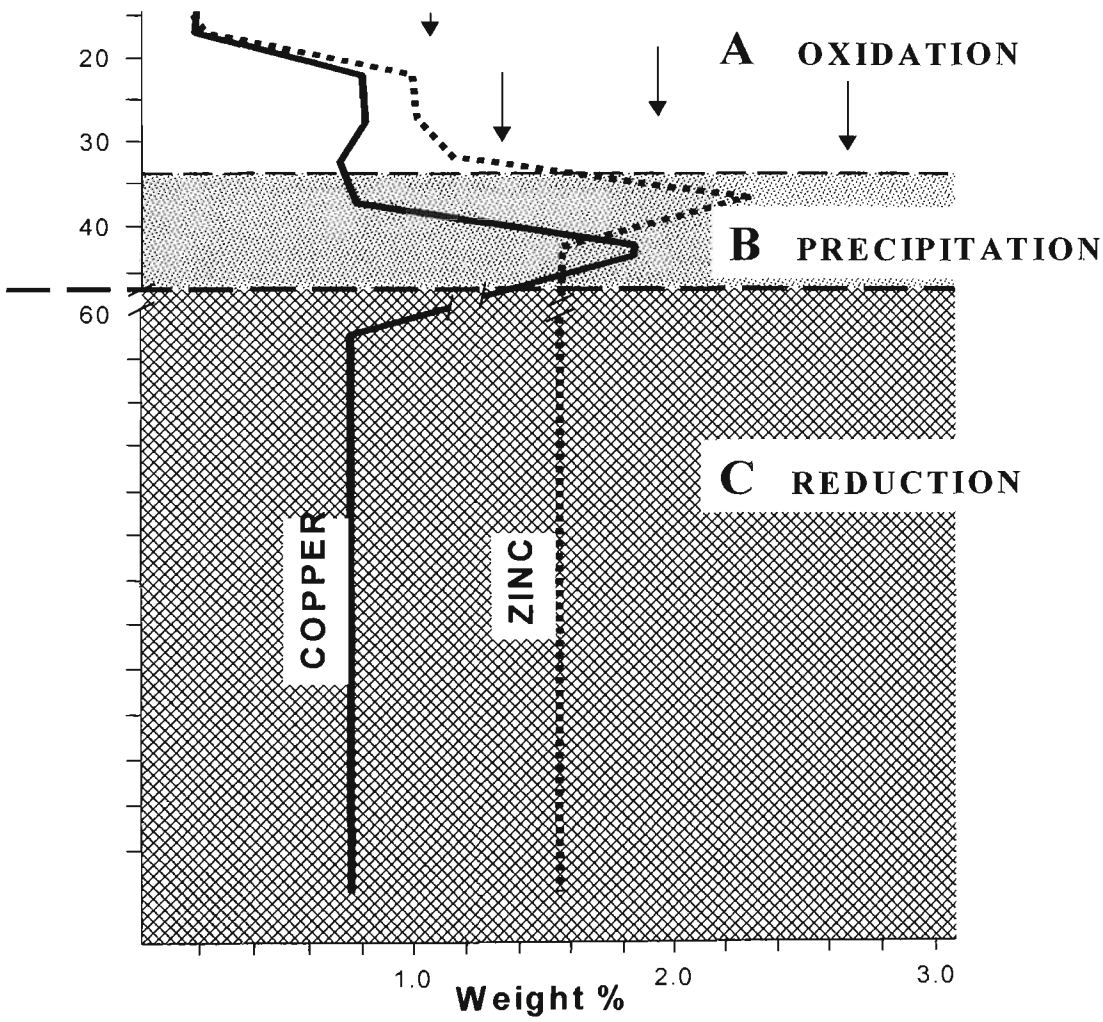


Figure 4.3. Mobilization and subsurface concentration of copper and zinc in mine tailings. Percolating ground water dissolves metals in the oxidising A zone and precipitates them in the B zone. This vertical zonation is not reflected in the results of the present study, since it did not sample subsurface layers. Depth scale is in cm. Taken from Gilluly *et al.* (1958).

Table 4.4 Woodland vegetation: tree species. Three dominant *Eucalyptus* species characteristics. The values shown are means (± se); tree numbers are indicated in thousand per hectare. GBH = girth at breast height; PFC = projective foliage cover; abs = absent; n.d. = not determined.

Tree species	Location	Number of trees (10^3/ha)	Height (m)	Crown diameter (m)	GBH (cm)	Biomass (t/ha)	PFC (%)
E. blakelyi	**M1**	17	10.1 ± 0.7	4.3 ± 0.5	52.1 ± 6.4	1,759	47
	M2	6	8.0 ± 1.8	3.7 ± 0.8	47.3 ± 7.8	455	18
	M3	18	12.2 ± 0.7	4.3 ± 0.4	58.3 ± 5.8	2,268	23
	M4	8	11.6 ± 0.7	3.3 ± 0.7	45.8 ± 3.9	533	30
	M5	abs	abs	abs	abs	abs	abs
	M6	7	10.1 ± 0.9	2.4 ± 0.6	45.9 ± 6.9	494	15
	Mean	**9.5±**	**10.4±**	**3.6±**	**49.9±**	**918.2**	**26**
E. polyanthemos	**M1**	abs	abs	abs	abs	abs	abs
	M2	abs	abs	abs	abs	abs	abs
	M3	abs	abs	abs	abs	abs	abs
	M4	3	7.7 ± 1.5	3 ± 0.6	43.3 ± 6.5	170	n.d.
	M5	27	6.5 ± 0.6	3 ± 0.3	27.6 ± 2.5	697	54
	M6	3	7.7 ± 1.5	5.7 ± 1.2	45.3 ± 8.6	194	n.d.
	Mean	**5.5±**	**7.3±**	**3.9±**	**38.7±**	**176.8**	**54**
E. macrorhyncha	**M1**	2	11.5 ± 0.5	2.5 ± 0	43.5 ± 0	109	n.d.
	M2	2	10 ± 0	3.0 ± 0	64.5 ± 0	260	n.d.
	M3	abs	abs	abs	abs	abs	abs
	M4	3	14.3 ± 0.7	5.7 ± 1.5	81.7 ±10.6	651	n.d.
	M5	3	13.3 ± 0.3	6 ± 1.0	86.3 ± 9.6	726	n.d.
	M6	3	12.0 ± 0.6	2.3 ± 0.9	58.7 ± 6.2	317	n.d.
	Mean	**2.2±**	**12.2±**	**3.9±**	**66.9±**	**343.8**	**-**

highest for *E. blakelyi*, with almost double the number of trees per unit area as *E. polyanthemos* and approximately four times as many as *E. macrorhyncha* (Table 4.4). Rowe (2001) conducted a similar study at nearby Peelwood mine site and documented *E. blakelyi, E. macrorhyncha, E. polyanthemos* and *Acacia dealbata* as the dominant woodland species.

In the understorey of the woodland, the main vegetation groundcover was Snow Grass (*Poa sieberiana* Sprengel) (Table 4.5). According to Lamp *et al.* (1990), Snow Grass is a tussock-forming perennial and is widespread in eastern Australia, preferring drier sites. Snow Grass grows in woodlands or grasslands and is usually 15-80 cm high, which is the typical height at Mt. Costigan. The Snow Grass was thickest and most common in parts of the northern and eastern woodland (M1 and M4 in Table 4.5), and relatively scarce in the southern woodland, where the plants were also smaller. A scattered ground cover of herbaceous dicots made up a sporadic and relatively minor component of the woodland vegetation. Sedge was present in modest amount in the southern woodland at M5. Small areas of lichen covered rocky surfaces and exposed ground in the southern woodland around M6.

The fact that the eastern woodland area (M3 and M4) is located topographically above the mineshaft level (Fig. 3.1, Chapter 3) has left it least disturbed by mining activities, as evidenced by the presence of large, naturally occurring boulders. The leaf litter cover was 81% on average here, and the Snow Grass grew to its maximum height. The woodland to the north and south of the site had definitely been cleared and the boulders removed. The litter cover was greater here, almost 90%, indicating high productivity, but the slope was flatter. The grass undergrowth was generally lower at these two locations than in the eastern woodland.

Variation among the northern, eastern and southern woodlands is summarized in Table 4.6 for the combined species. Although the tree density is highest in the south, this area has the lowest total biomass. Conversely, the eastern area has below average tree density as well as the lowest projective foliage cover, but has the highest biomass.

Table 4.5 Ground cover in the woodland, Mt Costigan. Projective foliage cover (PFC) is shown as a percentage, and heights are in cm. Sampling locations M1-M6 are shown on Fig. 3.1; n.r. = not recorded.

Location	Species/Cover	Ground cover PFC%	Height range (cm)	PFC (%) of tree canopy
M1	*Poa sieberiana* Litter	30 83	13-35 -	47
M2	*Poa sieberiana* Dicots Litter	50 3 93	40-60 n.r. -	18
M3	Lichen Litter	5 100	- -	23
M4	*Poa sieberiana* Dicots Litter	32 Trace 62	15-70 n.r. -	30
M5	Dicots Sedge Litter	Trace 15 100	n.r. 20 -	54
M6	*Poa sieberiana* Dicots Moss Lichen Litter	7 5 10 5 78	10-15 n.r. - - -	15

It is noteworthy that the native trees have revegetated these woodland areas and appear to be thriving, but will not grow at all just a few metres away on the barren site, presumably because of metal toxicity. In this regard, Mt. Costigan mine site differs from Peelwood, where Rowe (2001) showed that the four woodland tree species were colonizing the barren mine site. This is surprising because metal levels are significantly higher at Peelwood mine site; they are 50->100% higher for copper, zinc and manganese and ten times higher for cadmium (Table 4.7). Conductivities are also higher at Peelwood. As discussed in Section 8.1.3, bioavailability is more important that absolute soil-metal content in restricting plant growth on the Mt. Costigan barren slope. Time is probably another important factor, since the Mt. Costigan barren slope has been regraded in recent years.

Table 4.6 Woodland vegetation: variation among locations. Density of all tree species per hectare, projective foliage cover (PFC) as a percentage, total biomass in tonnes per hectare, and ground-cover biomass index in cubic metres cover per square metre of surface.

Vegetation parameters	Woodland			
	North	East	South	Mean
Tree density of combined species (10^3 ha^{-1})	27	32	50	36 ± 7
PFC (%)	38	27	35	33 ± 4
Total tree biomass (tha^{-1})	430	603	404	480 ± 60
Ground-cover biomass index (m^3m^{-2})	2.3	0.08	0.005	0.8 ± 0.8

Table 4.7 Characteristics of barren-site and woodland soils from Peelwood mine site. Adapted from Rowe (2001).

Parameters	Location	
	Barren Site	Woodland
pH_{water}	5-7.3	4.8 – 5.6
Conductivity (µS/cm)	260-1,060	110-440
Metal content (mg/kg)		
Cu	200-3,200	50-340
Zn	1,800-6,500	85-440
Pb	2,500-12,700	120-1,300
Mn	330-500	540-2,400
Cd	12-75	0-9

In summary, Mt. Costigan woodland soil has low conductivity, sulphate, and metal levels, and a lower pH than much of the barren site, plus a high level of organic matter. It would seem that all of these factors are important to native plant growth. Australian native plants have a remarkable ability to grow in poor soils, as was

evident in the woodland and base-of-slope areas of the Mt. Costigan study site. There is no single strategy for coping with low nutrients in soil; rather there are several, and these strategies frequently complement each other within a species (Bowen, 1981; Groves, 1994).

4.2.2 Barren-site vegetation

Vegetation of the Mt. Costigan barren site was assessed by examining both species diversity (Fig. 4.4) and vegetation cover (Fig. 4.5). Rowe (2001) did not find the same exotic plant diversity at the Peelwood mine site, but he did document the fact that the four dominant woodland tree species were colonizing the barren slope as well. At Mt. Costigan, there were a total of 39 plant species on the barren site (Appendix). There were 14 families of plants represented on the barren slope, all low-growing (< 5 cm), non-woody species. High species diversity was found at the very top of the slope (Site 1, 26 species), although the total area covered by vegetation was low overall, averaging <50% (Fig. 4.5). Maximum plant cover, averaging 58%, was S5 at the bottom of the slope, an area of high species diversity, with an average of 21 species represented.

4.2.3 Soil quality as a determining factor in site vegetation

Soil pH. Soil pH has a major influence on plant growth because it affects nutrient and metal availability (See section 1.4.1). Soils were acidic toward the top of the barren slope, more neutral in the centre, and alkaline at the base of the slope (see section 4.1.2 above and Chapter 7). The woodland soils were also acidic, with a pH similar to the barren top, but with higher levels of organic matter, attaining over 9% LOI compared with 6.1% LOI for the top of the barren site. Organic acids associated with leaf litter reduce pH and increase mobilization of heavy metals in soils (Kabata-Pendias and Pendias, 1984). The high pH of the slope base may be a result of lime from past remediation attempts that has been washed down the slope, although the low level of organic matter here, a mean LOI of only 3%, could also be a factor (see Chapter 7).

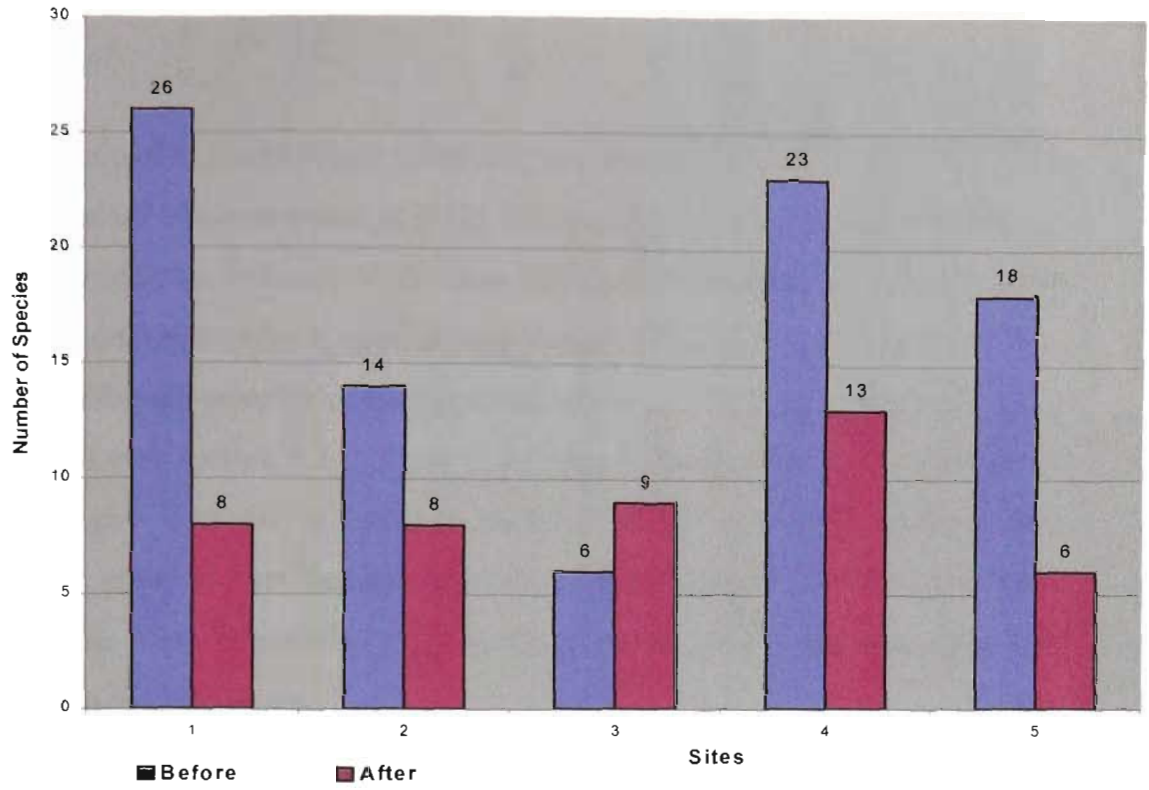


Figure 4.4 Barren-slope species diversity. Comparative plant diversity at five sampling locations (S1-S5 shown in Fig. 3.1) down the barren slope, conducted before and after remediation. Actual number of species per $10m^2$ is shown above each column.

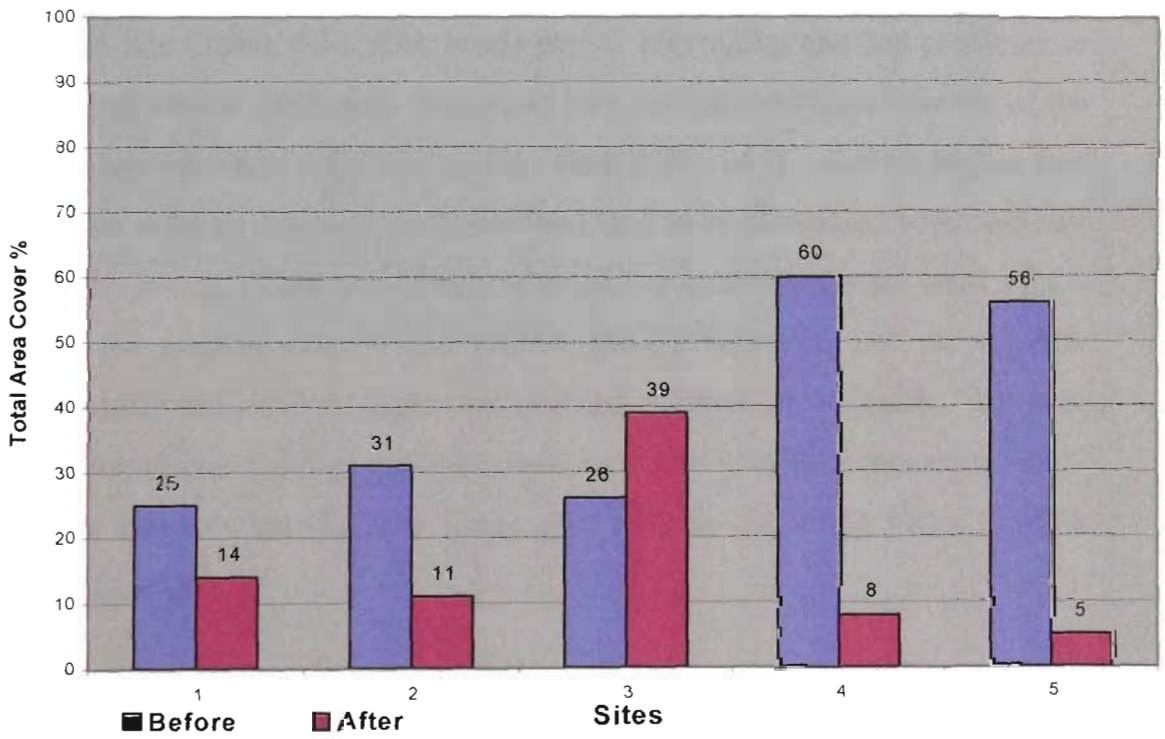


Figure 4.5 Barren-slope vegetation cover. Total vegetation ground cover at Sites S1-S5 on the barren slope is expressed as a percentage ground cover over $10m^2$ sampling areas before and after remediation.

Most Australian native plants prefer a low pH, but the concern with low pH is that it may increase metal concentrations in plant tissues (Society for Mining, Metallurgy, and Exploration, 1998). Because of the close similarities in pH and organic matter in the woodland and barren site, it appears that neither is likely to be a factor limiting plant growth, although possibly affecting metal uptake. Low pH of the mid to upper barren-site soils (see section 4.1.2 above and Chapter 7), similar to pH levels in the woodland, suggest that pH is unlikely to be a factor inhibiting plant growth. However, it is possible that the combination of low pH and much higher metal concentration in these barren-site soils inhibited plant growth because uptake of metals could reach toxic levels.

Sulphate. Soluble sulphates, which are present in low to moderate amounts in the barren-site soils at the top and base of slope (see Chapter 7), play an important role in soil equilibrium processes and are readily available to plants (Kabata-Pendias and Pendias, 1984). According to the United States Department of Agriculture (USDA, 2002), the ideal sulphate value in residential soils is around 50 mg/kg, comparable with the barren site (Table 4.1), with levels above 150 mg/kg causing problems to most plants. Sulphate is frequently associated with acidic conditions because of the oxidation and breakdown of sulphides and the availability of H^+. Levels higher than 150 mg/kg often indicate drainage problems, and tend to be associated with high soil acidity (USDA, 2002). This is certainly true of the water-discharge areas of the barren site. As pointed out by the USDA (2002) however, not all sulphate compounds acidify soil, for example calcium and magnesium sulphate. Because highly acidic conditions and high salinities in conjunction with sulphates are inimical to plant health and survival (USDA, 2002), they may be a limiting factor in plant growth at Mt. Costigan.

Salinity. Electrical conductivity (EC) of the soils, a measure of salinity and metal content, can limit plant growth (Kabata-Pendias and Pendias, 1994). EC shows a positive correlation with metal ion concentration, electrolytic composition, soil moisture, and conductivity of the mineral phase, and it is affected by depth to bedrock or clay layers (Hartsock *et al.*, 2000). Conductivity is also related to cation

exchange capacity (CEC), both of which tend to be high in clay. According to Hartsock *et al.* (2000), where water is ponded above bedrock, conductivity tends to increase markedly. This is probably true of the acid seep S3 location of the mid-slope (see section 4.1.2 above), where conductivity may be a factor restricting plant growth on the barren slope (Hartsock *et al.*, 2000).

Metals. The primary factor limiting plant growth on the barren site could have been elevated metal content compared with values in the adjoining woodland (Table 4.1). Furthermore, these metals presumably would have had an additive toxic effect. Copper and zinc inhibit root growth (Kabata-Pendias and Pendias, 1984; Marschner, 1986); manganese is particularly toxic in acidic soils (Marschner, 1986), such as are present at Mt. Costigan. Although relatively immobile, lead is a known plant toxin (Kabata-Pendias and Pendias, 1984). Cadmium uptake is rapid and phytotoxic (Kabata-Pendias and Pendias, 1984). Despite the fact that little iron is typically taken up by plants, excessive amounts of iron may induce deficiencies in other elements such as manganese and cadmium (Marschner, 1986), thereby restricting plant growth. On the other hand, Freedman (1995) observed that plant uptake of lead and zinc may actually be increased by the presence of other metals in excess, a process that he referred to as cotolerance.

In summary, any one or a combination of factors may have been responsible for the absence of woody vegetation on the barren site. A more comprehensive analysis of soil chemistry may provide a clearer answer.

4.3 Evaluation of site remediation

An earlier remediation effort by the DMR at Mt. Costigan in 1997 included the use of lime in order to raise pH so that exotic pasture mix grasses would grow, the construction of diversion banks for draining the site, initial revegetation attempts, and the filling of vertical shafts with contaminated soil. These measures were successful only for a short period before erosion and further site degradation by farm animals occurred (Johnson, personal communication) and, as shown by the results of this study, had little ameliorating effect on the site (Sections 4.1 and 4.2). In 2000,

after the pre-remediation assessment described in this chapter had been completed, further remediation was carried out by the DMR. This involved construction of soil terraces and berms (Figs. 3.1 and 4.6) to divert runoff into a concrete culvert (Fig. 4.7), which flowed to a collection dam (Fig. 4.8) at the bottom of the site. The slope was then covered with a layer of organic biosolid, added at 120 m^3/ha and averaging 2.5 cm thick, to provide nutrients. Straw bales were set up in rows, perpendicular to the slope, to retard runoff. Immediately following these measures, a "pasture mix" of grasses was sown for quick soil stabilization (Fig. 4.9). The grasses germinated well, but grazing by sheep and native fauna led to further erosion of the site and partial removal of the biosolid layer, despite fencing. Furthermore, acid seepage in mid-slope area prevented any sustained plant growth. Notwithstanding the increase in plant diversity and percentage cover shown in Figs. 4.4 and 4.5, these plants were shallow-rooted in the surface biosolid. They would probably not have survived as they grew larger and their roots extended into the toxic substrate.

4.3.1 Barren site soils after remediation

Soil quality parameters at the site showed some changes after remediation – the pH decreased and conductivity increased (Table 4.1), but in neither case was the change significant. Nevertheless, the remediation efforts achieved a reduction in soil metal content of the barren site (Table 4.2). Iron showed the smallest decrease, possibly because any reduction was offset by the 110 mg/kg iron in the added biosolid (Australian Native Landscapes, 2000). Significantly, levels of manganese fell, even though the biosolid contained 12 mg/kg of this metal. This is anomalous, as according to Kabata-Pendias and Pendias (1984), manganese levels have been known to increase from 242 to 555 ppm (dwt) in sewage sludge-amended soil over a period of five years.

