The Economic Significance of the Phytoextraction of Nickel, Cobalt and Gold from Metalliferous Soils.

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Abstract

Phytoextraction of heavy metals is a relatively new technology that has potential applications for the remediation of many contaminated sites around the world. The technology has significant applications in the minerals industry for the treatment of low-grade ores and metalliferous mine waste.

This study concerns the investigation of the potential to remove heavy metals, in particular nickel, cobalt and gold, from artificial and lateritic substrates. Four experiments comprise this study of the phytoextraction of nickel, cobalt and gold using both accumulator and non-accumulator species. Nickel and cobalt bioavailability was determined by ammonium acetate extraction for both artificial and laterite substrates. It was found that ammonium acetate extractability was predictive for nickel accumulation from a nickel-only artificial substrate. Cobalt bioavailability did not predict the accumulation response of either *Alyssum bertolonii* or *Berkheya coddii* grown of artificial substrates.

The potential for phytoextraction of nickel and cobalt was investigated using the known nickel hyperaccumulators *A. bertolonii* and *B. coddii*, grown on artificially prepared substrates. The substrates were nickel-only (4 mg/kg to 1000 mg/kg), cobalt-only (4 mg/kg to 1000 mg/kg) and nickel-cobalt mixed (1:1 ratio, 4 mg/kg to 500 mg/kg) amendments of sulphates to commercial potting mix. Hyperaccumulation from nickel-only and cobalt-only substrates resulted in typical logarithmic metal uptake by both species. The cobalt-only substrates were phytotoxic to *B. coddii* above a concentration of 15-20 mg/kg. Phytotoxicity significantly reduced biomass production in *B. coddii* without effecting the bioaccumulation coefficient. No corresponding cobalt phytotoxicity was observed in *A. bertolonii* over the experimental range, although biomass production appears to favour substrate concentrations below 30 mg/kg. The bioavailability and hyperaccumulation of cobalt from the mixed nickel-cobalt substrates dramatically reduced the nickel accumulation potential of both species at substrate concentrations below 300 mg/kg. At higher substrate metal concentrations both species return to nickel dominant hyperaccumulation.

Induced gold accumulation in *B. coddii* and *Iberis intermedia* was investigated using, sequential ammonium thiocyanate and ammonium thiosulphate chelation to, a 5 mg/kg gold artificial substrate. An attempt to determine gold bioavailability by ammonium thiocyanate and ammonium thiosulphate extraction was made on the substrate. It was found that neither chelator extraction could be correlated with plant accumulation induced by the same concentration of the reagent. Ammonium thiocyanate induction resulted in plant gold accumulation at or below the substrate concentration. Ammonium thiosulphate induced gold accumulation in *I. Intermedia* reached 48.8 mg/kg when treatment with a 1% solution. *B. coddii* accumulated 9.3 mg/kg gold for the same treatment.

Five consignments of metalliferous lateritic materials from Western Australia were investigated. Three substrates originated from Project Murrin Murrin nickel and cobalt mine operated by Anaconda Nickel Ltd. and two substrates originated from Boddington Gold Mine operated by Worsley Alumina Ltd. Nickel and cobalt accumulation by *A. bertolonii* and *B. coddii* was found to be significantly lower than observed using artificial substrates. Nickel and cobalt bioavailability, determined by ammonium acetate extraction, failed to predict the accumulation responses from laterite substrates. This is attributed to elemental interference by, and possibly ammonium acetate chelation of, other mobile heavy