4.3.2 Barren-site vegetation after remediation

The post-remediation vegetation studies were conducted in April 2000 using the same sampling pattern as for the pre-remediation assessment in order to provide a valid basis for comparison. The assessment found that there was a reduction in plant

Figure 4.6 Remediation at Mt. Costigan: soil terraces and berms. Barren site, viewed towards the north.

Figure 4.7 Remediation at Mt. Costigan: concrete culvert. Viewed towards the west. Patchy biosolid is visible to the right of the culvert.

Figure 4.8 Runoff collection dam at the base of the barren slope after remediation. Viewed towards the west.

Figure 4.9 The barren slope after the second remediation attempt. Brown soil colouration in the foreground is biosolid. Light green cereal pasture mix was used for site stabilization. The chalk-coloured surface behind the figures is the acid seep. The "lone gum" (See Fig. 3.1) is present in the centre-left of the slope. Viewed towards the northeast.

diversity and overall plant cover on the barren site after remediation. Post-remediation species diversity (Fig. 4.4 and Appendix) decreased at all sites except for S3). The 14 species that disappeared post remediation included *Trifolium campestre*, *Hydrocotyle laxiflora*, *Plantago lanceolata*, *Briza minor* and *Lomandra filiformis* (Appendix). Only two new species were found on the barren site, blackberry and aster (both weeds), while the variety of thistles increased. The largest reduction in species, 60-70%, was at the top and base of the slope. Some species were displaced from one location to another, with the mid-slope Site S3 gaining mixed grasses (including *Agrostis* spp.) and *Chondrilla juncea*. However, only one assessment has been conducted pre- and post-remediation.

A comparable post-remediation ground-cover trend was observed, with an increase at mid-slope Site S3 and a pronounced reduction at all other sites, with a maximum decrease of 90% at the base of slope Site S5. After remediation, the greatest cover (39%) was at Site S3, and the lowest (5%) at the base, which was almost a complete reversal of the original, pre-remediation cover (Fig. 4.5). In the mid-slope section (S3), total ground cover increased by 50%, mainly in the form of tussock grass and dicots, while species diversity increased 33%.

These results need to be interpreted with caution since the pre- and post-remediation assessments were carried out in different seasons (Spring 1999 and late Autumn 2000).

The observed lack of success in revegetation may have been due to a number of factors:

- Decrease in soil pH, so that only those species that were adaptable were able to re-colonize;
- Seasonal fluctuations in growth of the vegetation;
- The length of time required for species to establish at the site. It had been three years since the last remediation when the initial survey was done, and only four months since the second remediation attempt when the post-remediation

assessment was carried out. Insufficient time has elapsed since the second remediation for the full possible range of species to become established.

4.3.3 Biosolid amelioration

The amelioration measures at the barren site resulted in overall decrease in pH, without much impact on salinity, while organic content was unchanged (Table 4.1). However, there was an overall decrease in metal levels at the site after remediation. The differences in plant distribution and abundance across the site before and after the remediation are not conclusive because of likely seasonal effects. It is significant however, that the only increase in the number of plant species at the site occurred in the mid-region, despite the presence of the "acid seep". Furthermore, the electrical conductivity of these soils was still high (497 μS/cm) and organic matter remained relatively low (5.4%). It would have been expected that addition of sludge would greatly increase bioavailability and thus, toxicity of the soils to plants (Freedman, 1995). According to Freedman (1995), the increase for zinc can be as much as thirty-fold in sandy loam, and 3-10 fold for other metals such as copper, lead, zinc and cadmium in clay soil and loam. In this study, it is possible that the seedlings were rooted entirely in the biosolid layer (and not in contact with contaminated soil), and only later would the rootlets have penetrated the toxic site soil and accessed the metal contaminants that would have been liberated in large amount by the biosolid.

It is possible that the layered biosolid provided an artificial topstratum that was quite distinct from the *in-situ* soil. The plants may therefore have been surviving in the thin (25 mm) top layer of biosolid. However, this layer would be subject to rapid moisture deficiency during drought, and the plants would also be vulnerable to highly acidic conditions created by capillary action or ground-water seepage (D.K. Hobday, personal communication). The layered nature and limited thickness of biosolid application may not have the desired effect of enhancing revegetation over the longer term. The manufacturer of the biosolid (Australian Native Landscapes, 2000) recommends application to a thickness of 100 mm, followed by mixing with soil to a depth of 200 mm. At Mt. Costigan, however, the biosolid layer was left exposed at the surface (Fig. 4.5), and subject to erosion and oxidation.

The questions raised during the course of these field evaluations of soils and vegetation at the Mt. Costigan abandoned mine site pointed to the need for glasshouse/laboratory experiments. Hypotheses regarding the toxicity of site soils and the effectiveness of biosolid amelioration were tested and the outcomes of these are presented in the chapters that follow.

Section B: Glasshouse/Laboratory Studies

Chapter 5. Laboratory studies: introduction

5.1 Aims and rationale

Field observations in Chapter 4 provided the physico-chemical and biological framework to conduct further studies of plant response to Mt. Costigan soils in a controlled environment. The glasshouse/laboratory studies described in this Section complement the field investigations and thus complete the triad approach in the assessment of Mt. Costigan site.

Glasshouse studies were carried out using a series of bioassays and were directed towards:

- Evaluating the toxicity of site soils to a range of test plant species that were mainly native species;
- Evaluating the effectiveness of the biosolid used in site remediation as an ameliorating agent;
- Evaluating the potential applicability of phytoremediation process at Mt. Costigan; and
- Comparing results among the tested plant species to determine their suitability for future remediation of the barren slope.

In Chapter 4 were outlined many of the problems associated with the Mt. Costigan mine site, the remediation methods attempted to date, and their results. The DMR was concerned that their past remediation attempts had not been successful, and sought to determine whether more effective methods were available. Use of plants was favoured because of their potential to phytostabilize, and possibly ameliorate soil toxicity.

A finding of the site field assessment (Chapter 4) was that the soils of the naturally revegetated woodland were similar in pH and organic content to the barren slope,

but had less metal contamination and lower salinity (EC and SO_4). One of the major aims of this study was, therefore, to assess the extent of toxicity of the soils on the barren slope, in comparison with those of the woodland.

The bioassays designed to achieve this were based on OECD Guideline 208 (Section 5.5.1), Terrestrial Plant Growth Test (OECD, 1984). They were conducted using sieved soils from Mt. Costigan and diluting them with varying proportions of commercial washed river sand to assess the level of toxicity of the soils to plants. After initial trials involving four species of eucalyptus and four species of acacias, those with the most reliable germination characteristics were selected for testing the soil toxicity and experiments on metal uptake.

5.2 The role of toxicity tests (bioassays) in pollution assessment

The terms "toxicity test" and "bioassay" are often used interchangeably, though they have slightly different connotations. Both involve analytical determinations of contaminant-uptake levels in plants from glasshouse, laboratory and field experiments in order to derive dose-response relationships for the contaminant. Bioassays entail empirical determination of contaminant levels in plants and thus are an integral part of pollution assessment, ecotoxicology, and triad approach advocated by the United States Environmental Protection Agency (USEPA). The USEPA (2002 a) defines bioassay as "a test to determine the relative strength of a substance by comparing its effect on a test organism with that of a standard preparation". Toxicity testing extends beyond the scope of bioassays to include the ecological impacts that occur when contaminants exceed maximum allowable limits (Lacatusu, 2002).

The ecotoxicological approach advocated by the USEPA (2002 a) comprises both bioassays and toxicity tests to determine the composition, concentration, distribution and bioavailability of contaminants, as well as their impact on organisms. Determination of toxicity thresholds is a crucial part of this exercise (Lacatusu, 2002). Procedures and standards for deriving and presenting data

elements for the United States' national toxicological databases (aquatic, terrestrial) are provided by the USEPA (2000 b). The national database includes:

- Chemical fields comprising the contaminants,
- The species fields of tested organisms,
- Test-condition fields,
- Exposure fields, and
- The measured impacts of contamination.

Ecotoxicology seeks to quantify the effects of these relationships in ecosystems, to evaluate physical and functional changes in organisms exposed to natural or anthropogenic compounds, and predict future impacts (USEPA, 2002 a and b). The USEPA's computer-based "ECOTOX" system provides specific toxicity values for contaminants impacting aquatic life, terrestrial plants, and terrestrial wildlife. These data provide a basis for assessment of impact and ecological risk, and consistent ecosystem management decisions. The "PHYTOTOX" database provides data relating to lethal and sublethal responses of terrestrial plants to chemical contaminants. Standards and quality assurance criteria are also included.

A limitation of bioassays/toxicity tests, as conducted by the USEPA, is that they focus on single variables and ignore the effects of combining more than one chemical compound or metal element ("stressor") in the experiments. For example, the uptake of many metals is strongly affected by the presence of other elements (Tolra *et al.*, 1996), so single-element stressor data from the USEPA may be misleading. Further, microbial factors are not considered, and these may have a pronounced effect on biouptake (Kabata-Pendias and Pendias, 1984). The USEPA (2002 a) concedes that linking biological effects with their causes is particularly complex when multiple stressors impact an organism.

The USEPA (2002 a) concludes that "investigation procedures are needed that can successfully identify the stressors and lead to appropriate corrective measures through habitat restoration, point and non-point source controls, or invasive species control." To this end, the USEPA (2002 a.) utilizes the "Stressor Identification" (SI) component in bioassessment/biocriteria programs to enable resource managers to understand and control stressors affecting biota, and thus better protect the biological integrity of ecosystems.

5.3 Phytotoxicity testing

Phytotoxicity tests used in this study adopted the triad approach developed by the USEPA for ecological risk assessment, using laboratory, field, and glasshouse experiments (Pascoe and DalSoglio, 1994). This involves:

i) Chemical analysis of soils and plant material to establish the distribution and concentration levels of toxic metals;

ii) Ecological field assessment of plant distribution and speciation, abundance relative to metal contamination, and heavy metal levels over entire site, to determine potentially negative ecological impacts; and

iii) Glasshouse bioassays studying toxicity of soils from various parts of the site, and the adjacent woodland as a "reference" site to document possible links between contaminant distribution, chemistry and bioavailability and their ecological impacts.

The USEPA guide for conducting ecological assessments at hazardous waste sites describes this triad of information as essential to establish a solid relationship between toxic wastes and ecological effects, and sets out the procedures employed (Pascoe and DalSoglio, 1994).

Use of plant bioassays lags behind other advances in phytotoxicity testing for several reasons. Firstly, there is the difficulty in simulating natural conditions. Secondly, as Freedman (1995) and Kabata-Pendias (1995) have shown, toxicity

measures must take into account all factors affecting bioavailability, and the fact that the presence of certain metals in excess affects plant uptake and tolerance mechanisms. Bioassays alone are therefore of limited value. It is necessary to look at bioassays within the broader context of soils and ecology (Lacatusu, 2002). For example, the Dutch system (Ewers, 1991) recognizes three soil categories based on metal content: A (reference levels), B (maximum permissible levels), and C (levels requiring remediation). Reference levels are not fixed for the various metals, but vary according to mathematical formulae that take into account differences in soil chemistry that affect bioavailability (See Section 4.2). Lacatusu (2002) takes this a step further by determining the ratio between the reference level according to the Dutch system and the metal content of a contaminated site.

Apart from testing and comparing chemical toxicities, plant phytotoxicity testing can contribute to the selection of plants for biomonitoring and bioremediation. In 1989, the USEPA initiated a baseline risk assessment to characterize potential human health and ecological risks associated with soil contaminants. The foundation for this document was provided by the 1980 Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and the 1990 National Contingency Plan (Pascoe and DalSoglio, 1994).

5.3.1 OECD Guideline

This study is based on the OECD Guideline 208, Terrestrial Plant Growth Test (OECD, 1984), which sets out test procedures, recommends plant species, and specifies reporting standards. The Guideline is designed to investigate the toxicity of soil-incorporated substances on the emergence of seedlings and plant growth after a single application. In this study, the Guideline was adapted to design a series of bioassays for the already contaminated soils from Mt Costigan site.

The OECD (1984) sets out standards for sieving (<5 mm), carbon (<1.5%), total organics (<3%), and fines (10-20%). The method incorporates various concentrations of the test substance in the soil in which seeds are sown, and identifies critical concentrations affecting plant growth. The number of seedlings is

recorded, and at least two weeks after 50% of the seeds have emerged, the plants are harvested and weighed. The test is considered valid if at least 80% of the control seeds produce healthy seedlings that exhibit normal growth. At least three species should be selected for testing, including one or more from each of the categories of grasses, vegetables (mustard, rape, radish, etc.), and a group containing legumes, lettuce and red clover (OECD, 1984). At least five seeds are planted in prescribed sand-soil mixtures containing three concentrations of the test substance amounting to 0 (control), 1, 10 and 100 mg/kg of oven-dried soil. The number of seedlings and their wet and dry weights are recorded. The effects of the test substance are expressed as EC 50 and LC 50, the concentrations at which the plant growth and seedling emergence are 50% of that of the control.

5.3.2 Species selection

Most of the phytotoxicity protocols have a list of suggested test species that are sometimes grouped according to their physiology or agricultural use.

The OECD Guideline 208 states that a minimum of three species should be selected for testing, from a list of test species provided (OECD, 1984). As with the other protocols, these are internationally available crop species that are grouped into three distinct groups comprising monocots, dicots and plants belonging to the cabbage family. However, the Guideline states that "Other species may be used if the rationale for their selection is justified in the test report." (OECD, 1984).

In this study, oats (*Avena sativa*) were selected from the OECD list, and a number of native species, acacias and eucalypts, were considered. The rationale for selecting the particular Australian species was that, being native to the Mt. Costigan region, they would be most adaptable to the harsh climate and would not pose an environmental threat if they were to spread beyond the confines of the mine site. In addition, they could perhaps be used in any future site stabilization or remediation.

5.4 The use of Australian plants for phytotoxicity testing

One of the major problems in using native plants as test species arises from their inherent genetic variability. Thus, unlike the cultivated crop species, germination times tend to vary over a longer period, and this creates difficulties in determining the achievement of full germination in the control groups set up for phytotoxicity testing (OECD, 1984). The other problems that are frequently encountered with some native seeds are the poor and inconsistent germination rates and the tiny seed size that make it very difficult to count out and plant for toxicity tests (A. Pulkownik, personal communication).

Many native tree seeds such as *Eucalyptus* spp. germinate readily when exposed to a warm, moist environment, and do not need any form of pretreatment before sowing. However, seed of other native species such as *Acacia* spp. contain built-in mechanisms to delay germination for months or even years (Gorrie and Doran, 1999). In nature this dormancy aids survival. Therefore, before such seeds are planted they must be pre-treated to break the dormancy (Gorrie and Doran, 1999.). Many native Australian plants produce seed with a hard shell, a characteristic of some legumes, e.g. of the *Acacia* and *Cassia* genera. Their hard seed coats must be broken manually, scarified to expose the seed embryo, or cracked or softened in boiling water before the seeds are planted (Gorrie and Doran, 1999).

5.4.1 Treatments to improve germination success

A number of seed pre-treatments have been developed specifically to improve germination success among Australian native plant species (Gorrie and Doran, 1999):

Scarification. Scarification is the method by which hard-coated seed is pierced, nicked or filed to allow moisture to reach the embryo. This may be done using a sharp knife, nail clippers, file, sandpaper, or a needle mounted on a suitable handle.

Heat treatment. Many of the Australian native plants have adapted to bush fires and either require bush fire treatment to germinate (*e.g. Banksia* spp.) or have their

normal germination enhanced by bushfires (*e.g. Acacia* spp.) (Groves, 1994). The requisite heating event may be reproduced by soaking the seed in boiling water or by controlled dry heating. This method is widely used because large numbers of seeds can be treated quickly and simply.

The few acacia species that produce a semi-hard or soft coat needed special consideration, as intense heating would kill these seeds. All acacia species in this bioassay were tested in these treatments to determine which was most suitable for the individual species.

Sulphuric acid treatment. A less common method for seeds that are thick-shelled and fully mature is to soak them in concentrated sulphuric acid (Turnbull, 1986). This technique was not used in this bioassay.

5.5 Rationale for the experimental design

5.5.1 Modification of the OECD Guideline

Since the OECD Guideline 208 (OECD, 1984) was designed for testing chemicals on crop species, its application to assess the toxicity of site soils required the following modifications:

i) Because the metals were already present in soil collected from the Mt. Costigan mine site, the test soils did not require them to be spiked with chemicals. Instead, the soils were diluted with sand to produce a series of (presumably) decreasing concentrations of toxic components. The controls required by the Guideline were, therefore, set up with sand only.

ii) The Guideline requires at least three species, selected from the three lists of common crop species to be tested. In this study, only one of these was tested, *Avena sativa*, with other species being tested from among native Australian tree species common to the area of study, for the reasons stated previously.

iii) The growth period recommended by the Guideline was extended for two reasons. Firstly, a longer test period was required because the native species did not have a discrete germination period endpoint. Secondly, the longer growth period was required because the focus of the study was not only on germination and survival, but also on accumulation of metals in plant tissue. For this a large enough plant biomass was required, and thus a longer growing period.

5.5.2 Plant and soil analysis

An objective of this study was to determine the dose-response relationship between metal levels in plants and soils and the corresponding growth response curves obtained in glasshouse trials using soils with different levels of metals. While soil analyses indicate the potential availability of metals that roots may take up under conditions favorable for root growth and activity, plant analyses reflect the actual levels of metals in the plants, and help identify those metals that most severely limit growth. Marschner (1986) therefore recommends a combination of soil and plant analyses to provide meaningful conclusions.

According to Marschner (1986), the nutritional status of a plant is generally better reflected in the metal content of the leaves than in any other part of the plant. Consequently, Marschner (1986) suggests that the leaves are best for use in plant analysis. With certain plant species and metals, levels may differ between leaf blades and petioles, and in some situations the petioles are a better indicator of nutritional status. In this study, the leaf blades and petioles were combined in the samples. On the other hand, if the function of the selected plants is to be phytostabilization, then metal accumulation in the roots is also important.

5.5.3 Selection of suitable plant species

One of the many aims that a remediation program might have is the return of the site to a condition as close as possible to its original or natural state. Ideally, this restricts the choice of plants to indigenous species. Because Mt. Costigan is in a

remote location and is surrounded by natural woodland, factors of climate, local ecology and conservation had to be considered in plant selection.

The study area has an arid, inland climate, and the high altitude results in frost and low winter temperatures. According to the Bureau of Meteorology, Climate Averages Table for the nearby Crookwell township, the summer (January) average temperature range is 11-27°C, with the maximum sometimes rising to 38°C, while the winter (July) range is 0-10°C, with extreme lows of -9° C. Precipitation is fairly evenly distributed throughout the year, totaling 866 mm on average. Snow is not uncommon in the winter months. Many exotic plants cannot adapt to these climatic conditions without special care. Furthermore, exotic plants pose a potential threat of spreading to adjoining agricultural lands and creating a weed problem. On the other hand, native tree species used in this study are suited to the environment and would not cause unintended environmental problems in the ecosystem.

Site revegetation generally involves a choice between transplanting tube stock or sowing seeds. In this study, the feasibility of using seeds was investigated. Reliable germination is very important for a bioassay, as it provides a basis for comparing the site soils with the control. Consequently, the study included an investigation of treatments to improve germination rates for the hard-shelled acacia seeds.

5.6 Experimental objectives

The bioassay experiments described in this Section were designed with a number of specific objectives:

- To determine the relative toxicities of the various site soils to a range of plant species using sequentially diluted soils;
- To investigate and compare the extent of metal accumulation and partitioning in the test plants grown under bioassay conditions;

- To assess, from the results of metal accumulation studies, the potential of the tested species to be useful as phytoremediators or phytostabilizers at the sites;
- To investigate the effectiveness of the biosoil as a soil ameliorating agent; and
- To compare the effectiveness of the application methods of biosolid used at the site in separate bioassays.

Chapter 6. Laboratory studies: materials & methods

6.1 Materials

6.1.1 Seed sources

The eight species from which seeds were used are listed in Table 6.1, along with their natural distribution in NSW. All of the acacia seeds and seeds of *E. sideroxylon* were obtained from the Australian Seed Company, Hazelbrook, NSW. The remaining eucalyptus seeds were obtained from Royston Petrie Seeds, Kenthurst, NSW. Seeds of *Avena sativa* (oats) were obtained from a local grains supplier. Fig. 6.1 shows the considerable variation in the size and shape of seeds among similar species.

6.1.2 Growth media

Commercial growth media. Species-screening tests were conducted in two commercially bagged soil types, namely Green-Gold Professional topsoil, purchased from The Mosman Gardener and Florist, Mosman, NSW and Supersoil Garden Mix, purchased from Australian Native Landscapes, Sydney. For bioassays, commercial washed river sand and Nitrohumus biosolid were purchased from Australian Native Landscapes, Sydney.

Mine-site soil. Soil was collected from the five sampling locations down the barren slope (Sites S1 to S5, see Fig. 3.1) and from the southern woodland site, as a reference soil. Approximately 40 kg of soil was collected from each of these sites, after digging out with a shovel to a dept of 20 cm and sieving through 7 mm sieve at the site.

6.1.3 Other materials

Seedling punnets (15 cm x 10 cm x 5 cm deep) for germination experiments and pots (100 mm or 4 inch tall) for bioassays were purchased from a garden supplier. Liquid

Table 6.1. Species used in glasshouse trials and their natural distribution.

Plant species	Common name	Regional distribution in NSW	Use in this study
Eucalyptus sideroxylon ssp. *sideroxylon* Cunn. ex Woolls	Red Ironbark; Mugga	Western Slopes	Germination potential; bioassays
Eucalyptus macrorhyncha F.Muell. ex Benth	Red Stringybark	Lower Tablelands	Germination potential
Eucalyptus polyanthemos Schauer	Red Box	Tablelands	Germination potential
Eucalyptus blakelyi Maiden	Blakely's Red Gum	Tablelands	Germination potential
Acacia dealbata Link	Silver Wattle	Tablelands and Southwestern Slopes	Germination potential
Acacia salicina Lindley	Cooba; Native Willow	Central-West	Germination potential; bioassays
Acacia verniciflua Cunn	Varnish Wattle	Central and Southern Tablelands, and Western Slopes	Germination potential
Acacia hakeoides Cunn. ex Benth	Western Black Wattle; Hakea-leaf Wattle,	Central Tablelands and Western Slopes	Germination potential; bioassays

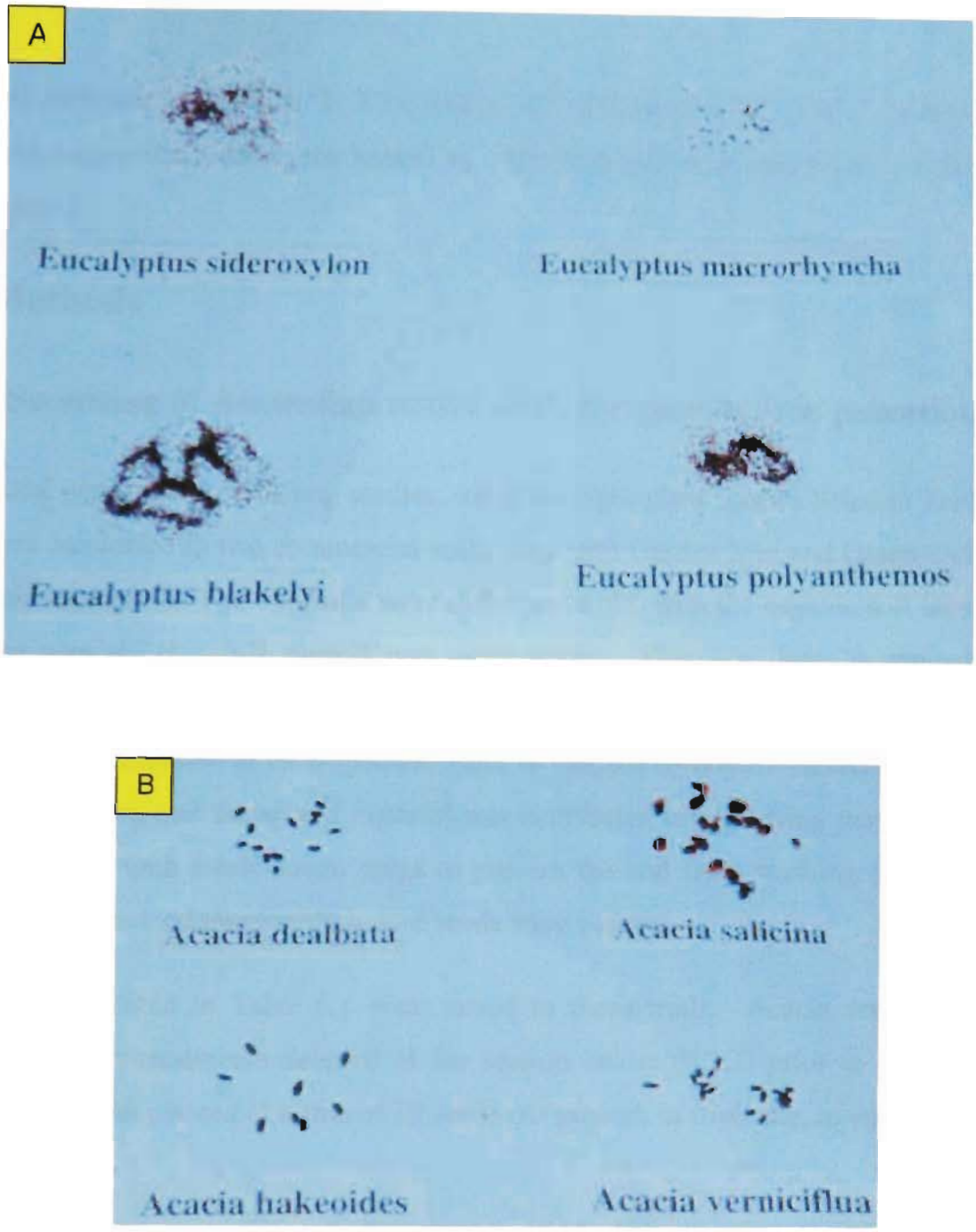


Figure 6.1 Visual comparison of the seed sizes and shapes. These four *Eucalyptus* spp. (A) and *Acacia* spp. (B) were trialed for species selection. As an indication of scale, *E. sideroxylon* seeds are approximately 1 mm in length; *A. salicina* and *A. hakeoides* seeds are around 5 mm and 3 m in length respectively.

seaweed fertilizer (N.P.K. 7: 3: 2.1), Maxicrop® (Multicrop, Bayswater, Victoria) was used occasionally during the bioassays. All other materials used were described in Chapter 3.

6.2 Methods

6.2.1 Screening of Australian native seeds for germination potential

The initial germination screening studies, using the eight plant species listed in Table 6.1, were conducted in two commercial soils, Supersoil Garden Mix and Green-Gold Professional topsoil. The two soils were different in pH, with the organic soil being close to neutral pH, while topsoil was more acidic. This was done to compare germination at two different pH levels, in order to establish which pH was more suitable for germination of each species. Prior to use, the soils were sieved using a 5 mm plastic sieve, and the sieved material was distributed into seedling punnets that had been lined with shade gauze strips to prevent the soil from washing out. The punnets were watered thoroughly before seeds were planted.

All the seeds listed in Table 6.1 were tested in these trials. Acacia seeds were subjected to pre-treatments detailed in the section below (6.2.2) prior to planting. Each species was planted at a rate of 10 seeds per punnet, in triplicate, in each series of two soils.

The acacia seeds were sown 2.5 cm apart and 1 cm deep, while eucalyptus seeds were placed on the surface of the soil and only covered lightly with soil. In the neat soils, a 2mm thick layer of washed river sand was applied as a "mulch" to assist in eucalypt germination. The punnets were placed on a heated bed insulated by a layer of aquarium gravel and covered with a layer of plastic sheeting. This was set up in the glasshouse in a north-facing position to increase insolation and further stimulate germination. Regular watering with tap water was conducted. The numbers of seedlings germinated were recorded cumulatively every second day, based on the emergence of the seedling above the soil surface. After several weeks of growth,

when no further seedlings emerged, the seedling survival rate was calculated by dividing the total number of seeds surviving by the number germinated.

6.2.2 Testing the effect of seed pre-treatment on germination success in *Acacia* spp.

Batches of seeds of *Acacia* spp. were subjected to four different pre-treatment protocols advocated by Gorrie and Doran (1999) to ascertain the best method to break any dormancy of these particular acacia species. The pre-treated seeds were then planted out as described in the preceding section and germination success was calculated similarly. The four pre-treatments were:

i) Seeds were clipped on the shoulder opposite to the end where the seed was attached to the pod, using nail clippers (scarification). The treated seed was then stored in moist paper towels for 24 hours, or planted directly and watered immediately;

ii) Seeds were soaked in water at 90° C for one minute. This brief treatment is reported to be especially suited to seeds with only a semi-hard coat;

iii) Seeds were covered with boiling water in a bowl and allowed to cool slowly to room temperature. This method is reported to be best suited for hard-coated seed; or

iv) Seeds were sown directly without treatment, as a basis for comparison. This is reported to be usually best for soft-coated species.

6.2.3 Preparation of soils for bioassays

Pretreatment of field soils. Soils that were collected from the five locations (Sites S1-S5) on the barren slope (Fig. 3.1), and a reference soil from the southern woodland, had already been pre-sieved at the site. The soils from the two upper locations (S1 and S2) on the barren slope were combined and used as a single soil source, referred to as T (top) site soil. The soils from the two lower locations (S4 and S5) were also combined and used as B (base) site soil, since these soils had

similar characteristics (see Chapter 4). Soil from Site S3 was used alone, and was referred to as M (middle) site soil. These, together with the reference soil from southern woodland, gave rise to four groups of soils that were used in the bioassays (top, middle, base of barren slope, and woodland).

Prior to use in glasshouse experiments, the soils collected from the barren slope were sieved once more using a 5 mm plastic sieve over three large plastic trays. This ensured that the samples collected from each location were evenly mixed, thus making the consolidated samples from the T and B subsites completely homogeneous. Several scoops were taken from each of the trays to fill the individual bags for the preparation of soil mixes.

Preparation of soil-dilution series. Washed river sand was mixed in various proportions with the sieved site soils in preparation for bioassay. Compositions ranged from 100% river sand, which was used as a control, to admixtures of 25:75%, 50:50%, 75:25%, and 0:100% by volume of sand:site-soil mixtures. The five dilutions were prepared in 10 kg lots and mixed thoroughly using a cement mixer lined with a plastic garbage bin, which was replaced after each soil type was used in order to avoid cross contamination.

Treatment with biosolids. Because the DMR site-remediation process involved the use of a biosolid (Nitrohumus), a parallel experiment with two extra treatments was set up for both species. A layer of biosolid 2.5 cm thick was placed on one series to mimic the site application, and an equivalent amount of biosolid was mixed into each site soil in another series to determine whether this produced better results. These treatments were, for each of the four site soils:

- 100% site soil, with a 750g/2.5 cm top layer of biosolid (as used on the mine site)

- 100% site soil, with a 750g/2.5 cm layer of biosolid mixed throughout the soil (using the cement-mixer method).

6.2.4 Setting up bioassays

Seeds. All seeds were visually graded for uniform shape and colour before planting. *E. sideroxylon* was the only eucalyptus seed used in the bioassays. Its seed was about 2 mm long, orange-brown and crescent-shaped; these seeds were planted 5 mm deep. *A. salicina* was used for all of the barren site soil bioassays. Its seeds had shiny, dark-brown to black, oval to oblong seeds 4-6 mm long and 3-4 mm broad. A fleshy appendage, known as an aril, was attached to the seed end. This seed was available in limited quantities because of a poor growing season, therefore *A. hakeoides* was used for the bioassays of woodland soils. Its seeds were darker and smaller, 3-4 mm long, 2-3 mm broad without a notable appendage (Fig. 6.1). All acacia seeds were scarified prior to planting, and were sown 10 mm deep. In addition to the native plants, oats (*Avena sativa*) was also included in the bioassays as the standard plant used in phytotoxic tests. Oats were planted 1 cm deep into the soil.

Experimental design of the bioassay. Both eucalyptus and acacia species were tested for survival and growth in a range of site soil dilutions. For each of the T, M, B and woodland locations, the experimental growth media comprised neat soil plus three dilutions (25, 50 and 75%), as well as the washed river sand control. This was conducted for each species, with each treatment comprising five replicate pots per dilution. Further tests were performed using mixed and layered biosolid preparations. For each location, therefore, there were seven growth media, representing the neat soil and river sand end member and the three dilutions, plus the two biosolid soils, with five pots, giving a total of 105 pots.

In June 1999, the first series of bioassay were conducted. The three barren-site soils and their dilutions were sown with *A. salicina*, *E. sideroxylon* and *A. sativa*. The site woodland soils were collected and prepared later, thus the growth trials in the woodland soils began in October 1999. *A. hakeoides* and *A. salicina* were both grown at this time, concurrently with *E. sideroxylon* and *A. sativa*.

There were 30 pots per dilution (10 per group). Seed from *E. sideroxylon* was sown in 105 pots (10 seeds/pot), and *A. salicina* seed was planted in another 105 pots (only 9 per pot because seeds were more costly). *A. sativa* (oats) were planted in 100% and 0% dilutions in another 30 pots (10 seeds/pot), made up of 15 pots per dilution and 5 pots per soil group. The acacia seeds were scarified before being sown 10 mm deep. The eucalypt seed was sown directly, to a depth of 5 mm. The oat seeds were pushed into the soil, 1 mm deep.

In the woodland soils, seed of *E. sideroxylon* (10 seeds/pot) and *A. hakeoides* (9 seeds/pot) was sown. For a summer growth comparison, and as seed was limited, additional 0%, 50% and 100% dilutions were set up in these soils using *A. salicina* (4 seeds per pot). Seedlings were later thinned to three acacias and four eucalypts per pot to reduce competition for nutrients and increase light exposure.

Growth conditions. Pots were randomized and set up at the northern side of the glasshouse. They were placed in rows as illustrated in Fig. 6.2 and rotated midway through the growth period to ensure equal exposure to sun. During the winter trials the pots were placed on a glasshouse bench on a heating bed, with the thermostat for controlling winter soil temperatures set at 20°C. This was recommended by Gorrie and Doran (1999) as the ideal temperature for germinating native seeds. Because the thermostat controlled the heat (bottom heat) generated by metal coils buried in the aquarium gravel, the actual soil temperature in the pots fluctuated by a few degrees from night to day. Gravel covered the heating elements in the heating bed and a clear plastic sheet covered the gravel to prevent possible contamination from the bioassay soils above. A fine spray of tap water was used on the plants twice a day in hot weather, and once every second day in cooler weather, keeping the soil evenly moist, thereby avoiding "go-stop-go" germination (Gorrie and Doran, 1999). Maxicrop seaweed fertilizer (1.9mL/L) was applied lightly on a fortnightly basis to all treatments other than those involving biosolid. *A. salicina* and *A. hakeoides* were grown for 15 weeks before harvest. *E. sideroxylon* was grown for an additional four weeks (19 weeks in total) because of its slower growth rate.

Figure 6.2 The setup of pots for the bioassay experiment. The randomized pots for the glasshouse trials on a heated bed, set up during winter, 1999.

Harvest. At the end of the growth period plants were counted and their heights measured before being harvested. To reduce contamination from the soils, plants were harvested in the order of ascending levels of soil-metal content. Shoots were clipped at the soil surface, and roots separated from the soil and pot liners. The separated roots and shoots of individual plants were washed in tap water and processed as follows. Fresh masses of roots and shoots were determined immediately after harvest. The leaves were removed from the shoots, flattened, and placed between clear plastic sheets and then run through a leaf-area meter (Li-Cor Leaf Area Meter, Lincoln, Nebraska, USA). Total leaf area for each plant was recorded. Dry plant root and shoot mass was calculated after oven drying (70° C, 4 days).

6.2.5 Soil and plant analyses

Site soils from the T, M, B and woodland locations had been analyzed previously (Chapter 3). After the procedures outlined in Sections 6.2.4 had been completed, the soils used were collected from different treatments, individually bagged and air-dried before being used for subsequent soil analyses. The air-dried soils were analyzed for pH, salinity (electrical conductivity), soil organic matter and heavy metal content using the same procedures as described in Chapter 3. Similarly, the metal content of plant materials (roots and shoots) was determined by the same methodology as in Chapter 3. Biosolid-treated soils contain metals introduced by the Nitrohumus, and were not analyzed.

Chapter 7. Laboratory studies: results & discussion

This chapter describes the results obtained in germination trials of several eucalypt and acacia species, and the selection of suitable test species for toxicity assessment of mine-site soils. The outcomes of the bioassays are also discussed and the success of the remediation of the site is evaluated.

7.1 Germination and growth of eucalypt and acacia species

The germination trials of four eucalypts and four acacia species were designed to determine which species of each genus would be most suitable for toxicity tests. Desired qualities included high germination rates, reproducible germination and good growth of seedlings. The germination trials were carried out in two diverse soils, organic mix of neutral pH and a slightly acidic topsoil.

7.1.1 Eucalypts

The results of germination trials with the eucalypt species showed that a higher percentage of seeds of all species germinated in the organic mix than in topsoil (Fig. 7.1). *E. blakelyi and E. sideroxylon* produced superior results, with no significant difference ($p<0.05$) between the germination of these two species in organic mix. *E. polyanthemos* was less responsive in both soils (Fig. 7.1). Germination of *E. macrorhyncha* was very poor in organic mix, and failed to germinate at all in topsoil. The low germination rate of these species in topsoil indicates that this medium was not suited for eucalypt germination. These results indicate that *E. sideroxylon* and *E. blakelyi* were the most tolerant of the four eucalypt species to the soil types tested.

While the germination results suggested that organic mix was the better of the two soils, post-germination growth of seedlings displayed the opposite trend. Growth was more robust in topsoil for all species (Table 7.1). Only *E. sideroxylon* seedlings producing healthy growth in both soils, whereas the other three species produced stunted, yellowing seedlings in organic mix.

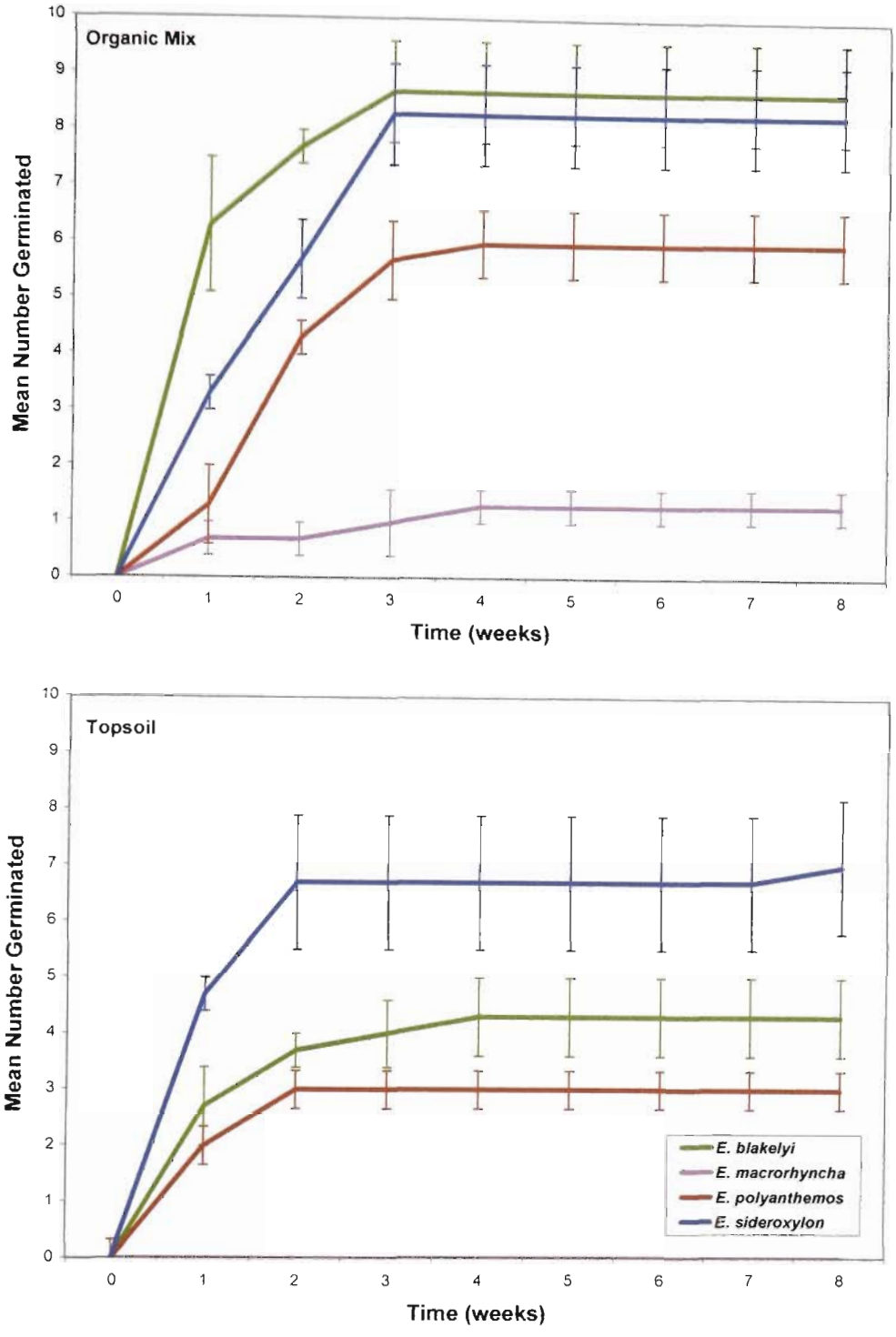


Figure 7.1 Germination trials of *Eucalyptus* species. Germination in organic-mix soil (top) and topsoil (bottom) over eight weeks is shown as the number (mean ± s.e., n = 3) of emerged seedlings from 10 seeds planted in each punnet. No significant differences were found between the germination rates in organic soil for *E. blakelyi* and *E. sideroxylon*, as determined by paired t-test.

Table 7.1 *Eucalyptus* and *Acacia* spp. seedling characteristics. Record of growth characteristics and foliage between germination and week 15 of growth to compare response of plants grown in topsoil versus those grown in organic garden mix.

Plant species	Growth medium	
	Topsoil	Organic mix
Eucalyptus		
E. sideroxylon	Large seedlings, healthy appearance	Fairly sturdy seedlings
E. polianthemos	Large seedlings, healthy appearance	Small, stunted, yellowing of leaves
E. macrorhyncha	Large seedlings, healthy appearance	Small, stunted, yellowing of leaves
E. blakelyi	Large seedlings, healthy appearance	Small, stunted, yellowing of leaves
Acacia		
A. salicina	Sturdy seedlings; broad, healthy leaves	Sturdy seedlings
A. hakeoides	Seedlings struggle to break out of split seed coat, unless shell kept moist to soften	Seedlings struggle to break out of split seed coat, unless shell kept moist to soften
A. dealbata	Compact leaves, medium sized plant	Maintained very modest growth
A. verniciflua	Fragile, small plant	Very delicate foliage

7.1.2 Acacias

All acacias benefited from some form of pre-treatment of seed, though there were a number of differences among the species (Fig. 7.2). Only a small proportion of untreated seeds germinated, and these also took the longest time to germinate. Scarification of seeds improved germination success for most species except for *A. verniciflua*. Scarification also reduced the lag time between planting and germination. Heat treatment was effective in most acacia species grown in topsoil, but far less so with the organic mix. Heat-treated seeds of *A. salicina* did not perform significantly ($p<0.05$) better than seeds without any treatment in organic mix, and no significant difference ($p<0.05$) in germination were detected in topsoil between scarified and heat-treated seeds of *A. salicina* or *A. verniciflua* (Fig. 7.2).

Soil type had little effect on germination of untreated acacia seeds. Maximum germination was higher after scarification in seeds planted in topsoil, with the exception of *A. verniciflua,* which was considerably higher in organic mix. Heat treatment proved highly effective in topsoil, although there was generally a time lag with respect to scarified seeds. *A. hakeoides* in organic mix most clearly displays the delayed response to heat treatment (Fig. 7.2).

Growth characteristics of acacia seedlings, shown in Fig. 7.3, did not indicate significantly different responses to the two growth media (Table 7.1). *A. salicina* and *A. dealbata* grew well in both soils, but the other two species produced less-robust seedlings. The extent of growth after 15 weeks is shown in Fig. 7.4.

7.1.3 Selection of test species for bioassays

The results of germination of the eucalypt species clearly indicated that *E. sideroxylon* was the superior with respect to germination, tolerance of soil type and seedling growth. *E. sideroxylon* was therefore chosen as the eucalypt test species for bioassays.

The choice of a suitable acacia species was made difficult by the diverse results among the species (Table 7.1). Clearly, the seeds required pretreatment to increase

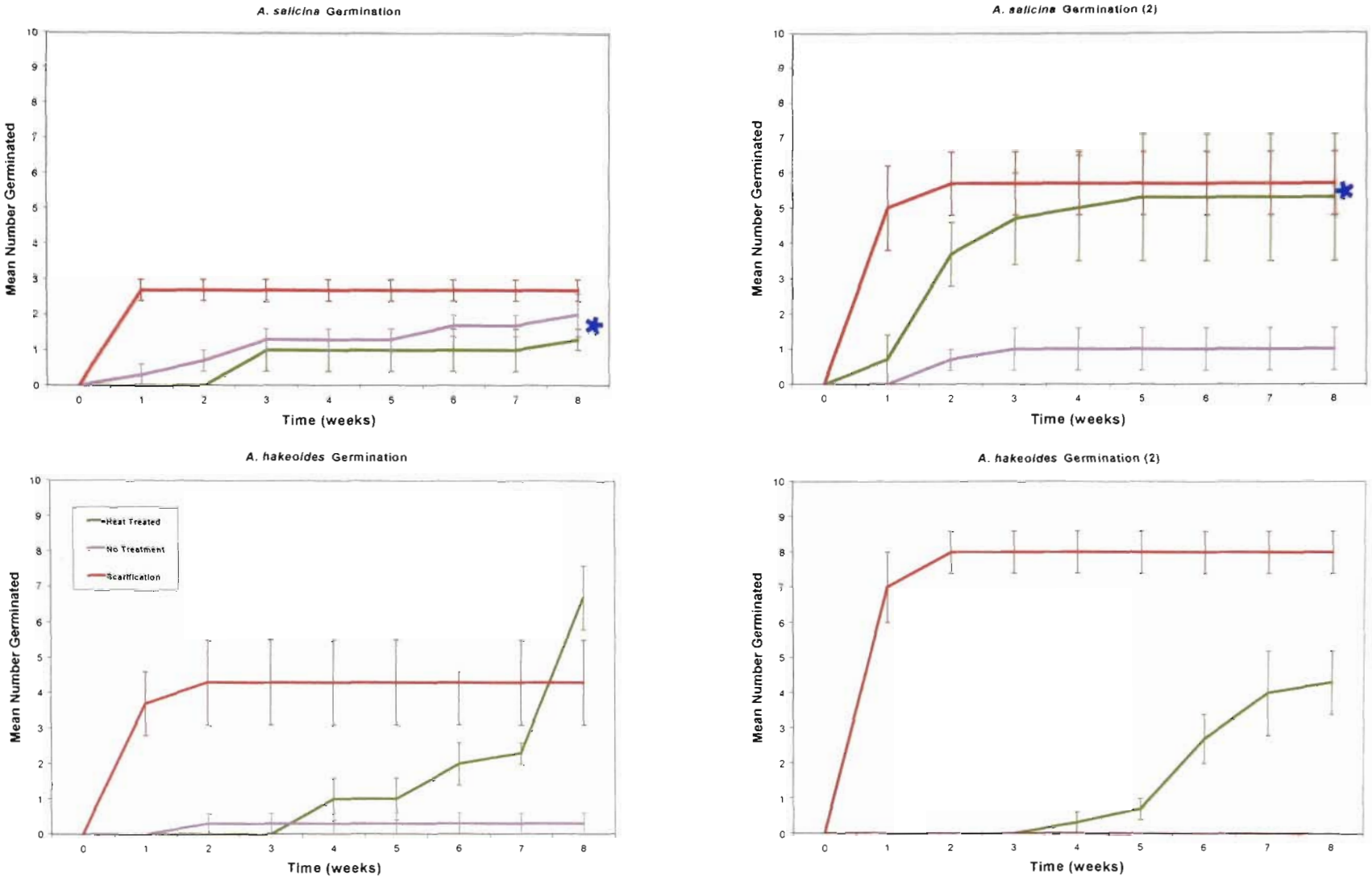


Figure 7.2 Germination trials of *Acacia* species. Germination in organic-mix soil (left) and topsoil (right) over eight weeks is shown as the number (mean ± s.e., n = 3) of emerged seedlings from 10 seeds planted in each punnet. Each set of graphs compares the germination of seeds of the same species pre-treated in one of the following ways: no treatment, heat treatment, and scarification. * indicates where no significant differences were detected (using a paired t-test) between treatments.

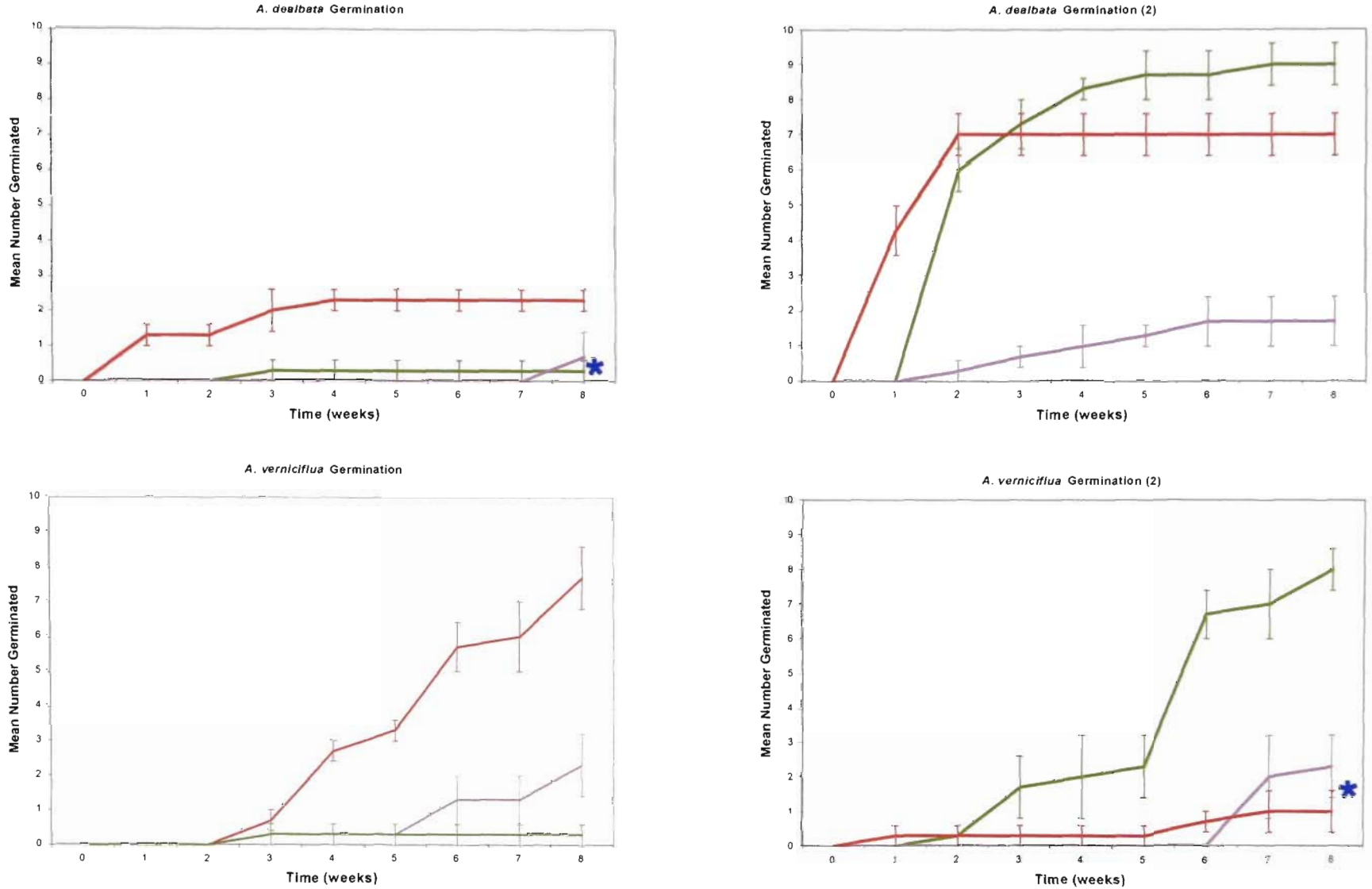


Figure 7.2 (continued). Germination trials of *Acacia* species.

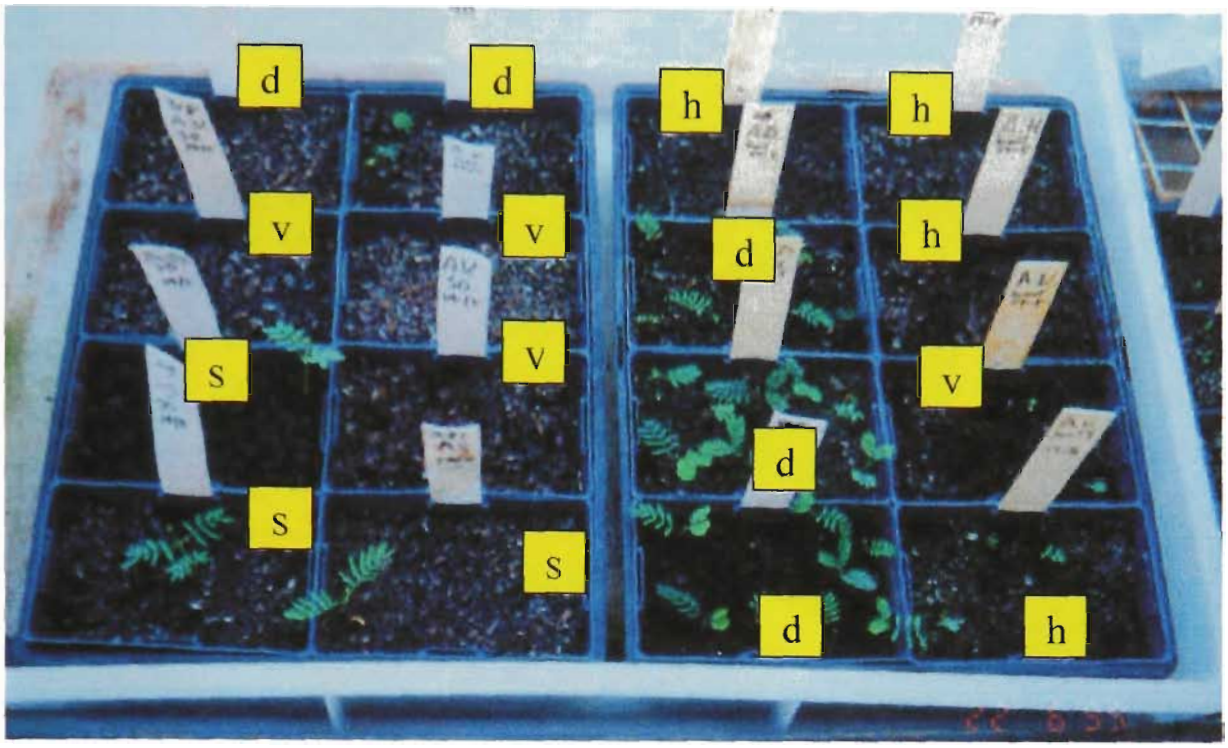


Figure 7.3 Setup of acacia germination trials. Laboratory experiment to compare germination and growth response in garden mix and topsoil growth media. S = *A. salicina;* d = *A. dealbata;* v = *A. verniciflua;* h = *A. hakeoides.*

Figure 7.4 Glasshouse bioassay showing extent of growth of test species. *Acacia salicina* seedlings are growing in the foreground and *Avena sativa* seedlings in the background, as they appeared in late August, 1999.

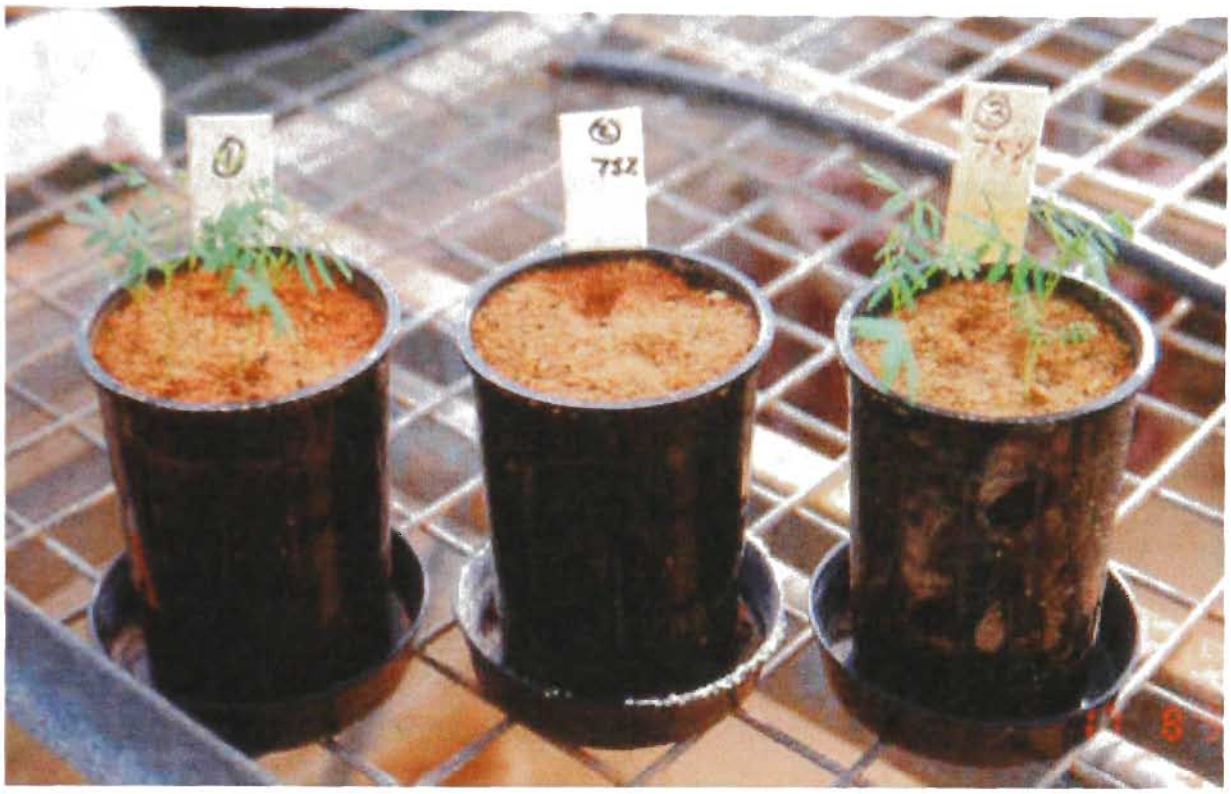


Figure 7.5 A comparison of *Acacia salicina* germination and survival results. From left to right are soils from the top (T subsite), middle (M subsite), and base (B subsite) of the barren site. The growth medium consisted of 75%:25% mixture of site soil and washed river sand. Note that no plants germinated and survived in M subsite soil, where white zinc precipitate on the edge of the drainage pan under the pot attests to the high salinities.

germination, and scarification was chosen since this gave the most consistent results. Within this treatment category, *A. hakeoides* was the best germinator but seedling growth was poor. *A. salicina*, produced satisfactory germination and the most robust seedlings in both soil types (Table 7.1), and was chosen as the test species for the bioassays. Thus, when there was a poor seed crop among acacias in 1999, and *A. salicina* was scarce, *A. hakeoides* was substituted in bioassay experiments.

The dominant tree species at Mt. Costigan were *Eucalyptus blakelyi, E. polyanthemos* and *E. macrorhyncha,* but these were not selected for bioassays because of the better germination results and soil tolerance of *E. sideroxylon.*

7.2 Bioassay of site soils: soil characteristics

Soils that were sieved and mixed with various proportions of river sand for plant germination and growth trials were analyzed in terms of metal content. The soil metal results are presented in Tables 7.2 to 7.7, along with the metal content of plant tissue from these soils. Separate analyses were performed for eucalypt and acacia soils and soil/sand blends. Soil metal content reflects the degree of dilution with washed river sand.

7.3 Bioassay of site soils: germination and growth

7.3.1 Eucalypts

Table 7.8 compares the germination and seedling survival rates of *E. sideroxylon* in barren-site and woodland soil bioassays. Germination results demonstrated moderate to high rates in the top (T) and base (B) barren-slope subsite soils. However, a markedly different response was obtained in the mid-slope (M) soils, where germination was zero, except in the control. Organic matter alone was probably not a critical issue, since broadly comparable germination results were obtained from pure river sand and various blends of river sand and woodland soil (Table 7.8). *E. sideroxylon* recorded a germination time delay in the three barren-

Table 7.2 Bioassay of site soils: copper. Concentration in soils and plant tissues of acacia and eucalyptus. Metal concentrations are expressed as mg/kg^{-1} or $\mu g/g^{-1}$ dry weight. Concentration factors (ratios of metal concentration in plant tissues to that in the soil) are indicated in parentheses; E.R., or enrichment ratio of the highest metal contamination to that of the control, is shown. The values are means ± se (n = 5), except where the values are means of duplicate determinations. The limit of detection for copper was 7 mg/kg.

Acacia spp.	Copper concentration (mg/kg)									Woodland (mg/kg)		
	Top			Middle			Base					
Soil dilution (%)	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves
100% sand	<7	52.0 ± 3 (74.3)	<7	<7	52.0 ± 3 (74.3)	<7	<7	52.0 ± 3 (74.3)	<7	<7	34.1 ± 3.4 (48.7)	27.2 ± 0.3 (38.9)
25	165	407 ± 0 (2.5)	<7	-	-	-	26.6	85.5 ± 14.3 (3.2)	<7	<7	42.0 ± 4.9 (7.8)	34.0 ± 1.3 (6.3)
50	225.6	209 ± 4.7 (0.9)	<7	-	-	-	65.8	98.6 ± 3.8 (1.5)	<7	7.3	41.2 ± 3.4 (5.6)	37.4 ± 0.8 (5.1)
75	336.2	217 ± 48.3 (0.6)	<7	-	-	-	104.7	93.4 ± 15.3 (0.4)	<7	12.7	28.0 ± 0.5 (2.2)	34.7 ± 1.5 (2.7)
100% soil	492	232 ± 0 (0.5)	<7	-	-	-	157.2	64.9 ± 16.4 (0.4)	7.1 ± 0 (<0.1)	25.9	29.3 ± 3 (1.1)	31.7 ± 0.5 (1.2)
E.R.	>70	7.8	-	-	-	-	>22	1.8	>1	>3.7	1.2	1.4
E. sideroxylon												
100% sand	<7	82.8 ± 7.3 (118.3)	7.3 ± 0.7 (10.4)	<7	82.8 ± 7.3 (118.3)	7.3 ± 0.7 (10.4)	<7	82.8 ± 7.3 (118.3)	7.3 ± 0.7 (10.4)	<7	57.2 ± 3.8 (81.7)	10 ± 0.3 (14.3)
25	-	-	-	-	-	-	40.3	117.4 ± 0 (2.9)	12.3 ± 0.6 (0.3)	<7	28.4 ± 3.4 (8.9)	7.7 ± 0.1 (2.4)
50	-	-	-	-	-	-	43.3	160 ± 0 (3.7)	18.4 ± 0 (0.4)	7.6	23.1 ± 1.2 (3.0)	<7
75	-	-	-	-	-	-	111.9	134.8 ± 0 (11.3)	17.6 ± 0 (0.2)	13.5	43.2 ± 4.6 (3.2)	9.8 ± 0.1 (0.7)
100% soil	491.7	168 ± 0 (0.3)	22.3 ± 0 (<0.1)	-	-	-	-	-	-	25.8	38.3 ± 6.6 (1.5)	7.7 ± 0.3 (0.3)
E.R.	>70	2.0	3.1	-	-	-	>16	1.9	2.5	>3.7	0.8	1

Table 7.3 Bioassay of site soils: zinc. Concentration in soils and plant tissues of acacia and eucalyptus. Metal concentrations are expressed as mg/kg^{-1} or $\mu g/g^{-1}$ dry weight. Concentration factors (ratios of metal concentration in plant tissues to that in the soil) are indicated in parentheses; E.R., or enrichment ratio of the highest metal contamination to that of the control, is shown. The values are means ± se (n = 5), except where the values are means of duplicate determinations. The limit of detection for zinc was 17 mg/kg.

***Acacia* spp.**	**Zinc concentration (mg/kg)**									**Woodland (mg/kg)**		
	Top			**Middle**			**Base**					
Soil dilution (%)	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**
100% sand	<17	216 ± 10.6 (12.7)	18.9 ± 1.6 (2.9)	<17	216 ± 10.6 (33.2)	18.9 ± 1.6 (2.9)	<17	216 ± 10.6 (33.2)	18.9 ± 1.6 (2.9)	<17	219 ± 24.9 (33.2)	44.4 ± 1.3 (44.4)
25	308	3,300 ± 0 (10.7)	550 ± 0 (1.8)	-	-	-	68.8	193 ± 18.5 (2.8)	25.2 ± 1.2 (0.4)	<17	222 ± 49.3 (17.9)	59.8 ± 7.5 (4.8)
50	429	1,826 ± 0 (4.3)	397 ± 47 (0.9)	-	-	-	167	275 ± 12.7 (1.6)	28.1 ± 0.2 (0.2)	28.6	315 ± 39.7 (11.0)	57.4 ± 1.2 (2.0)
75	647	1,530 ± 232 (2.4)	206 ± 11 (0.3)	-	-	-	353	361 ± 43.1 (1.0)	38.7 ± 0.9 (0.1)	32.0	234.1 ± 15.4 (7.3)	62.1 ± 2.2 (1.9)
100% soil	910	1,290 ± 0 (1.4)	159 ± 3.6 (0.1)	-	-	-	379	193 ± 41.4 (0.5)	46.5 ± 2.1 (0.1)	76.7	219 ± 24.9 (2.9)	52.0 ± 0.6 (0.7)
E.R.	>54	15.3	29.1	-	-	-	>22	1.7	2.5	>4.5	1.4	1.4
E. sideroxylon												
100% sand	<17	147 ± 10 (22.6)	40.2 ± 2.9 (6.2)	<17	147 ± 10 (22.6)	40.2 ± 2.9 (6.2)	<17	147 ± 10 (22.6)	40.2 ± 2.9 (6.2)	<17	344 ± 32.8 (52.9)	22.7 ± 1.8 (3.5)
25	-	-	-	-	-	-	81	186 ± 0 (2.3)	59.8 ± 4.7 (0.7)	<17	243 ± 21.3 (22.3)	42.6 ± 1.6 (3.9)
50	-	-	-	-	-	-	210	269 ± 0 (1.3)	92.1 ± 0 (0.4)	19	231 ± 18.2 (12.2)	26.4 ± 1.7 (1.4)
75	-	-	-	-	-	-	356	280 ± 0 (0.8)	142 ± 0 (0.4)	35.4	194.4 ± 11.2 (5.5)	32.3 ±1.2 (0.9)
100% soil	915	472 ± 0 (0.5)	140 ± 0 (0.2)	-	-	-	-	-	-	76.7	195 ± 16.7 (2.5)	32.7 ± 1.6 (0.4)
E.R.	>54	3.2	3.5	-	-	-	>21	1.9	3.5	>4.5	0.6	1.9

Table 7.4 Bioassay of site soils: iron. Concentration in soils and plant tissues of acacia and eucalyptus. Metal concentrations are expressed as mg/kg^{-1} or $\mu g/g^{-1}$ dry weight. Concentration factors (ratios of metal concentration in plant tissues to that in the soil) are indicated in parentheses; E.R., or enrichment ratio of the highest metal contamination to that of the control, is shown. The values are means ± se (n = 5), except where the values are means of duplicate determinations. The limit of detection for iron was 82 mg/kg.

Acacia spp.	Iron concentration (mg/kg)									Woodland (mg/kg)		
	Top			Middle			Base					
Soil dilution (%)	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves
100% sand	2,050	1,120 ± 56.6 (0.5)	<82	2,050	1,120 ± 56.6 (0.5)	<82	2,050	1,120 ± 56.6 (0.5)	<82	2,050	2,900 ± 563 (1.4)	<82
25	13,500	5,630 ± 0 (0.4)	<82	-	-	-	5,200	2,530 ± 420 (0.5)	<82	2,940	8,140 ± 1,910 (2.8)	<82
50	19,000	3,360 ± 0 (0.2)	<82	-	-	-	10,500	3,250 ± 288 (0.3)	<82	5,090	8,509 ± 1,165 (1.7)	<82
75	27,400	3,510 ± 630 (0.1)	98.7 ± 36.2 (<0.01)	-	-	-	15,200	3,490 ± 678 (0.2)	119 ± 5.5 (<0.01)	8,890	4,460 ± 238 (0.5)	<82
100% soil	41,400	4,000 ± 0 (0.1)	<82	-	-	-	21,200	1,940 ± 470 (0.1)	82.2 ± 4.3 (<0.01)	13,400	4,920 ± 1,380 (0.4)	104 ± 3.7 (<0.01)
E.R.	20	3.6	>1	-	-	-	10	1.7	>1	6.5	6.5	>1
E. sideroxylon												
100% sand	2,050	2,400 ± 254 (1.2)	<82	2,050	2,400 ± 254 (1.2)	<82	2,050	2,400 ± 254 (1.2)	<82	2,050	3,230 ± 174 (1.6)	<82
25	-	-	-	-	-	-	5,830	3,120 ± 0 (0.5)	123 ± 26 (<0.01)	3,230	4,510 ± 1,240 (1.4)	<82
50	-	-	-	-	-	-	8,940	3,600 ± 0 (0.4)	105 ± 0 (<0.01)	4,970	2,330 ± 257 (0.5)	<82
75	-	-	-	-	-	-	16,400	3,060 ± 0 (0.2)	258 ± 0 (<0.01)	7,370	5,870 ±1,120 (0.8)	<82
100% soil	40,200	6,710 ± 0 (0.2)	183 ± 0 (<0.01)	-	-	-	-	-	-	13,400	6,470 ± 1,990 (0.5)	<82
E.R.	20	2.8	>2	-	-	-	-	-	-	6.5	2.0	-

Table 7.5 Bioassay of site soils: lead. Concentration in soils and plant tissues of acacia and eucalyptus. Metal concentrations are expressed as mg/kg^{-1} or $\mu g/g^{-1}$ dry weight. Concentration factors (ratios of metal concentration in plant tissues to that in the soil) are indicated in parentheses; E.R., or enrichment ratio of the highest metal contamination to that of the control is shown. The values are means ± se (n = 5), except where the values are means of duplicate determinations. The limit of detection for lead was 12 mg/kg.

Acacia spp.	Lead concentration (mg/kg)									Woodland (mg/kg)		
	Top			Middle			Base					
Soil dilution (%)	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves
100% sand	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	15.8 ± 2.3 (7.2)	<12
25	756	820 ± 0 (1.1)	<12	-	-	-	36.6	18.4 ± 3.0 (0.5)	<12	13.1	85.7 ± 17.1 (6.5)	<12
50	847	465 ± 0 (0.5)	<12	-	-	-	88.6	30 ± 2.9 (0.3)	<12	24	84.8 ± 12.1 (3.5)	<12
75	1,220	428 ± 82.5 (0.4)	12.9 ± 2.4 (<0.01)	-	-	-	107.5	25.7 ± 9.6 (0.2)	<12	36.4	61.6 ± 4.1 (1.7)	15.3 ± 0.9 (0.4)
100% soil	1,970	483 ± 0 (0.2)	<12	-	-	-	228	25.3 ± 6.9 (0.1)	<12	80.4	44 ± 10.3 (0.5)	<12
E.R.	>165	>68	>1	-	-	-	>19	>2		>6	>5	>1
E. sideroxylon												
100% sand	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12
25	-	-	-	-	-	-	45.2	31.5 ± 0 (0.7)	<12	<12	70.7 ± 8.8 (7.5)	<12
50	-	-	-	-	-	-	68.3	40.9 ± 0 (0.6)	<12	22.5	43.3 ± 1.6 (1.9)	<12
75	-	-	-	-	-	-	114	<12	<12	46.6	68.8 ± 6.7 (1.5)	<12
100% soil	1,790	67.1 ± 0 (<0.01)	<12	-	-	-	-	-	-	80.4	59.6 ± 10.7 (0.7)	<12
E.R.	>150	>5	-	-	-	-	>9	>3	-	>9	>5	

Table 7.6 Bioassay of site soils: manganese. Concentration in soils and plant tissues of acacia and eucalyptus. Metal concentrations are expressed as mg/kg^{-1} or $\mu g/g^{-1}$ dry weight. Concentration factors (ratios of metal concentration in plant tissues to that in the soil) are indicated in parentheses; E.R., or enrichment ratio of the highest metal contamination to that of the control is shown. The values are means ± se (n = 5), except where the values are means of duplicate determinations.

***Acacia* spp.**	**Manganese concentration (mg/kg)**									**Woodland (mg/kg)**		
	Top			**Middle**			**Base**					
Soil dilution (%)	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**
100% sand	18	939 ± 27.1 (52.2)	1,130 ± 27.5 (62.8)	18	939 ± 27.1 (52.2)	1,130 ± 27.5 (62.8)	18	939 ± 27.1 (52.2)	1,130 ± 27.5 (62.8)	18	683 ± 44.2 (37.9)	274 ± 54.3 (15.2)
25	86.2	93.5 ± 0 (1.1)	148 ± 0 (1.7)	-	-	-	35.7	46 ± 7 (1.3)	16.2 ± 0.7 (0.5)	24.6	307 ± 31.8 (12.5)	418 ± 37.7 (17.0)
50	44.9	39.5 ± 0 (0.9)	98.9 ± 3.1 (2.2)	-	-	-	47.9	27.9 ± 0.6 (0.6)	13.6 ± 0.2 (0.3)	35.7	341 ± 44.1 (9.6)	453 ± 23.2 (12.7)
75	48.2	23.3 ± 2.1 (0.5)	32.9 ± 0.9 (0.7)	-	-	-	72.8	24.9 ± 5 (0.3)	15.9 ± 0.3 (0.2)	46.6	153 ± 13.4 (3.3)	461 ± 9.3 (9.9)
100% soil	66.6	18.7 ± 0 (0.3)	20 ± 0.3 (0.3)	-	-	-	77.6	12 ± 2.9 (0.2)	12.6 ± 0.6 (0.2)	69.8	104 ± 25.2 (1.5)	260 ± 11.8 (3.7)
E.R.	3.7	<0.01	<0.01	-	-	-	4.3	<0.01	<0.01	3.9	0.2	0.9
E. sideroxylon												
100% sand	18	662 ± 27.6 (36.8)	1,670 ± 57.5 (92.8)	18	662 ± 27.6 (36.8)	1,670 ± 57.5 (92.8)	18	662 ± 27.6 (36.8)	1,670 ± 57.5 (92.8)	18	119 ± 6.4 (6.6)	429 ± 29.6 (23.8)
25	-	-	-	-	-	-	45.1	37.7 ± 0 (0.8)	60.5 ± 5.2 (1.3)	19.2	432 ± 69.3 (22.5)	1,950 ± 36.3 (101.6)
50	-	-	-	-	-	-	55.2	31.9 ± 0 (0.6)	53.1 ± 0 (1.0)	29.9	533 ± 42.4 (17.8)	1,790 ± 148 (59.9)
75	-	-	-	-	-	-	74.8	28.7 ± 0 (0.4)	69.3 ± 0 (0.9)	41.3	385 ± 47.2 (9.3)	1,760 ± 13.9 (42.6)
100% soil	51.3	41.7 ± 0 (0.8)	79.8 ± 0 (1.6)	-	-	-	-	-	-	69.8	251 ± 32.3 (3.6)	1,190 ± 22.6 (17.0)
E.R.	2.9	0.1	0.1	-	-	-	-	-	-	3.9	2.1	2.8

Table 7.7 Bioassay of site soils: cadmium. Concentration in soils and plant tissues of acacia and eucalyptus. Metal concentrations are expressed as mg/kg^{-1} or $\mu g/g^{-1}$ dry weight. Concentration factors (ratios of metal concentration in plant tissues to that in the soil) are indicated in parentheses; E.R., or enrichment ratio of the highest metal contamination to that of the control is shown. The values are means ± se (n = 5), except where the values are means of duplicate determinations. The limit of detection for cadmium was 5 mg/kg.

***Acacia* spp.**	**Cadmium concentration (mg/kg)**									**Woodland (mg/kg)**		
	Top			**Middle**			**Base**					
Soil dilution (%)	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**	**Soil**	**Roots**	**Leaves**
100% sand	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
25	<5	51.7 ± 0 (28.7)	<5	-	-	-	<5	5.8 ± 1.1 (3.9)	<5	<5	<5	<5
50	<5	43.1 ± 0 (19.6)	<5	-	-	-	<5	8.1 ± 0.5 (3.7)	<5	<5	<5	<5
75	<5	38.9 ± 10.2 (389)	<5	-	-	-	<5	19.9 ± 3.7 (8.0)	<5	<5	<5	<5
100% soil	<5	40.7 ± 0 (23.9)	<5	-	-	-	<5	8.3 ± 2.3 (4.0)	<5	<5	<5	<5
E.R.	-	>10	-	-	-	-	-	>4	-	-	-	-
E. sideroxylon												
100% sand	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
25	-	-	-	-	-	-	<5	7.0 ± 0 (8.8)	<5	<5	<5	<5
50	-	-	-	-	-	-	<5	11.5 ± 0 (7.7)	<5	<5	<5	<5
75	-	-	-	-	-	-	<5	18.3 ± 0 (5.4)	<5	<5	<5	<5
100% soil	<5	15.5 ± 0 (5.7)	<5	-	-	-	-	-	-	<5	<5	<5
E.R.	-	>3	-	-	-	-	-	>3	-	-	-	-

Table 7.8 Maximum germination and seedling survival of *E. sideroxylon* in a bioassay of site soils. The soils were diluted with washed river sand in the proportions indicated. Results are shown for neat soils (100%), their dilutions with sand and sand alone (0%). Germination results are given as means ± se (n = 3) of the number of seedlings that have emerged, while survival is expressed as % of the emerged seedlings that have survived.

	Barren site soils						Woodland soil	
	Top		Middle		Base			
Soil dilution (% soil)	Max. Germ.	Survival (%)	Max. Germ.	Survival (%)	Max. Germ.	Survival (%)	Max. Germ.	Survival (%)
0	5.2 ± 0.4	54	4.8 ± 0.4	52	4.8 ± 0.5	56	9.8 ± 0.2	90 ± 4.7
25	5.6 ± 0.5	0	0	0	9 ± 0.3	27	7.4 ± 1.3	70 ± 10.6
50	5.6 ± 0.8	0	0	0	2.6 ± 0.5	65	8.6 ± 0.5	86 ± 6.0
75	5 ± 0.4	0	0	0	3.2 ± 0.4	41	10 ± 0	90 ± 1.6
100	4 ± 0.4	0	0	0	4.4 ± 0.5	23	8.6 ± 0.5	89 ± 3.5

slope soils of about 7 days (Fig 7.1), but this was faster than most acacias. Eucalypt germination rate was highest and most rapid in neat woodland soils and blends.

Apart from their complete lack of germination in any M subsite soils, eucalypt seedlings were also unable to survive in any soil from the T subsite, even in dilutions with as little as 25% soil (Table 7.8). Survival of *E. sideroxylon* in B subsite media is consistent with earlier observations concerning the less acidic soil conditions on the lower slope. Even here, however, the eucalypt survival rates are substantially lower than acacias in the same soils, again reflecting the lower tolerance by the eucalypts of barren-site conditions.

7.3.2 Acacias

Table 7.9 shows the patterns of germination of *A. salicina* in the three barren-slope soils, and of *A. hakeoides* in woodland soil, over a period of 25 days. Unlike the eucalypts, acacias showed greater tolerance and germinated in all soils. A large proportion of *A. hakeoides* germinated in neat woodland soil, similar to the germination rates in the river sand control. Germination was consistently lower in the site soils and soil/sand mixtures, especially in mid-slope M samples, where rates decreased as the proportions of site soil increased. Nonetheless, *A. salicina* showed a reasonable ability to germinate even in the contaminated M soil, in spite of some time delay.

Like the eucalypts, no acacia seedlings survived at all in the M subsite growth medium, even when diluted by 75% river sand (Table 7.9). However, acacia seedling survival was high in woodland soils, as well as in neat soil from the T and B subsites. Furthermore, acacias showed little or no response to dilution with river sand, with over 90% survival in neat site soils.

These results suggest that a critical factor inhibiting acacia seedling survival is the combination of low pH, high salinity, and metal toxicity present in the mid-slope M subsite in the vicinity of the seep. High metal loadings alone are not critical, given the results obtained from the T and B subsite soils. It appears that other factors are at play that affect bioavailability of metals and their toxic impact. Furthermore, the

Table 7.9 Maximum germination and seedling survival of *A. salicina* in a bioassay of site soils, and *A. hakeoides* in woodland soil. The soils were diluted with washed river sand in the proportions indicated. Results are shown for neat soils (100%), their dilutions with sand and sand alone (0%). Germination results are given as means ± se (n = 3) of the number of seedlings that have emerged, while survival is expressed as % of the emerged seedlings that have survived.

Species	*Acacia salicina*						*Acacia hakeoides*	
	Barren site soils						Woodland soil	
	Top		Middle		Base			
Soil dilution (% soil)	Max. Germ.	Survival (%)	Max. Germ.	Survival (%)	Max. Germ.	Survival (%)	Max. Germ.	Survival (%)
0	5.8 ± 0.4	90	6.2 ± 0.6	68	6 ± 0.7	93	8.6 ± 0.2	100 ± 0
25	3.8 ± 0.4	79	3.2 ± 0.4	0	5.4 ± 0.5	93	7.8 ± 1.2	98 ± 2.2
50	5.0 ± 0.3	80	2.4 ± 0.5	0	5.8 ± 0.6	76	8.8 ± 0.2	100 ± 0
75	4.6 ± 0.5	83	1.2 ± 0.4	0	4 ± 0.8	95	8.6 ± 0.2	100 ± 0
100	4.2 ± 0.6	95	2 0.6	0	6.8 ± 0.8	94	8.8 ± 0.2	98 ± 2.2

presence of organic matter is not essential for seedling survival, since it is very low in river sand.

7.3.3 Acacia and eucalyptus growth characteristics

Germination and survival rates of both *E. sideroxylon* and *A. hakeoides* were high in woodland soil, and were not significantly affected by dilution with river sand. Eucalypt leaf area and height are substantially greater in woodland than site soils, with the difference increasing in proportion to the ratio of site soil to sand (Table 7.10). Acacias, on the other hand, show no notable difference between leaf and height values in woodland versus different blends of and site soils (Table 7.10). In fact, eucalypt growth characteristics in neat B subsite soil are very similar to the results obtained in woodland soil.

Apart from the M subsite, where no eucalypt seeds germinated, no seedlings survived in the T subsite medium. In soil from the B subsite, diminishing eucalypt seedling survival rates accompanying increasing proportions of site soil reflect toxicity effects. All of these poor results for eucalypts in barren-site media stand in marked contrast to their strong affinity to woodland soils.

Acacias were far more tolerant of barren-slope soils. Apart from the fact that no seedling survived in M subsite soil, both T and B subsites showed moderately good germination and high survival rates (Fig. 7.5). Surprisingly, there is no preference for river sand over these T and B site soils, indicating a remarkable tolerance by acacias for the metals present in these soils.

Reinforcing this adaptation, overall biomass was markedly higher in the acacias than eucalypts in barren-site soils. There were interesting differences in acacia response to the T and B subsites. Contrary to expectations, acacia leaf area and height were higher in neat, metal-rich T subsite soil than in diluted blends. The opposite was true of B site soil, where the biomass declined with increasing soil metal content.

Table 7.10 Bioassay of site soils. Mean height and leaf area of *Eucalyptus sideroxylon* and *Acacia salicina* seedlings in three barren site soils, and *A. hakeoides* in woodland soil: Top (T), Middle (M) and Base (B) and Woodland (W) soil. Values are means ± se (n = 3).

Dilution (%)	Leaf area (cm^2)				Height (cm)			
	T	M	B	W	T	M	B	W
	Eucalyptus sideroxylon							
0	62.4 ± 5.2	50.1 ± 16.1	103.8 ± 29.8	36.9 ± 5.7	19.4 ± 2.3	18.4 ± 2.5	24.1 ± 2.6	16.3 ± 3.2
25	0	0	19.4 ± 4.3	39.6 ± 7.6	0	0	9.5 ± 1.5	10.6 ± 2.3
50	0	0	18.6 ± 5.2	62.0 ± 6.2	0	0	10.1 ± 2.4	18.2 ± 1.4
75	0	0	3.1 ± 2.1	67.0 ± 5.2	0	0	3 ± 1.0	24.1 ± 2.4
100	5.1 ± 2.4	0	1.4	112.2 ± 8.1	4.5 ± 1.3	0	2.5 ± 0	27.6 ± 1.2
	***Acacia salicina* (barren site) and *A. hakeoides* (woodland)**							
0	57.1 ± 6.7	60.6 ± 4.8	50.3 ± 6.2	16.3 ± 1.3	19.0 ± 1.5	16.2 ± 1.4	15.5 ± 3.1	8.6 ± 0.9
25	8.9 ± 1.6	0	51.7 ± 5.4	35.6 ± 3.3	3.4 ± 0.4	0	21.2 ± 1.7	13.4 ± 1.2
50	13.3 ± 2.1	0	51.9 ± 2.6	52.6 ± 3.1	6.1 ± 0.9	0	22.2 ± 2.9	18.7 ± 1.2
75	24.9 ± 3.3	0	41.9 ± 5.2	48.1 ± 6.0	10.0 ± 1.6	0	20.7 ± 1.8	17 ± 1.7
100	31.6 ± 4.7	0	31.9 ± 3.4	47.9 ± 4.9	13.5 ± 2.0	0	16.9 ± 1.5	18.1 ± 1.6

E. sideroxylon. in contrast, *showed* a strong preference for woodland soil, with a biomass approximately double that of *A. hakeoides* in the same medium. Although the acacias had the largest biomass overall and were clearly the more adaptable species, the greatest biomass of all was recorded in *E. sideroxylon* in the woodland soil.

The root:shoot ratio (Figs. 7.6 and 7.7) provides an important basis for comparison of growth conditions, as changes in the ratio can reflect changes in the plant's vigour (A. Pulkownik, personal communication). In all trials involving site soil, acacia shoot biomass exceeded that of the roots by ratio by a clear margin (Fig. 7.7), showing the healthy adaptation of the acacias to site conditions. On the other hand, *E. sideroxylon* does twice as well as the acacias in woodland soils in terms of overall biomass and far more vigorous growth characteristics. Notably, *A. salicina* achieves maximum biomass not in the woodland soil but in diluted site soils.

Eucalypt leaf area and height were both extremely low in the T and B site soils compared with the woodland and sand control (Table 7.10). In contrast, the acacias flourished in these site soils, with leaf areas subequal to, or only a little below, those in grown in woodland soil and river sand.

In summary, A. *salicina* root and shoot biomass, leaf area and height confirm that this species is better suited to Mt. Costigan barren-slope conditions than are the euclypyts. *A. salicina* is highly tolerant of all but the most highly contaminated soils. On the other hand, *E.* sideroxylon does twice as well as the acacias in woodland soils, where it shows vigorous growth characteristics. However, its response to barren-slope soils in terms of low germination rate, poor survival and biomass confirms that it is a good indicator of toxicity, even to low metal levels in those diluted site soils, where acacias flourish.

Variable results in the river-sand control medium may have stemmed from several factors including: seasonal difference (barren-site controls were grown in the cool season, whereas woodland controls were grown at a warmer time of year); rapid drying out of the sand, which lacked appreciable organics or clays to retain

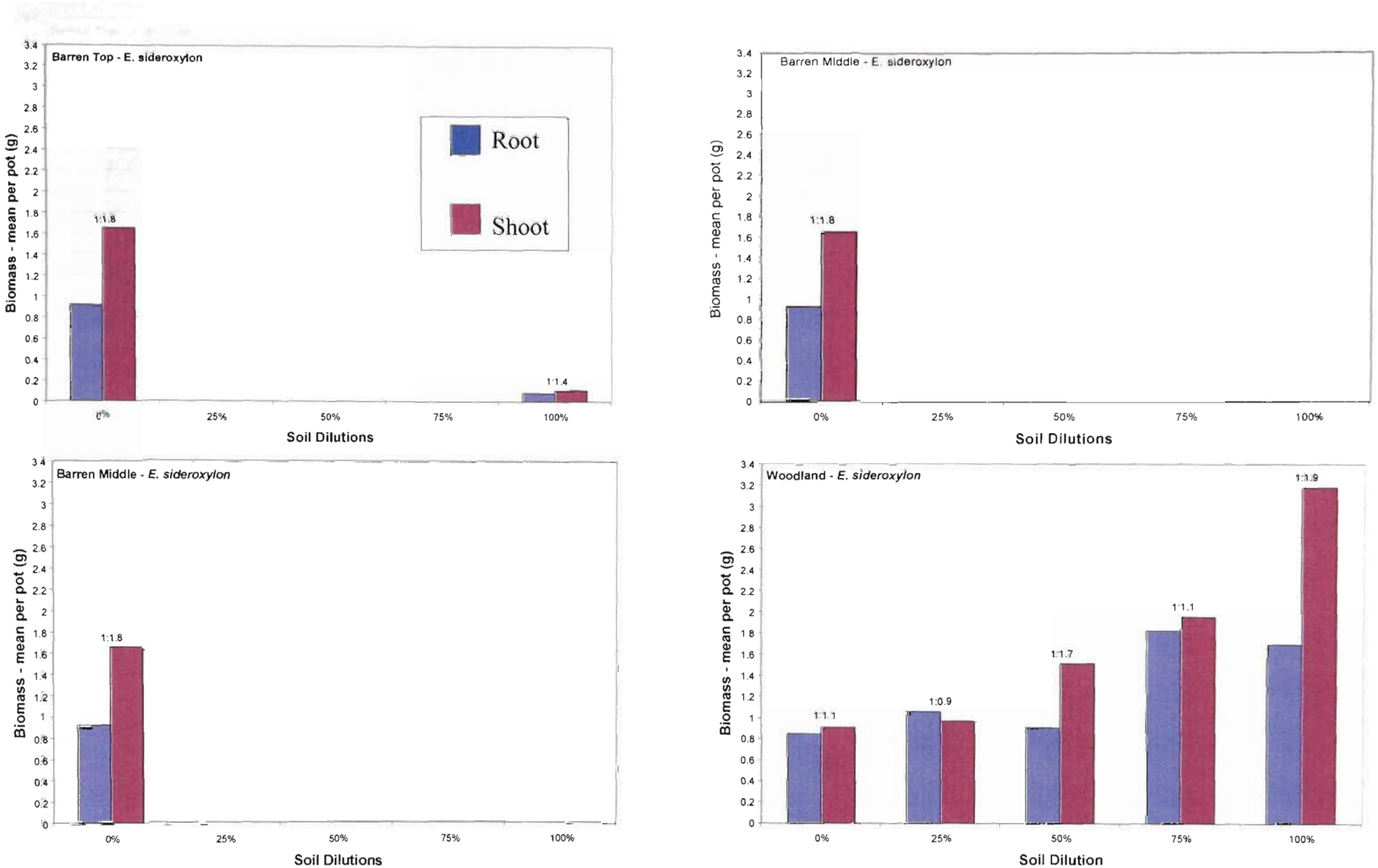


Figure 7.6 Root and shoot biomass of eucalypts grown under bioassay conditions. 100% refers to neat soil; 0% refers to neat sand. The numerical ratio of root to shoot biomass is indicated at the top of the columns.

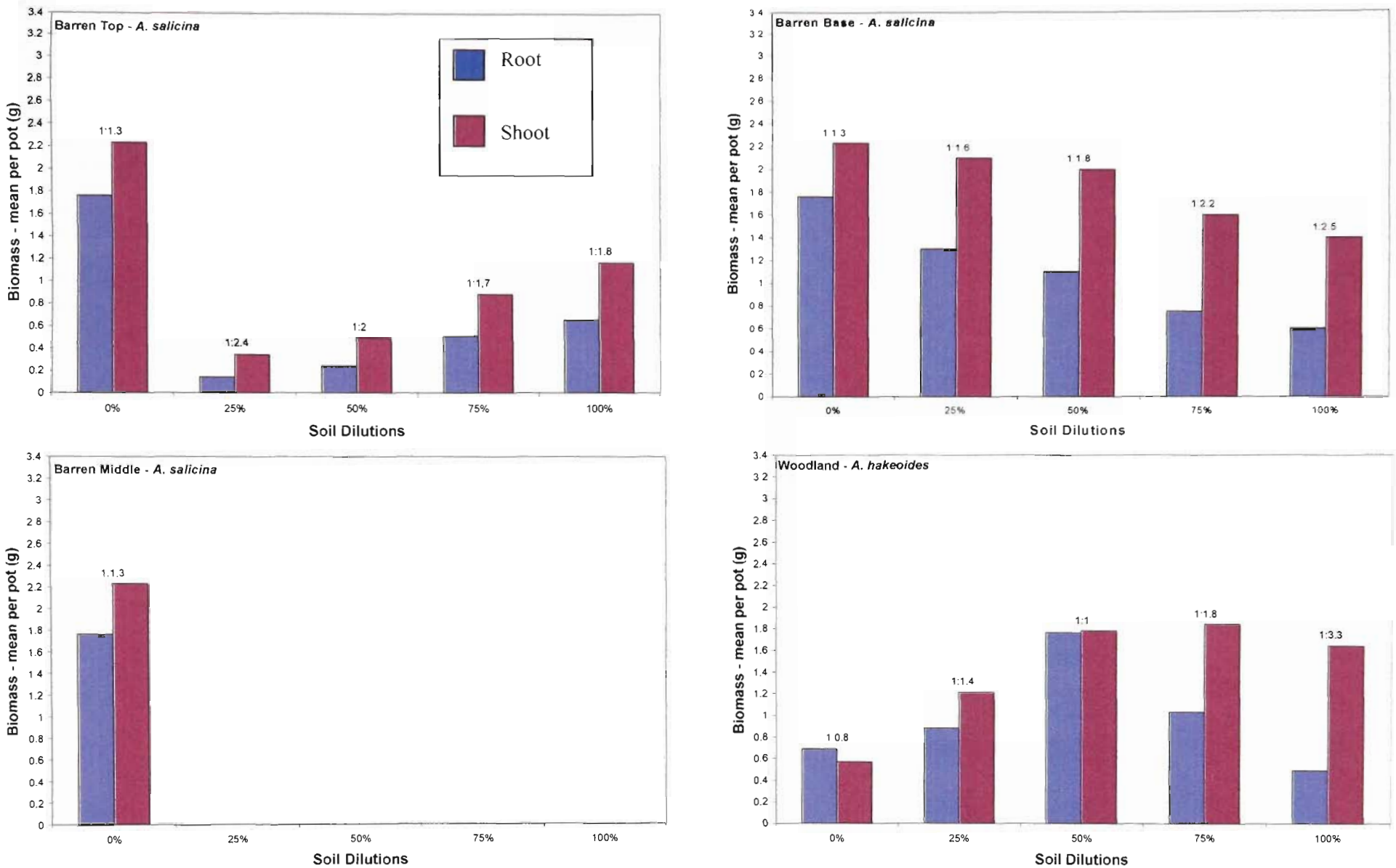


Figure 7.7 Root and shoot biomass of acacia species grown under bioassay conditions. Plants were grown in mixtures of river sand and soil from the top (T), middle (M) and base (B) of the barren site, and woodland. 100% refers to neat soil; 0% refers to neat sand. The numerical ratio of root to shoot biomass is indicated at the top of the columns.

moisture; or irregular rotation of pots, which may not have had equal exposure to sunlight. Some pots were more affected than others by dense, green algal colonization on the soil surface.

7.3.4 *Avena sativa* (Oats)

Oats was used as a control-crop species. Table 7.11 shows that plants grown in undiluted woodland soil produced better growth (plant mass, height and leaf area) than those in the sand alone. The T and B subsites achieved relatively good height and leaf area, not far behind the woodland and control, but biomass was low in both cases. Oats grown in soil from the M subsite, in contrast, was only one-tenth of the height of the specimens in woodland soil, and extremely low in terms of mass and leaf area. However, unlike the acacias and eucalypts used in this study, oats germination did occur in all soil types.

Table 7.11 Growth characteristics of oats (*Avena sativa*) grown in soils from the barren mine site and adjacent woodland. The control represents washed river sand; others are all neat soil samples (100%) from the Top, Middle and Base subsites of the barren site, as well as from the woodland. Plant weight is expressed in grams, heights are in centimetres, and leaf areas are in cm^2. The values shown are means (± se), n=5.

Soil Type	Dilution	Root Weight	Shoot Weight	Plant Height	Leaf Area
Control	0%	0.06 ± 0	0.06 ± 0	33.6 ± 0.74	16.8 ± 0.53
Top	100%	0.07 ± 0.01	0.04 ± 0	26.4 ± 1.03	11.9 ± 0.68
Middle	100%	0.02 ± 0	0.01 ± 0	3.5 ± 0.5	0.45 ± 0.08
Base	100%	0.08 ± 0.01	0.05 ± 0	30.8 ± 0.46	18.6 ± 0.32
Control	0%	0.13 ± 0.02	0.16 ± 0.01	43.6 ± 0.51	15.4 ± 0.51
Woodland	100%	0.30 ± 0.05	0.23 ± 0.01	40.4 ± 0.81	24.0 ± 1.25

The results as a whole indicate that the oats species could be used as more relevant surrogate test species for purposes of environmental phytotoxicity assessment and restorative ecology. The fact that they survive in all soils would permit comparisons on growth rate, biomass and overall plant health.

7.4 Bioassay of site soils: Metal accumulation and partitioning in native plants and in oats

Metal levels determined at harvest are shown in Tables 7.2 to 7.7 (native plants) and Table 7.12 (oats), utilizing the means of results from five samples, including duplicates. Acacias have greater relevance than eucalypts, which had only survived in site soils from one location (B), whereas acacias provided data from T and B subsites. General trends for metal uptake at the barren site and woodlands were:

7.4.1 Copper in native species

With regard to woodland soils, roots of both acacias and eucalypts were capable of taking up moderate amounts of copper (Table 7.2), with acacias showing more leaf uptake of the metal than eucalypts. Concentration factors (C.F.) declined with increasing concentrations of copper in the soil, and the enrichment ratio (E.R.) for both roots and shoots was close to 1.0 for both species. In barren-site soils, absolute values for copper were highest in the roots of acacias grown in T media, both neat and diluted with sand, with an E.R. of 7.8 compared with 2 for the eucalypts. Maximum leaf uptake, on other hand, was far higher than that of the acacias at this location. Only acacias survived in pure B subsite soil, with substantially higher uptake of copper in roots than leaves. However, eucalypt uptake of copper was higher than acacias in dilutions of B subsite soil.

7.4.2 Zinc in native species

Both acacia and eucalypts showed a tendency to concentrate zinc in roots, with far smaller amounts in leaves (Table 7.3). Metal uptake from the B subsite was roughly equivalent to uptake from woodland soils. However, in the soils from the T subsite, with the highest zinc contamination, acacia root and leaf uptake of metal in

Table 7.12 Bioassay of site soils: metal levels in roots and leaves of *Avena sativa*. Metal concentrations are expressed in mg/kg. The values shown are means ± se. (n = 5) for barren subsites and (n = 3) for woodland. Detection limits for copper, zinc, iron, lead, manganese and cadmium were 7, 17, 82, 12, 4 and 5 mg/kg respectively.

Copper		**100% Sand**	**100% Soil**
Top	Roots	23.9 ± 2.3	547 ± 27.1
	Leaves	9.9 ± 0.2	45.8 ± 5.0
Middle	Roots	23.6 ± 1.1	2,320 ± 180
	Leaves	9.4 ± 0.8	1,200 ± 344
Base	Roots	31.5 ± 4.2	202 ± 14.0
	Leaves	9.3 ± 0.2	31.0 ± 1.3
Woodland	Roots	<7	<7
	Leaves	<7	<7
Zinc			
Top	Roots	279 ± 30.6	2,190 ± 90.8
	Leaves	38.8 ± 3.5	641 ± 22.3
Middle	Roots	371 ± 63.6	11,800 ± 841
	Leaves	34.1 ± 0.9	14,300 ± 1,630
Base	Roots	345 ± 39.5	520 ± 29.6
	Leaves	38.1 ± 2.3	98.0 ± 3.6
Woodland	Roots	<17	<17
	Leaves	<17	<17
Iron			
Top	Roots	6,190 ± 822	18,500 ± 2,000
	Leaves	269 ± 27.7	488 ± 34.7
Middle	Roots	5,840 ± 264	3,430 ± 539
	Leaves	217 ± 18.2	383 ± 78.1
Base	Roots	6,150 ± 1,360	9,320 ± 4,130
	Leaves	150 ± 13.6	347 ± 68.7
Woodland	Roots	<82	<82
	Leaves	<82	<82

Table 7.12 Continued

Lead		100% Sand	100% Soil
Top	Roots	<12	1,750 ± 113
	Leaves	<12	37.8 ± 2.8
Middle	Roots	<12	365 ± 72.6
	Leaves	<12	57.6 ± 14.1
Base	Roots	<12	31.3 ± 19.1
	Leaves	<12	<12
Woodland	Roots	<12	<12
	Leaves	<12	<12
Manganese			
Top	Roots	839 ± 63.8	75.8 ± 7.1
	Leaves	1,110 ± 38.4	103 ± 10.8
Middle	Roots	577 ± 190	169 ± 13.1
	Leaves	1,110 ± 31.5	248 ± 50.0
Base	Roots	721 ± 118	72.1 ± 3.6
	Leaves	1,190 ± 44.0	101 ± 6.6
Woodland	Roots	<4	<4
	Leaves	<4	<4
Cadmium			
Top	Roots	<5	23.6 ± 2.0
	Leaves	<5	9.7 ± 0.6
Middle	Roots	<5	35.8 ± 2.7
	Leaves	<5	45.4 ± 4.8
Base	Roots	<5	17.7 ± 0.9
	Leaves	<5	5.1± 0.5
Woodland	Roots	<5	<5
	Leaves	<5	<5

neat site soil was high, and uptake increased further with progressive dilution of this soil. T subsite zinc levels were highest by far in the acacia roots, with only modest zinc concentrations in leaves. In diluted soils from the B subsite, eucalypt leaves were more effective in taking up zinc than acacias, but root accumulation of zinc was considerably higher than leaves in both species (Table 7.3).

7.4.3 Iron in native species

The absolute amount of iron present in plant tissue of both species exceeded 8,000 mg/kg, but C.F. values were low (Table 7.4). In those neat soils in which they survived, eucalypts were more effective in iron uptake than acacias, with most of the metal accumulating in the roots. In this regard, iron and manganese are unlike the other metals, for which acacia uptake is higher.

7.4.4 Lead in native species

Lead uptake from site soils was low in the two plant species (Table 7.5), both in absolute terms and as a C.F., with acacias more effective than eucalypts in soil metal uptake from the highly contaminated T subsite. Uptake of lead from woodland soils was even lower, but here the C.F. values of 1-7 were markedly higher than from the site soils, where they attained a maximum of just over 1.

Interestingly, there was a tendency towards increased lead uptake in site soils diluted with sand, reaching a maximum in the range of 25 to 75% soil. The plants were unable to increase uptake of lead in neat soil (Table 7.5). This observation also holds for both species in woodland soil.

7.4.5 Manganese in native species

Although manganese uptake was very high in plants grown in the sand control, and moderately high in woodland soil (Table 7.6), uptake in site soil was relatively low. The differences in C.F. values reflect the contrasts in metal uptake, in the 50-100 range for acacias and eucalypts grown in sand, to 1-20 for neat woodland soils, and 0.2-1.6 in site soils. Factors other than the manganese content of the growth media

clearly control uptake, since the amount of the metal present in neat site and woodland soils is very similar.

7.4.6 Cadmium in native species

Soil concentrations of cadmium were very low, but both species showed an ability to concentrate the metal from contaminated site soil in plant tissue, specifically in their roots. A high C.F. of 24 was recorded in acacia roots from T subsite soil, and an extreme C.F. of almost 400 in a 75% T soil blend (Table 7.7). This high root uptake of cadmium is consistent with observations by Azadpour and Matthews (1996).

7.4.7. Metal uptake in oats

Table 7.12 shows the levels of the six tested metals in the oats roots and leaves. The lowest levels of metal accumulation were in the woodland soil, but metal uptake was generally much higher from the contaminated mine subsite soils. Although there was not much growth in the barren M subsite soil, metal uptake was relatively high, especially for copper, zinc, manganese and iron. Results from the T subsite soils were higher for iron and lead. Oat roots were generally more effective than leaves in accumulating metals, but with several exceptions. These included manganese, zinc and cadmium in M subsite and zinc in B subsite soils, where higher levels of metal uptake were recorded in the oat leaves. Manganese uptake from the sand control medium was surprisingly high.

7.5 Bioassay of site soils: influence of biosolid addition

7.5.1 Soil characteristics

Table 7.13 compares the pH (in H_20 and KCl) and conductivity of neat barren-site and woodland soil (100%) with soil and biosolid thoroughly mixed ("100%+Mix"). The impact of biosolid on conductivity was more pronounced than on pH. Biosolid produced a minor reduction in acidity in the woodland soil for both acacias and eucalypts, and slightly neutralized the T subsite soils. In eucalypt soils, the mildly

Table 7.13 Bioassay of site soils: soil characteristics after addition of biosolid. Soil pH (in H_2O and KCl) and conductivity in neat soils ("100%"), and soils mixed with biosolid ("100% + Mix") after complete growth of acacia and eucalyptus. Conductivity measured in µS/cm. n.s. = no survivors; soils not sampled.

Acacia spp.	Barren slope						Woodland	
	Top		Middle		Base			
	100% + Mix	100%	100% + Mix	100%	100% + Mix	100%	100% + Mix	100%
pH (H_2O)	6.5	6.1	n.s.	n.s.	7.8	7.7	4.8	4.5
pH (KCl)	5.7	5.3	n.s.	n.s.	7.3	7.4	4.0	3.6
Mean pH	6.1	5.7	-	-	7.6	7.6	4.4	4.0
Conductivity (EC)	358	475	n.s.	n.s.	337	505	329	258
E. sideroxylon	**100% + Mix**	**100%**	**100% + Mix**	**100%**	**100% + Mix**	**100%**	**100% + Mix**	**100%**
pH (H_2O)	7.1	6.8	n.s.	n.s.	7.6	7.8	4.7	4.5
pH (KCl)	6.0	5.4	n.s.	n.s.	7.4	7.7	3.8	3.6
Mean pH	6.6	6.1	-	-	7.5	7.8	4.3	4.0
Conductivity (EC)	287	329	n.s	n.s.	413	352	271	258

alkaline B subsite soils generally became slightly more neutral with biosolid, but acacia soils were practically unchanged with regard to pH. This indicates that plants affect soil quality.

The effect of biosolid on salinity was highly varied. Biosolid had the effect of raising conductivity of woodland soils, while lowering conductivity in T subsite soils. Furthermore, the two plant species had opposite effects on conductivity in B subsite soils; biosolid raised conductivity in the case of eucalypts, while reducing conductivity in the same medium for acacias.

7.5.2 Germination and growth of *E. sideroxylon* and *A. salicina* with biosolid

Germination, survival and growth parameters of acacias and eucalypts grown in neat site soils, as compared with soils and biosolid are shown in Table 7.14. The biosolid was either mixed ("100% Mix"), or placed as a 2.5 cm layer (1 inch, shown as "100% + 1") on top of the neat soils ("100%"). Maximum germination took place in neat woodland soil, with or without mixed or layered biosolid, and eucalypts did slightly better here than the acacias. On the other hand, overall germination was higher for acacia seeds planted in barren-site soil than it was for the eucalypts, but neither species germinated at all in the biosolid mixed with soils from the M subsite. Nor did eucalypts survive in neat B subsite soil. Survival rates were generally high, with biosolid improving survival in a few cases, specifically in eucalypts in B subsite soils, and to a lesser degree with the T subsite samples. Furthermore, layered biosolid permitted a high survival rate for acacias in the M subsite soil, but only marginal survival for eucalypts.

Maximum height and leaf area for both acacias and eucalypts (Table 7.14) were found in the neat woodland soil trials, with eucalypts superior overall, with more than double the height of acacias and considerably more foliage. Mixed biosolids produced lower readings in woodland soils, and layered biosolid proved least effective. Minimum values for plant height and leaf area were from the M subsite with layered biosolid, where eucalypt growth was particularly stunted, growing to a height of only 1 cm. Total biomass was highest for eucalypts in neat woodland soil,

Table 7.14 Effect of biosolid on plant growth. Acacia and eucalyptus were grown in site soils with three different preparations: 1" (2.5 cm) biosolid layer on top (1"), soil with biosolid mixed throughout ("Mix"), and neat soil ("100%"). The values shown are means (± se), maximum germination. n = 9 for acacia, and n = 10 for eucalyptus is shown as number of seeds that have germinated after 15 weeks. Leaf area is given per entire plant, height is mean seedling height, biomass is stated on per dry weight basis.

Acacia spp.	Barren Slope									Woodland		
	Top			Middle			Base					
	1"	Mix	100%	1"	Mix	100%	1"	Mix	100%	1"	Mix	100%
Maximum germination	4.8 ± 0.6	4.4 ± 0.4	4.2 ± 0.6	4.8 ± 0.2	-	2 ± 0.6	5.6 ± 0.7	4.2 ± 0.6	6.8 ± 0.8	6.2 ± 0.7	8.8 ± 0.2	8.8 ± 0.2
Survival (%)	83	92	95	96	-	-	93	88	94	94 ± 3.8	100 ± 0	98 ± 2.2
Leaf area (cm^2)	18.1 ± 1.7	15.9 ± 1.7	31.6 ± 4.7	5.4 ± 0.6	-	-	15.7 ± 0.9	12.0 ± 0.9	31.9 ± 3.5	25.1 ± 7.3	32.4 ± 5.1	47.9 ± 4.9
Height (cm)	10.6 ± 0.9	9.0 ± 0.9	13.5 ± 2	3.3 ± 0.2	-	-	8.3 ± 0.8	5.7 ± 0.6	16.9 ± 1.5	10.7 ± 2.9	11.0 ± 1.4	18.1 ± 1.6
Root biomass (g)	0.1 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0	-	-	0.2 ± 0	0.1 ± 0	0.3 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0
Shoot biomass (g)	0.2 ± 0	0.2 ± 0	0.3 ± 0	0.1 ± 0	-	-	0.2 ± 0	0.1 ± 0	0.5 ± 0	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Total biomass (g)	0.2 ± 0	0.3 ± 0	0.5 ± 0	0.1 ± 0	-	-	0.3 ± 0	0.2 ± 0	0.7 ± 0	0.3 ± 0.1	0.5 ± 0	0.5 ± 0.1
Root:shoot ratio	1:3	1:1.7	1:2	1:1.2	-	-	1:1.1	1:1.2	1:1.4	1:3.1	1:1.9	1:5.3
E. sideroxylon												
Maximum germination	3.2 ± 0.8	4.8 ± 1.2	1.6 ± 0.4	1 ± 0	-	-	2.8 ± 0.5	2.8 ± 0.9	1 ± 0	9.2 ± 0.9	10.0 ± 1.0	8.8 ± 0.5
Survival (%)	64	60	40	2	-	-	69	79	5	83 ± 3.7	92 ± 6.5	89 ± 3.5
Leaf area (cm^2)	6.7 ± 1.7	1.3 ± 0.3	5.1 ± 2.4	0.4 ± 0	-	-	9.9 ± 3.1	1.1 ± 0.4	1.4 ± 0	90.3 ± 8.4	95.8 ± 12.8	112.2 ± 8.1
Height (cm)	6.9 ± 0.8	3.1 ± 0.4	4.5 ± 1.3	1.0 ± 0	-	-	7.3 ± 1.2	3.3 ± 0.9	2.5 ± 0	18.3 ± 2.2	24.3 ± 3.4	27.6 ± 1.2
Root biomass (g)	0	0	0	0	-	-	0	0	0	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
Shoot biomass (g)	0.1 ± 0	0	0	0	-	-	0.1 ± 0	0	0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0
Total biomass (g)	0.1 ± 0	0	0.1 ± 0	0	-	-	0.1 ± 0	0	0	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.1
Root:shoot ratio	1:8	1:0.4	1:4	1:1.3	-	-	1:7.9	1:1.8	1:2.3	1:0.3	1:0.4	1:0.3

or with biosolids added as layers or mixtures. However, acacias proved superior to eucalypts in site soils, especially when grown in B subsite soils, where biosolids actually proved disadvantageous. Shoot biomass generally tended to be approximately equal to or larger than the roots, except for eucalypts grown in pure or mixed woodland soils, where root biomass was significantly higher.

In summary, eucalypts proved more suited to woodland soils and responded favourably to biosolids in site soils; acacias were better adapted to site soils, where biosolids assisted with germination and survival but did not contribute materially to growth.

7.5.3 Metal accumulation in plants

Plant metal levels determined at harvest are shown in Tables 7.15 and 7.16, which compare the metal levels in the neat soil (100%) with the metal levels in the soil with the biosolid mixed throughout. Addition of a biosolid mix had varied effects on metal content in soil, with no consistent pattern observed. In some cases the metal values were increased by mixing biosolid with the soil, in others they decreased.

Where metal content is highest, in the T subsite for most metals, neat soil provided maximum root levels of copper, zinc and cadmium among the acacias, and manganese in eucalypts. Among the eucalypts, only manganese root uptake was markedly higher in pure soil than in the biosolid growth media. On the other hand, in the case of acacias, root amounts of manganese and lead were lower in pure soil than with biosolid mix.

Layered biosolid was less effective than mixed biosolid with regard to root uptake of metals. Exceptions were iron in woodland acacias, manganese in acacias from B subsite soils, and zinc, iron and lead in eucalypt roots from B subsite soils. Root uptake of metals was generally higher with the biosolid mix than with layered biosolid, but only copper showed this tendency consistently. Metal levels in leaves of both acacias and eucalypts tended to be higher with the layered biosolid and soils from the B subsite.

Table 7.15 Bioassay of site soils: influence of biosolid addition. Metal concentration in soils and plant tissues of acacia. Soils with a top layer of biosolid (Nitrohumus) (100% + 1"), biosolid mixed throughout (Mix), and neat soil only (100%). Soil treatments are indicated as percent dilution of the neat sieved soils, and metal concentrations are expressed as mg/kg. Concentration factors (ratios of metal concentration in plant tissues to that in the soil) are indicated in parentheses. The values shown are means ± se (n = 5), except in soils where the values are means of duplicate determinations. Detection limits for copper, zinc, iron, lead, manganese and cadmium were 7, 17, 82, 12, 4 and 5 mg/kg respectively; n.s. = no survivors.

	Barren Site									Woodland		
	Top			Middle			Base					
	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves
Cu												
100% + 1"	<7	73.8 ± 6.2	7.65 ± 1.3	<7	594 ± 0	21.9 ± 0	<7	3.9 ± 0	18.3 ± 3.5	<7	30.6 ± 0.1	31.1 ± 2.0
Mix	550	217 ± 47.4 (0.3)	8.1 ± 0.5 (<0.1)	<7	n.s.	n.s.	164	168 ± 0 (1.0)	9.7 ± 2.3 (0.1)	41.1	44.6 ± 4.6 (1.1)	31.5 ± 2.2 (0.8)
100%	492	232 ± 0 (0.5)	<7 (<0.1)	<7	n.s.	n.s.	157	64.9 ± 16.4 (0.4)	7.1 ± 0 (0.1)	25.9	29.3 ± 3.0 (1.1)	31.7 ± 0.5 (1.2)
Zn												
100% + 1"	<17	361 ± 20.5	51.6 ± 13.9	<17	5,540 ± 0	831 ± 0	<17	17.2 ± 0	417 ± 99.6	<17	176 ± 9.8	50.1 ± 3.4
Mix	993	1,170 ± 149 (1.2)	183 ± 8.3 (0.2)	<17	n.s.	n.s.	415	415 ± 0 (1)	63.3 ± 15.7 (0.2)	67.3	206 ± 10.6 (3.1)	52.9 ± 3.4 (0.8)
100%	910	1,290 ± 0 (1.4)	159 ± 3.6 (0.2)	<17	n.s.	n.s.	379	193 ± 41.4 (0.5)	46.5 ± 2.1 (0.1)	76.7	219 ± 24.9 (2.9)	52 ± 0.6 (0.7)
Fe												
100% + 1"	<82	2,560 ± 459	<82	<82	13,600 ± 0	181 ± 0	<82	<82	247 ± 19.7	<82	16,800 ± 1,660	221 ± 18.2
Mix	40,600	4,090 ± 782 (0.1)	<82	<82	n.s.	n.s.	20,000	5,190 ± 0 (0.3)	<82	12,800	9,240 ± 32.2 (0.7)	181 ± 17.9 (<0.1)
100%	41,400	4,000 ± 0 (0.1)	<82	<82	n.s.	n.s.	21,200	1,940 ± 470 (0.1)	<82	13,400	4,920 ± 1,380 (0.4)	<82

Table 7.15 Continued

	Barren Site									Woodland		
	Top			Middle			Base					
	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves
Pb												
100% + 1"	<12	21.9 ± 4.87	<12	<12	38.9 ± 0	0	<12	0	32.7 ± 7.1	<12	44.2 ± 2.7	<12
Mix	2,870	529 ± 120 (0.2)	13.0 ± 0.4 (<0.1)	<12	n.s.	n.s.	165	68.1 ± 0 (0.4)		90.9	57.9 ± 9.0 (0.6)	<12
100%	1,972	483 ± 0 (0.2)	<12	<12	n.s.	n.s.	228	25.3 ± 6.9 (0.1)		80.4	44 ± 10.3 (0.5)	<12
Mn												
100% + 1"	<4	17.8 ± 2.1	16.1 ± 1.4	<4	62.5 ± 0	33.1 ± 0	<4	1,130 ± 0	39.6 ± 7.5	<4	68.1 ± 4.3	175 ± 8.0
Mix	57.7	30 ± 5.9 (0.5)	24.9 ± 1.0 (0.4)	<4	n.s.	n.s.	73.7	34.8 ± 0 (0.5)	16.4 ± 3.9 (0.2)	79.3	88 ± 7.6 (1.1)	252 ± 16.1 (3.2)
100%	66.6	18.7 ± 0 (0.3)	20 ± 0.3 (0.3)	<4	n.s.	n.s.	77.6	12 ± 2.9 (0.2)	12.6 ± 0.6 (0.2)	69.8	104 ± 25.2 (1.5)	260 ± 11.8 (3.7)
Cd												
100% + 1"	<5	11.8 ± 0.6	<5	<5	20.5 ± 0	<5	<5	<5	<5	<5	<5	<5
Mix	<5	27.7 ± 7.3 (19.8)	<5	<5	n.s.	n.s.	<5	18.8 ± 0 (9.9)	<5	<5	<5	<5
100%	<5	40.7 ± 0 (23.9)	<5	<5	n.s.	n.s.	<5	8.3 ± 2.3 (4.0)	<5	<5	<5	<5

Table 7.16 Bioassay of site soils: influence of biosolid addition. Metal concentration in soils and plant tissues of eucalyptus. Soils with a top layer of biosolid (Nitrohumus) (100% + 1"), biosolid mixed throughout (Mix), and neat soil only (100%). Soil treatments are indicated as percent dilution of the neat sieved soils, and metal concentrations are expressed as mg/kg. Concentration factors (ratios of metal concentration in plant tissues to that in the soil) are indicated in parentheses. The values shown are means ± se (n = 5), except in soils where the values are means of duplicate determinations. Detection limits for copper, zinc, iron, lead, manganese and cadmium were 7, 17, 82, 12, 4 and 5 mg/kg respectively; n.s. = no survivors.

	Barren Site									Woodland		
	Top			Middle			Base					
	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves
Cu												
100% + 1"	<7	287 ± 0	10.7 ± 0	<7	92.2 ± 0	7.8 ± 0	<7	227 ± 0	13.5 ± 0.9	<7	43.2 ± 2.9	<7
Mix	510	506 ± 0 (1.0)	23.6 ± 0 (0.1)	<7	n.s.	n.s.	170	224 ± 0 (1.3)	14.8 ± 0 (0.1)	34.4	45.9 ± 5.6 (1.3)	<7
100%	492	168 ± 0 (0.3)	22.3 ± 0 (0.1)	<7	n.s.	n.s.	157	n.s.	n.s.	25.9	38.3 ± 6.6 (1.5)	7.7 ± 0.3 (0.3)
Zn												
100% + 1"	<17	750 ± 0	68.4 ± 0	<17	468 ± 0	2,220 ± 0	<17	759 ± 0	209 ± 15.3	<17	185 ± 22.9	<17
Mix	966	1,280 ± 0 (1.3)	47.5 ± 0 (0.1)	<17	n.s.	n.s.	393	267 ± 0 (0.7)	124 ± 0 (0.3)	61.4	194 ± 7.6 (3.2)	24.3 ± 2.3 (0.4)
100%	915	472 ± 0 (0.5)	140 ± 0 (0.2)	<17	n.s.	n.s.	465	n.s.	n.s.	76.7	195 ± 16.7 (2.5)	32.7 ±1.6 (0.4)
Fe												
100% + 1"	<82	9,580 ± 0	303 ± 0	<82	2,940 ± 0	<82	<82	4,430 ± 0	198 ± 59.2	<82	4,410 ± 275	<122
Mix	42,500	8,640 ± 0 (0.2)	290 ± 0 (<0.1)	<82	n.s.	n.s.	22,500	2,990 ± 0 (0.1)	152 ± 0 (<0.1)	13,300	4,300 ± 230 (0.3)	<122
100%	40,200	6,710 ± 0 (0.2)	183 ± 0 (<0.1)	<82	n.s.	n.s.	24,900	n.s.	n.s.	13,400	6,470 ± 1,990 (0.5)	<122

Table 7.16 Continued

	Barren Site									Woodland		
	Top			Middle			Base					
	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves
Pb												
100% + 1"	<12	74.8 ± 0	<12	<12	13.6 ± 0	0 ± 0	<12	572 ± 0	19.3 ± 3.8	<12	52.3 ± 9.6	<12
Mix	1,830	1,120 ± 0 (0.6)	37.6 ± 0 (<0.1)	<12	n.s.	n.s.	211	20.9 ± 0 (0.1)	0 (<0.1)	86.6	49.2 ± 3.4 (0.6)	<12
100%	1,790	67.1 ± 0 (<0.1)	<12	<12	n.s.	n.s.	202	n.s.	n.s.	80.4	59.6 ± 10.7 (0.7)	<12
Mn												
100% + 1"	<4	64.3 ± 0	20 ± 0	<4	27.7 ± 0	90.6 ± 0	<4	22.1 ± 0	43.3 ± 0.6	<4	280 ± 9.7	676 ± 45.7
Mix	61.6	30.1 ± 0 (0.5)	66.7 ± 0 (1.1)	<4	n.s.	n.s.	80.5	27 ± 0 (0.3)	74.5 ± 0 (0.9)	65.2	290 ± 15.9 (4.4)	1,650 ±106 (25.3)
100%	51.3	41.7 ± 0 (0.8)	79.8 ± 0 (1.6)	<4	n.s.	n.s.	85.5	n.s.	n.s.	69.8	251 ± 32.3 (3.6)	1,190 ± 22.6 (17.0)
Cd												
100% + 1"	<5	10.1 ± 0	4.2 ± 0	<5	8.0 ± 0	10.0 ± 0	<5	24.7 ± 0	6.7 ± 0.3	<5	1.23 ± 0	0.4 ± 0.1
Mix	2.7	38.4 ± 0 (14.2)	8.4 ± 0 (3.1)	<5	n.s.	n.s.	3.4	17.8 ± 0 (5.2)	5.8 ± 0 (1.7)	0.8	1.4 ± 0.1 (1.8)	0.4 ± 0.2 (0.5)
100%	2.7	15.5 ± 0 (5.7)	3.8 ± 0 (1.4)	<5	n.s.	n.s.	4.0	n.s.	n.s.	0.9	1.0 ± 0.3 (1.1)	0.3 ± 0.1 (0.3)

Copper. Addition of biosolid increased the copper content of both soil and most plants. This is consistent with observations by Kabata-Pendias and Pendias (1984, p. 80) that sewage sludges increase the availability of copper to plants. Eucalypts proved more effective than acacias in taking up copper from contaminated soils with added biosolid.

Zinc. Mixing biosolid with soil did not result in any clearcut patterns of zinc uptake, apart from the observation that the zinc content of acacia and eucalypt leaves all increased in barren-site soils. On the other hand, layered biosolid had a pronounced effect on zinc levels in roots and leaves from the B subsite, as well as leaves from the T subsite. Soluble zinc-organic complexes in sewage sludge are very mobile in soils, and are easily available for plant uptake (Kabata-Pendias and Pendias, 1984, p. 104), but no consistent zinc enrichment was observed in this study.

Iron. With iron too, biosolid yielded inconsistent patterns of metal uptake. Mixed biosolid increased iron in the roots and leaves of acacias, especially from the B subsite. Layered biosolid significantly improved acacia leaf uptake of iron from the B subsite and woodland soil, but not from the T subsite. Biosolid increased the overall level of eucalypt uptake in the T subsite soil, but decreased the overall level in the woodland soil. Layered biosolid again improved the overall uptake of iron in leaves in most cases.

Lead. Biosolid mix had a pronounced effect on lead uptake, especially in acacia roots where the increase was more than tenfold. Layered biosolid provided varied results, raising lead levels in acacias leaves from the B subsite, and more significantly in eucalypts in T subsite soils, but decreasing levels in the roots from woodland soil. As with acacias, there was a lead increase in eucalypt leaves from the B subsite as a result of biosolid layering.

Manganese and cadmium. Neither manganese nor cadmium was significantly affected by the addition of biosolid.

7.6 Evaluation of soil toxicity

Barren-site soils are highly toxic to eucalypts. Rowe (2001) established similar toxic response in eucalypts at Peelwood mine site. Eucalypts were unable to germinate at all in the most toxic M soils at Mt. Costigan. Acacias germinated there, but did not survive in those neat soils. Unlike acacias, however, the eucalypt seedlings were unable to survive even in strong dilutions of T subsite soil, while in the less toxic B soils, eucalypt survival rates are substantially lower than acacias.

Zinc, iron and lead were selectively concentrated in root tissue of both plant species. Acacias concentrated high levels of copper and manganese in their leaves. Eucalypt leaves showed an exceptional ability to accumulate manganese, with C.F.s as high as 100 in mixtures of woodland soil and sand.

Both acacias and eucalypts proved effective in metal uptake from all soil/sand combinations where the species survived, but the lower germination and survival rate of eucalypts resulted in fewer observations. Metal uptake capacities and metal preferences therefore show considerable variation from one species to another, and depend upon growth medium and metal loadings, as well as other factors. For example, in many cases metal uptake is higher from woodland soils or site-soil dilutions than it is from neat site soils with very high metal content. Metal concentration factors (C.F.s) in plants generally tended to decline as the metal content in the growth medium increased. This tendency was most pronounced for copper, zinc and manganese. There was no cadmium in the sand control medium, and therefore practically no cadmium in plants.

Maximum plant uptake of metal therefore occurred from mixtures of site soil and river sand, except in the case of manganese, which also shows high uptake from pure sand. Optimal uptake varied from one metal to another in terms of soil/sand dilution levels, and also between the two plant species. In both plant species, roots tended to be far more effective than shoots in concentrating metals. The exception was manganese from barren site soils, and mixtures thereof. Manganese was

generally higher in the leaf tissue, especially in eucalypts, where it was approximately twice as high in leaves as it was in roots.

7.7 Outcomes

Acacias proved more viable than eucalypts in site soils, with better germination and survival and substantially greater heights and leaf areas. However, the reverse was the case in woodland soils, where eucalypts dominated. This is mirrored by the prevalence of eucalypts in the Mt. Costigan woodlands. There was a tendency for metals to accumulate in greater amount in the root tissues of both species, in all soils. However, copper and manganese were more evenly distributed between roots and leaves. Acacias and eucalypts both show accumulator tendencies (Section 1), with high concentration factors for copper, zinc and manganese from metal-poor woodland soil and river sand. Concentration factors decrease markedly with increasing metal content in the soil.

Bioassays of metal uptake showed considerable heterogeneity, but with some consistent trends. Contrary to other reports such as Kabata-Pendias and Pendias (1984), metal uptake showed no progressive increase with soil metal loadings, apart from cadmium in eucalypts from the base of slope (B subsite). In fact, manganese uptake was inversely related to soil levels. Bioassays of acacias grown in soil from the top of the barren slope (T subsite) showed maximum uptake of all metals except manganese in 25:75% mixtures of soil and sand. In addition to its ability to survive, *A. sativa* showed a stronger tendency than the eucalypts and acacias to take up metal from the most highly contaminated soils. Enrichment ratios in plants are highest for copper and lead from the top of the slope (T subsite), and are lowest there for manganese and cadmium.

Cadmium stands out for its high concentration factors (CFs) in both eucalypts and acacias. Acacias show strong excluder tendencies for cadmium. There is little cadmium uptake at low soil concentrations, but a strong increase at higher soil concentrations, for example reaching an extreme concentration factor of almost 400 in acacias grown in 75% T subsite soils. The cadmium CF of 24 for acacias in T

subsite soil eclipses values of 0.5, 1.4 and 0.2 for copper, zinc and lead respectively.

In general, however, plant concentration factors were inversely proportional to soil metal content. For example, copper uptake by both eucalypts and acacias was higher in pure woodland soils than neat barren-site soils, but was increased by dilution of contaminated site soil with river sand. The same was true for iron, which was present in large amounts in soils of the barren site and woodland, far exceeding all other metals in both acacias and eucalypts. Concentration factors for iron in plants grown in soil or soil mixtures from the barren site were very low however. Acacias were capable of concentrating lead from site soils in root tissue, but concentration factors were minimal compared with low-lead growth media, for example the woodland soil. *E. sideroxylon* shows indicator tendencies, with increasing absolute levels of zinc uptake with soil zinc content from the B subsite.

In summary, project outcomes in terms of the stated objectives in Chapter 1 were:

- Site soils proved highly toxic to eucalypts, completely inhibiting germination and survival in the most contaminated barren-site soils.

- In general, acacias were more effective in metal uptake from the highly contaminated top of the barren site, with most metal concentrated in the plant roots. Eucalypts, on the other hand, showed higher metal uptake from the base of the barren slope.

- Oats showed the best ability to survive in all site conditions and therefore might be used in site phytostabilization. Although acacias may be encouraged to grow over parts of the barren slope, they cannot tolerate the most toxic conditions (high metal content combined with low pH and high salinities) found in the mid-slope seep area. Eucalypts are more sensitive than euclaypts to high metal content, especially where this is accompanied by acid, saline conditions.

- Biosolid proved beneficial for eucalypts, but actually decreased the growth parameters for acacias. Layered and mixed biosolid applications improved eucalypt germination, survival rate, leaf area and height in barren-site soils.

Section C: Project Outcomes

Chapter 8. Outcomes and future directions

8.1 Overall significance of the results

The results of the present study have contributed to an improved understanding of the Mt. Costigan site, the nature and distribution of contaminants, the problems associated with these contaminants, and potential applications of phytoremediation. The findings are an incremental step towards achieving the remediation objectives at the site, since:

- Results of the study demonstrate the applicability of the triad approach as an appropriate assessment tool for the development of remediation strategies at contaminated mine sites;

- The work adds to the scientific foundation for future remediation and rehabilitation efforts at Mt. Costigan;

- Information has been provided on plant species and related techniques that could be useful for comparable mine-site restoration projects in similar climatic zones in southeastern Australia and elsewhere; and

- A contribution has been made to the development of innovative solutions to sustainable environmental management of metalliferous mine and industrial sites, utilizing indigenous plant species.

8.1.1 The mine-site assessment of soils and vegetation

Eucalypts including *Eucalyptus blakelyi, E. polyanthemos, E. macrorhynchia* and *E. sideroxylon* flourish in the woodland, which was once cleared but has undergone spontaneous regeneration. The woodland appears to display characteristics of the original ecosystem, and therefore provided a feasible reference for trials on the barren site. The barren slope bore only small, non-woody plant species such as tussock grass. In nearby Peelwood mine site, parts of the barren slope have been

revegetated (Rowe, 2001), notwithstanding poorer soil quality and higher metal content than at the Mt. Costigan mine site. Seeds of woodland tree species were sown across the Peelwood mine site and tube stock was planted (Johnson, personal communication). Reasons for the lack of regeneration at Mt. Costigan may include insufficient time or possibly the high bioavailability of metals. The overall reduction in metal content following DMR remediation was accompanied by a significantly lowered pH, but high electrical conductivities persisted. These highly acidic conditions are known to promote metal uptake (Section 4.3.3), possibly to toxic levels, with the high salinities also making the mine site inhospitable to woodland species. The decrease in pH may also increase hydrogen ion competition. Barren-site salinities (EC and SO_4) are high, particularly in the mid-slope seep area, and this correlates generally with elevated metal content. Peelwood differs in having less acid soils. In addition, indigenous trees were planted on the Peelwood site, whereas at Mt. Costigan only pasture mix was sown.

Metal levels in the Mt. Costigan woodland soil are comparatively low, and are inversely related to soil organic carbon content. On the barren site, however, both copper and lead content of the barren slope are within the upper range of typical mine-site soils, and exceed international and NSW EPA limits for residential areas by a factor of 20 (Table 4.3). Zinc exceeds EPA limits by a 10-20 fold margin. Cadmium reaches levels roughly double these limits. The highest levels for most metals were at the top of the barren slope around the old workings, and in the mid-slope section where dumps once existed, and where metals may also be precipitated by ground-water discharge (Chapter 2). Adverse soil quality (low pH, high conductivity and sulphate levels) is also associated with ground-water discharge. An increase in pH towards the base of slope may result from aqueous redistribution of lime, originally spread over the site (Section 4.1.1).

Site remediation of the barren slope by the DMR in 1999 resulted in more acidic soils and higher conductivities, but did achieve a significant overall reduction in metal content. Manganese and zinc levels were lowered over the entire site, whereas cadmium decreased substantially in the top and mid-slope section.

However, a decrease in copper and lead at the top of the slope was balanced by enrichment in these metals at the base of slope, possibly pointing towards physical or solution transport and precipitation.

The 1999 remediation exercise included application of a 25 mm biosolid surface layer. Following this, the number of plant species and percentage ground cover increased markedly in the mid-slope section. However, this improvement may only have been temporary, for the period when the small, shallowly rooted plants were able to survive in the thin biosolid layer.

Since the pre- and post-remediation assessments of site vegetation were carried out at different times of the year, any future assessment will need to exclude such seasonal differences.

8.1.2 Australian native plants as phytotoxicity test species

Field studies provided a basis for followup laboratory bioassays and toxicity testing. The initial tests focused on species selection, from species indigenous to the Mt. Costigan area. These trials demonstrated the considerable differences among the species in germination rates and response to different soils. Clearly, the desirable characteristics for a test species were not easily obtained in Australian native species. Such characteristics include reliability in germination, high germination rates and relative tolerance to soil type. In this study, three species were selected as best suited for toxicity studies, and they proved to be likely candidates for future remediation of the site: *E. sideroxylon* and *A. salicina,* with *A. hakeoides* as an alternative. In addition to these, oats (*Avena sativa*) was selected from the OECD list of recommended species. Bioassays followed OECD (1984) procedures, with minor adaptations with regard to species selection and duration of testing.

The selected species were then used for bioassays in site soils, to test their toxicity responses, to determine metal uptake and partitioning, to assess their potential applications to phytoremediation, and to investigate the effectiveness of biosolid. *E. sideroxylon* proved very sensitive to contaminated mine-site soils, with low

overall germination and survival rates. The acacias germinated better than the eucalypts in site soils, with a higher survival rate, except in the most contaminated soils from the mid-slope (M subsite). Acacias were therefore more useful than eucalypts for bioassays and toxicity tests. *Avena sativa* was more tolerant of site conditions and grew in soil from all locations.

Metal uptake showed little correlation with soil metal loadings, and in the case of manganese showed an inverse correlation with the soil metal content. Manganese uptake was very high in plants grown in metal-poor woodland soil, but relatively low from metal-rich barren-site soils. Furthermore, uptake of most metals was far higher from site soils diluted with river sand than it was from neat soil. For example, acacias showed maximum uptake from 25:75% blends of T subsite soil and sand. Metals such as copper and iron were taken up in greater amounts from woodland soil than from metal-contaminated site soil, reinforcing the fact that bioavailability is a key factor. Although present in only small amount, cadmium stands out for its very high concentration factors in both eucalypts and acacias, whereas the most abundant metals such as iron show lower concentration factors. Acacias proved more effective than eucalypts in terms of metal uptake from contaminated soils, but *A. sativa* was capable of higher uptake that either species.

8.1.3 Phytotoxicity testing as a measure of bioavailability of metals

During the course of this study, bioavailability was not determined by extractive means. However, because bioavailability, not the metal concentration in the soil, controls uptake by plants (Kabata-Pendias and Pendias, 1984), this implies that bioavailability is a critical determinant of the toxicity of soils to plants. For example, acacias grown in sand-diluted site soils showed greater evidence of zinc toxicity than did plants grown in neat site soils. It was therefore determined that the best way to investigate the bioavailability of metals in contaminated soils was to test the soils in toxicity trials. In this way, all of the factors operating in the soil, including metal concentration, will interact to determine bioavailability, and this will be reflected in the plant performance.

Root concentrations of metal tended to be significantly higher than in leaves, as is well documented in the literature (Kabata-Pendias and Pendias, 1984). This is most marked for the relatively immobile xenobiotics lead and cadmium, which were very low in the leaves of all plant species tested. Acacias in particular had high lead values in root tissue but very low values in leaves. However, because copper, zinc and manganese are micronutrients (Kabata-Pendias and Pendias, 1984), plants have a mechanism for transporting them through the plant. In the present study, acacias grown in woodland soil had maximum concentrations of copper in the leaves, showing that effective migration of copper took place from roots to shoots. Similarly, leaf concentration of manganese was high, especially in eucalypts grown in woodland soil. Contrary to the findings of Kabata-Pendias and Pendias (1984), the large concentrations of both zinc and copper at the barren site did not reduce the capacity for plant uptake of cadmium. However, the relative efficiencies of cadmium uptake by acacias and eucalypts varied markedly from on location to another, showing no consistent trend.

8.1.4 Advantages and disadvantages of biosolid use

In this study, the addition of biosolid as a nutrient and organic-matter source increased the amount of copper, zinc and lead in some of the bioassay soils, probably because of the metal contained in the biosolid itself. Waste sludge, a component of biosolid, contains high levels of potentially toxic metals, especially cadmium, copper and zinc (Ross, 1994). Decomposition of biosolid tends to increase soil acidity, mobility and bioavailability of metals, and metal buildup in sewage sludge-treated topsoils (Ross, 1994).

In the present study, uptake response to biosolid was complex. For example, biosolid increased zinc content in the acacias and eucalypts in T subsite soil, as well as the acacias in B subsite soil, but biosolid actually *decreased* zinc levels in the remaining soils. Thus, it appears that the addition of composted, organic-rich material can have both beneficial and undesirable effects on the soil.

A concern with biosolid use on slopes such as Mt. Costigan is that soil erosion and sheetwash soon after application can transport sludge metals to adjacent water bodies and into the ground-water system (Galloway *et al.*, 1979). Given that elevated metal concentrations are recorded in alluvial terraces downstream from old mines (Ross, 1994), an alternative nutrient medium would be advisable which would not risk further increasing the already high metal concentrations.

8.2 Possible measures to improve efficiency of plant-based remediation

Plant-based remediation offers site stabilization and containment while reducing contaminant levels. The results of this study and complementary research at Peelwood (Minfo, 1997; Rowe, 2001) demonstrate that phytoremediation could have a beneficial effect at Mt. Costigan and other mine sites in NSW with similar problems. In a parallel study, Peelwood was assessed using a similar, triad approach by Rowe (2001), whose overall conclusions confirmed those of the present study. For example, Rowe (2001) documented a similar suite of metals, copper, zinc, lead and cadmium, all exceeding background investigation levels; revegetation of the upper section of Peelwood mine site had been unsuccessful because of the combination of high metal content and acidic, poor-quality, soils. Furthermore, as at Mt. Costigan, direct toxicity studies confirmed that acacias were more tolerant of metal contaminants than eucalypts. Differences between the two mine sites include higher metal content and conductivity at Peelwood and less-acid soils.

The efficiency of these measures may be improved by sequential introduction of the test species, as suggested in Section 8.4 below, or by additional species that are more tolerant of site conditions. Furthermore, plant-based remediation may prove more effective when used in conjunction with other techniques (Section 8.3).

According to Minfo (1997), the lessons learned from the Peelwood project were:

i) Adequate funding and careful planning of staged remediation tasks are required to deliver favourable results;

ii) Successful remediation requires a multidisciplinary team effort requiring input from several sources;

iii) Remediation projects are slow, and the various phases may take many years to implement;

iv) It should not be expected that a contaminated site will necessarily be returned to a pristine condition; and

v) Part of the learning experience is failure, and this must be anticipated and incorporated in the learning experience.

Phytoremediation alone may not be the complete solution, however. Some of the hurdles that would need to be come in establishing a phytoremediation program at Mt. Costigan, and possible means of improving the process, are outlined below.

1. Establishing and maintaining a plant cover on the barren site, particularly the M subsite, would be difficult using native species. Results of this study show that oats might provide an initial cover and provide modest reduction in erosion and metal content. However, to establish other species, the problems of soil toxicity will need to be overcome. The most severe problems are associated with the mid-slope seep area of ground-water discharge. Here, the increased acidity of the M subsite since remediation appears to be a consequence of biosolid breakdown, and this problem is likely to diminish with time. The high salinity problem is likely to remain, however, without remedial action such drainage diversion. This might require the construction of flow barriers down to bedrock. These barriers would serve to divert subsurface flow to a lined discharge pit, where the contaminated discharge could be treated and neutralized prior to onsite disposal.

2. Plant-based remediation might take a decade or more to produce meaningful results at Mt. Costigan. However, the process might be accelerated by the implementation of complementary remediation techniques. For example, a chemical treatment such as increasing pH would initially transform the toxic

elements into non-bioavailable forms and encourage plant growth. At a later stage, once the tree cover is established, metal uptake might be increased by use of chelating agents (Huang *et al.*, 1997) such as EDTA.

3. Seedlings are vulnerable to drought and grazing animals at Mt. Costigan. More effective fencing and initial irrigation of young plants would be necessary to enhance survival. Planting would need be timed to coincide with the wet season, with mulching to provide a protective layer and conserve moisture. Progressive buildup of organic matter would help bind metals.

8.3 Recommendations for further work

The future use of phytoremediation at Mt. Costigan will be more effective if all of the factors influencing the contaminant problem are better understood. The physical, chemical and ecological processes at Mt. Costigan are closely interrelated. A "holistic" or multidisciplinary approach to the contaminant problem at the mine site is therefore recommended, using project procedures advocated by the Society for Mining, Metallurgy, and Exploration (1998) and Tearpock and Brenneke (2001).

Soil metal concentrations and plant uptake at Mt. Costigan are largely a function of geology and soil chemistry, modified by human activities. The geological framework determined the ore mineralogy. Chemical processes at the site are more complex, however. They include surface weathering and diagenetic mineral transformations, solubility reactions, hydration, changes in pH and oxidation-reduction potential (Eh), cation exchange and absorptive capacities, and chemical transport and re-precipitation of site contaminants (Galloway and Hobday, 1996). Plant growth and metal uptake are largely controlled by this chemical environment.

The tasks outlined below would serve to document the factors controlling contaminant distribution and help select and implement the most effective methods for amelioration of the Mt. Costigan mine site.

8.3.1 Improved site characterization and monitoring

Before further remediation measures are undertaken at Mt. Costigan, a more complete characterization of the site is necessary. A limitation in the present study and other contaminated sites is incomplete knowledge of factors controlling contaminant distribution and their changes over time. Understanding these processes should lead to better resolution of the problems. It is therefore necessary to identify the risk elements pose, and to monitor the responses to remediation. The monitoring process has been made easier by advances in remote sensing (Henderson, 2000).

Integrated risk-based corrective action uses inputs from all applicable disciplines (Society for Mining, Metallurgy, and Exploration, 1998). Site monitoring is a key element of these programs, and is constantly being improved by introduction of new technologies. For example, hyperspectral data from commercial, high-resolution satellites makes it possible to identify the composition, distribution and migration of contaminants within one-metre resolution (Henderson, 2000). These remote-sensing methods could make it possible to map and characterize the rocks, soils, minerals and vegetation at Mt. Costigan, and to monitor changes in contaminant levels, drainage and the health of the ecosystem over time

8.3.2 Assessing the broader impact

Mt. Costigan characterization and planning should take into account the broader environmental impact of the exogenous human factor beyond the mine site itself. Apart from the surface excavations and shafts, deforesting of the site to provide fuel for furnaces was highly destructive. The combination of toxic mine waste, denudation and destabilization of over-steepened slopes produces rapid surface runoff during storms, resulting in severe erosion. Apart from the impact on the site itself, the potential ecological damage is spread beyond the Mt. Costigan site into the Abercrombie River catchment basin. Future work should address the potential broader impact of contaminated sites and devise methods to address this.

8.3.3 Improved species selection and phytotechnology

The two plant species used in Mt. Costigan glasshouse experiments showed substantial differences in their ability to take up metals and to carry these metals from roots to plant shoots. There may be other plant species better suited to the site. For example, *Lomandra* sp. is present in the Western Slopes region and grows on the Sunny Corner mine site (Chaudhry *et al.*, 1998). This species appears to be tolerant of disturbed conditions and high metal content. Although the metal-uptake capacities of *L. longifolia* are somewhat lower than the species investigated in the present study, the species may adapt better to those parts of the barren slope where the eucalypt and acacia test species failed to survive. Native grass species should be further investigated, as grasses are better at holding the soil and resisting erosion during the early stages of revegetation. Exotic metallophyte species might also be considered. For example, Payson's sedge has proved successful on old mine sites in Montana (Society for Mining, Metallurgy, and Exploration, 1998). Furthermore, commercial development of metallophytes for remediation of mine wastes in the UK uses various metal- and acid-tolerant grasses. Although from climatically different areas, controlled use of plants such as these could be considered.

Selective plant breeding and genetic engineering reportedly have the capacity to increase metal uptake several fold, and may find application at Mt. Costigan. The goal of phytotechnology is to increase plant biomass and growth rates by the use of selective breeding, selection, or molecular biological techniques. Thus, a plant's natural metabolic capacity to hyperaccumulate may be increased by incorporating insect, bacterial, fungal, and even mammalian genes into the genome (Cunningham *et al.*, 1995).

8.3.4 Soil microbiology

Further organic/composting soil ameliorations should be coupled with an investigation of the role that microorganisms might play in encouraging revegetation of the site. Studies elsewhere have indicated the critical importance that soil microorganisms can have in mine-site remediation (Cunningham and Ow,

1996; Chaudhry *et al.,* 1998; Society for Mining, Metallurgy, and Exploration, 1998).

Fungi. Symbiotic associations of plants and mycorrhizal fungi can be advantageous to the plant and to its metal-accumulation capabilities, and might speed up remediation at Mt. Costigan. Two types of mycorrhizae occur naturally in the rhizosphere: ectomycorrhizae, primarily on conifers and some hardwoods, and endomycocorrhizae, primarily on hardwoods (Society for Mining, Metallurgy, and Exploration, 1998). Resultant increase in mineral and water absorption are of great benefit in mine-site conditions, and the protection given to the plant by mycorrhizae against soil toxicants and the secretion of detoxification enzymes is also of assistance (Chaudhry *et al.,* 1998). These rhizospheric fungi may be engineered to increase metal extraction (Cunningham and Ow, 1996), which will increase their practical applications as well as maintaining the health of the plants.

Inoculating a hyperaccumulator species with mycorrhizae or bacteria from a soil containing similar types of contaminants enhances their effectiveness. Microbial nutrients are released by plant roots and are food for microbes such as bacteria and fungi. These substances enhance biological activity in the rhizosphere (Chaudhry *et al.,* 1998).

For the past 20 years, the US Forest Service and mining companies have conducted trials on ectomycorrhizal fungi and other forms from native areas surrounding mining sites. *Pisolithus tintorius* is cultivated in vermiculite and peat moss, and is sold commercially as bulk spores, spore pellets, and spore-encapsulated seeds (Society for Mining, Metallurgy, and Exploration, 1998).

Bacteria. Acid mine drainage such as exists at Mt. Costigan can be ameliorated by suppression of bacterial action. For example, destruction of *Thiobacillus ferrooxidans* bacteria inhibits acid production from oxidation of mineral sulphides. Bactericides such as anionic surfactants destroy these bacteria, but must be used in conjunction with other methods such as a soil cap or new vegetation cover (Society for Mining, Metallurgy, and Exploration, 1998). The bactericide is applied to the

new vegetation to allow a healthy root system to develop, to reestablish heterotrophic soil bacteria and fungi, and stimulate root respiration. A trial of these methods at Sunnyside Mine, Colorado is reported to show promising results (Society for Mining, Metallurgy, and Exploration, 1998).

8.3.5 Physical conditions at the site

Geomorphology. No lasting improvement can be achieved at Mt. Costigan until site erosion is controlled. The barren slope requires stabilization and revegetation. This issue has already been addressed in part by the DMR. However, flow diversion and stabilization will be more effective if the geomorphological processes are understood. Critical issues to be addressed are:

- The rate of erosion and evolution of geomorphic processes, for example from rills and sheetwash to the large and expanding gullies on the site (D. Hobday, personal communication). What practical measures can be used to prevent erosion and reclaim erosional damage?

- The processes and rates of sediment transport, by traction, suspension and solution. To what extent are toxic contaminants transported along with the weathered rock debris? How does the buildup of certain metals at the base of slope take place? Is there selective, concentrated deposition of particulate toxic elements because of hydraulic factors, or is there precipitation from solution (D. Hobday, personal communication)?

- Has fine-grained organic matter been selectively winnowed and washed downslope by storm runoff, as described by Gilluly *et al.* (1958)?

- Is surficial sheetwash or ground-water flow responsible for downslope transport of lime originally added over the entire barren site by the DMR during an earlier remediation program?

Reclamation of contaminated sites requires management of surface drainage by slope modification, erosion-prevention structures, sediment and water retention

ponds, and revegetation. Vegetation is the key to successful reclamation of any site (Society for Mining, Metallurgy, and Exploration, 1998).

Soil-forming processes. Soil-forming processes, or pedogenesis, play a critical role in the mineral breakdown, mineral transformation, and release of metal elements from the Mt. Costigan bedrock, and therefore warrant serious attention. Leaching of the weathered surface zone at Mt. Costigan is causing physical and geochemical changes in the soil, with important implications for metal distribution and bioavailability. Because of the instability and reactivity of mafic mineral assemblages in the presence of oxygen, metal concentration by soil-forming processes is particularly rapid in old mine tailings such as Mt. Costigan (Ferm, 1974). The rate of accumulation of humic matter in soil and the effect of organic reactions on bioavailability are additional matters that deserve attention.

8.3.6 Soil geochemistry

Chemical composition and metal uptake by plants. As Kabata-Pendias and Pendias (1984) and Huang *et al.* (1997) have stressed, soil geochemistry exercises a primary control on metal uptake by plants. However, it is highly complex, with interdependent factors that are poorly understood. Altering one geochemical factor such as pH could have a negative impact on another soil attribute. A thorough geochemical review of local conditions should result in a coordinated plan to optimize conditions for remediation at Mt. Costigan.

Some metals are immobile, for example bound in clay lattices, the humic fraction, or oxide coatings, or in chemically inert native elements. Chemical methods are available to increase bioavailability of some of these metals. Electrical conductivity too may inhibit uptake, and this too can be mitigated by additives (Society for Mining, Metallurgy, and Exploration, 1998). However, further work may determine that it is better to achieve chemical or physical stabilization of contaminants by neutralization, precipitation, adsorption and chemical encapsulation. These techniques decrease bioavailability of contaminants to the level of acceptable risk (Society for Mining, Metallurgy, and Exploration, 1998).

In-situ chemical stabilization includes "toxicity characteristic leaching procedure" (TCLP analysis), a risk-based technique that assesses the risk of producing an undesirable byproduct. TCLP results can be favourably influenced by chemical stabilization of lead in particular (Society for Mining, Metallurgy, and Exploration, 1998), and therefore might be well suited to Mt. Costigan.

Chemical amendments. Chemical neutralization of rock waste containing sulphide minerals is of paramount importance because sulphides exposed to oxygen convert to sulphuric acid, which in turn affects salinities, pH and metal mobility at Mt. Costigan and other mines (Ferm, 1974). Chemical soil amendments include crushed limestone, hydrated lime, calcareous soils, ash or caustic waste, which react with excess acid (Society for Mining, Metallurgy, and Exploration, 1998). The hydrology must be well understood to ensure that ground water flow accesses the material. Silicate stabilization increases pH and precipitates metals as relatively insoluble hydroxides or carbonate salts. Phosphate stabilization is very effective with lead-contaminated soils, where relatively insoluble lead phosphate is precipitated. Even though the total lead content stays the same, TCLP analysis qualifies this as non-hazardous waste (Society for Mining, Metallurgy, and Exploration, 1998). The related process of phosphate armouring is used to seal off sulphide rock wastes from the atmosphere. This is achieved by precipitating an iron phosphate coating on the surfaces of sulphide particles such as pyrite, thus inhibiting oxidation (Society for Mining, Metallurgy, and Exploration, 1998).

A site-specific mix of chemical fertilizers and organic amendments could be developed for Mt. Costigan. This might include, for example, porous ceramic granules enriched with organic carbon, nitrogen compounds, micronutrients and microbial biomass. Similar soil amendments have been successfully tailored to site conditions, contaminants, organic matter content, nutrient capacity, and price considerations (Society for Mining, Metallurgy, and Exploration, 1998).

Remediation using carbon scavengers has been attempted at Mt. Costigan by the DMR (Johnson, personal communication). Organic matter in soil is a highly efficient oxygen scavenger, capable of preventing interaction of oxygen with

sulphide minerals, thereby reducing acid drainage. Organic matter placed in mine workings is decomposed aerobically by organisms that consume oxygen in converting the organic carbon to carbon dioxide. This removes most of the dissolved oxygen in mine waters, increases pH and decreases metal content and bioavailability in mine water (Society for Mining, Metallurgy, and Exploration, 1998). Advances in microspectroscopy (Astheimer *et al.*, 2000) suggest that it will be possible to identify humic substances with the strongest affinity for toxic substances, both organic and inorganic. This may help identify those organic substances that are most effective in neutralizing or sequestering soil contaminants at site such as Mt. Costigan.

8.3.7 Hydrogeological studies

The unconsolidated layer overlying bedrock at Mt. Costigan is thin and irregular, so flow systems will be shallow, complex and gravity-driven. There is the need for a hydrological survey of the Mt. Costigan mine site in order to understand the factors controlling metal distribution, and to resolve several questions. For example, does ground water account for differences between lead, cadmium and other metals on the barren slope? Future work should investigate the physico-chemical conditions under which the more soluble metals such as copper are taken into solution and transported, and what conditions are necessary for these metals to precipitate. Techniques could include surface sampling, borehole monitoring, geochemical sampling, and tracer studies to characterize ground-water flow systems. Future studies should also focus on surface runoff and its impact on metal distribution, perhaps associated with erosion and sediment traction.

Hydrological studies could also assess the viability of small, natural wetlands, where mine-site drainage could be directed through a shallow wetland containing a dense biomass of aquatic plants. Such wetlands ideally contain plant communities that support build-up of organic material and bacteria, both of which mediate chemical reactions resulting in metal precipitation. Such systems are self-sustaining

with little or no maintenance (Society for Mining, Metallurgy, and Exploration, 1998).

8.3.8 Engineering methods

The Society for Mining, Metallurgy, and Exploration (1998) outlines procedures for physical containment and isolation of mine waste. These include wet covers, e.g. at Island Copper Mine in British Columbia; capillary barrier covers (alternating layers of coarse sand and clay; self-sealing containment systems made up of layered materials that react with one another to form a barrier); and biomass liners such as manure, sewage sludge, and peat which are compacted to form a relatively impermeable barrier. An organic barrier comprising tree bark, wood pulp and saw dust has been used as part of the containment system for mine wastes at East Sullivan Mine, Quebec (Society for Mining, Metallurgy, and Exploration, 1998).

Conventional "pump and treat" and "dig and haul" methods may be prohibitively expensive at Mt. Costigan. An alternative is *in-situ* remediation using chemical or biological treatment. One method suggested by the Society for Mining, Metallurgy, and Exploration (1998) involves minerals such as calcite, which are emplaced in the ground-water flow path. Water passing through the calcite is made less acidic and metals are precipitated. Alternatively, specially designed lixiviants can be injected into the contaminated waste to selectively remove the targeted elements (Society for Mining, Metallurgy, and Exploration, 1998).

Integrated risk analysis. Risk-based remediation is finding broad acceptance. Risk-based corrective actions (RBCAs) are site-specific and are a better tool than the traditional deterministic methods using standardized techniques (Society for Mining, Metallurgy, and Exploration, 1998). This integrated evaluation method stands in contrast to the normal practice of dividing environmental systems into operational units or separate disciplines (Tearpock and Brenneke, 2001).

Risk assessment first characterizes the factors contributing to environmental contamination, as outlined in Section 8.3, and contributes to the most effective

remedial design. Computer modeling and simulation of integrated remediation actions helps identify longer-term outcomes (Society for Mining, Metallurgy, and Exploration, 1998).

8.4 Conclusions: recommended measures

Determination of metal contaminant concentrations and toxic impact of site soils on selected native plant species, coupled with an evaluation of the DMR measures, leads to the conclusion that plant-based remediation has a potentially important future role at Mt. Costigan. Not all of the possible measures in Section 8.3 can be trialed immediately, and some may prove impractical at Mt. Costigan, or not feasible on a cost-benefit basis. Some measures may be more relevant than others, and they are prioritized below. In order to assess their effectiveness, a pilot study should combine several carefully selected, independent methods that might prove more powerful in combination than they are in isolation. This trial process would then fine-tune the larger-scale project design.

Future phytostabilization and phytoremediation planning at Mt. Costigan should involve careful deployment of species best adapted to the specific conditions on the barren site and woodlands, in combination with other complementary physical, chemical or biological measures, as required. *Avena sativa* or some other tolerant species could be planted over the entire site. *Acacia salicina* could be planted on the B subsite and parts of the T subsite.

At the same time, conditions in the M subsite could be ameliorated by non-botanical methods. *Acacia salicina* would then be introduced to the M subsite as soon as it could survive. As conditions improve, *Eucalyptus sideroxylon* could be introduced. Mixing of biosolid would improve germination, survival and biomass of the eucalypts. Thus, the woodland would be encouraged to expand progressively over the barren site, so that with time the two native species could occupy their preferred ecological niches. In this way, Mt. Costigan would be restored to a more ecologically viable condition, and would no longer constitute a threat to the water quality of the catchment.

Appendix

Appendix 1a. Pre-remediation assessment, September 1999. The recorded (field) name, botanical name and family are listed. Some species could not be identified because of immature stage or lack of diagnostic flowers/seeds. Species present are indicated by X; those not present at a site are indicated as abs. Barren slope Sites S1-S5 are shown on Fig. 3.1.

Recorded Name	Botanical Name	Family	Site S1	Site S2	Site S3	Site S4	Site S5
Pink Dicot	Unknown		X	X	abs.	X	X
Orange Malvaceae	*Anagallis arvensis*	Primulaceae	X	X	X	X	X
Fine grass, not Briza	*Agrostis* spp.	Poaceae	X	X	abs.	X	X
Yellow Clover/Medic	*Trifolium campestre*	Fabaceae	X	X	abs.	X	X
Hydrocotyle	*Hydrocotyle laxiflora*	Apiaceae	X	abs.	abs.	X	X
Feathery Leaf Dicot	*Acaena novae-zelandiae*	Rosaceae	X	abs.	abs.	X	abs.
Dandelion	*Chondrilla juncea*	Asteraceae	X	abs.	abs.	X	X
Wheaty Grass	*Bromus mollis*	Poaceae	X	abs.	abs.	X	X
Plantago	*Plantago lanceolata*	Plantaginaceae	X	X	X	abs.	X
Briza	*Briza minor*	Poaceae	X	abs.	abs.	X	X
Nutgrass/Csirpus	Unknown	Poaceae	X	abs.	abs.	X	abs.
Subclover	Unknown	Fabaceae	X	X	abs.	X	abs.
Serrated Tussock	*Nassella trichotoma*	Poaceae	X	X	abs.	X	X
Iridaceae	*Lomandra filiformis*	Xanthorrhoeace	X	abs.	abs.	abs.	abs.
Sandpaper Sedge	Unknown	Poaceae	X	abs.	abs.	abs.	X
Cocks Foot Grass	*Dactylis glomerata*	Poaceae	X	abs.	abs.	X	abs.
Spike of Orange Blossoms	*Plantago gaudichaudii*	Plantaginaceae	X	abs.	abs.	abs.	abs.
Upright Segmented Grass	Unknown	Poaceae	X	abs.	X	X	abs.
Dianella-Like Fruit	*Dianella* spp.	Liliaceae	X	abs.	abs.	abs.	abs.
Petrorhagia (Pink)	*Petrorhagia* spp.	Caryophyllacea	X	X	abs.	abs.	abs.
Skinny Wheaty Grass	*Bromus* spp.	Poaceae	X	X	abs.	X	abs.
Thistle	*Cirsium* spp.	Asteraceae	X	abs.	X	abs.	abs.
Rabbit Tobacco	*Gnaphalium* spp.	Ateraceae	X	abs.	abs.	X	X
Pink Flower with Big Seedpod	Unknown		X	abs.	abs.	abs.	abs.
Blue Malvaceae	*Erodium* spp.	Geraniaceae	X	abs.	abs.	abs.	abs.
Moss	Unknown		X	X	abs.	abs.	abs.
Scaevola	*Goodenia dimorpha*	Goodeniaceae	abs.	abs.	abs.	X	abs.
Narrow Leaf Dicot	Unknown		abs.	X	abs.	X	abs.
Madly Branching	*Rumex acetosella*	Polygonaceae	abs.	X	abs.	X	X
Branching, larger, w' fruit and finely divided leaves	*Apium leptophyllum*	Apiaceae	abs.	X	abs.	abs.	X
Green clustered leaves branching above rosette	Unknown		abs.	X	abs.	abs.	abs.
Umbrella Grass	*Chloris truncata*	Poaceae	abs.	abs.	X	abs.	abs.
Pink Flower, Long Ovary	*Epilobium billardierianum*	Onagraceae	abs.	abs.	X	abs.	abs.
Ribby-Leaved Rosette	Unknown		abs.	abs.	abs.	X	abs.
Lambstongue, furry	Unknown		abs.	abs.	abs.	X	X
Purple Groundcover Malvaceae	*Geranium molle*	Geraniaceae	abs.	abs.	abs.	X	abs.
Blue Small Dicot	*Anagallis arvensis* var.	Primulaceae	abs.	abs.	abs.	abs.	X
Seedling Acacia	*Acacia* spp.	Fabaceae	abs.	abs.	abs.	abs.	X
Clover	*Trifolium* spp.	Fabaceae	abs.	abs.	abs.	abs.	X
Total Species			26	14	6	23	18

Appendix 1b. Post-remediation assessment, May 2000. Incoming species indicated by bold lettering.

Recorded Name	Botanical Name	Family	Site 1	Site 2	Site 3	Site 4	Site 5
Pink Dicot	Unknown		X	X	X	abs.	X
Orange Malvaceae	*Anagallis arvensis*	Primulaceae	X	abs.	X	abs.	X
Fine grass, not Briza	*Agrostis* spp..	Poaceae	X	X	X	X	X
Yellow Clover/Medic	*Trifolium campestre*	Fabaceae	abs.	abs.	abs.	abs.	abs.
Hydrocotyle	*Hydrocotyle laxiflora*	Apiaceae	abs.	abs.	abs.	abs.	abs.
Feathery Leaf Dicot	*Acaena novazelandiae*	Rosaceae	abs.	abs.	abs.	abs.	abs.
Dandelion	*Chondrilla juncea*	Asteraceae	X	abs.	X	abs.	X
Wheaty Grass	*Bromus mollis*	Poaceae	abs.	abs.	abs.	abs.	abs.
Plantago	*Plantago lanceolata*	Plantaginaceae	abs.	abs.	abs.	abs.	abs.
Briza	*Briza minor*	Poaceae	abs.	abs.	abs.	abs.	abs.
Nutgrass/Csirpus	Unknown	Poaceae	abs.	abs.	abs.	abs.	abs.
Subclover	Unknown	Fabaceae	X	X	X	X	X
Serrated Tussock	*Nassella trichotoma*	Poaceae	X	X	X	abs.	X
Iridaceae	*Lomandra filiformis*	Xanthorrhoeaceae	abs.	abs.	abs.	abs.	abs.
Sandpaper Sedge	Unknown	Poaceae	abs.	abs.	abs.	abs.	abs.
Cocks Foot Grass	*Dactylis glomerata*	Poaceae	abs.	abs.	abs.	abs.	abs.
Spike of Orange Blossoms	*Plantago gaudichaudii*	Plantaginaceae	abs.	abs.	abs.	abs.	abs.
Upright Segmented Grass	Unknown	Poaceae	abs.	abs.	abs.	abs.	abs.
Dianellaabs.Like Fruit	*Dianella* spp.	Liliaceae	abs.	abs.	abs.	abs.	abs.
Petrorhagia (Pink)	*Petrorhagia* spp.	Caryophyllaceae	abs.	abs.	abs.	abs.	abs.
Skinny Wheaty Grass	*Bromus* spp.	Poaceae	abs.	abs.	abs.	abs.	abs.
Thistle	*Cirsium* spp.	Asteraceae	X	X	X	X	X
Rabbit Tobacco	*Gnaphalium* spp.	Ateraceae	X	abs.	X	X	X
Pink Flower with Big Seedpod	Unknown		abs.	abs.	abs.	abs.	abs.
Blue Malvaceae	*Erodium* spp.	Geraniaceae	X	X	X	abs.	X
Moss	Unknown		X	X	abs.	abs.	abs.
Scaevola	*Goodenia dimorpha*	Goodeniaceae	abs.	abs.	abs.	abs.	abs.
Narrow Leaf Dicot	Unknown		abs.	abs.	abs.	abs.	abs.
Madly Branching	*Rumex acetosella*	Polygonaceae	abs.	abs.	abs.	abs.	abs.
Branching, larger, w' fruit and finely divided leaves	*Apium leptophyllum*	Apiaceae	abs.	abs.	abs.	abs.	abs.
Green clustered leaves branching above rosette	Unknown		abs.	abs.	abs.	abs.	abs.
Umbrella Grass	*Chloris truncata*	Poaceae	abs.	abs.	abs.	abs.	abs.
Pink Flower, Long Ovary	*Epilobium billardierianum*	Onagraceae	abs.	abs.	abs.	abs.	abs.
Ribbyabs.Leaved Rosette	Unknown		abs.	abs.	abs.	abs.	abs.
Lambstongue, furry	Unknown		abs.	abs.	abs.	abs.	abs.
Purple Groundcover Malvaceae	*Geranium molle*	Geraniaceae	abs.	abs.	abs.	abs.	abs.
Blue Small Dicot	*Anagallis arvensis* var.	Primulaceae	abs.	abs.	abs.	abs.	abs.
Seedling Acacia	*Acacia* spp.	Fabaceae	abs.	abs.	abs.	abs.	abs.
Clover	*Trifolium* spp.	Fabaceae	X	X	X	X	X
Blackberry	***Rubus* spp.**	**Rosaceae**	abs.	X	abs.	abs.	abs.
Aster	***Aster* spp.**	**Asteraceae**	X	X	abs.	X	abs.
Total Species			12	10	9	6	10

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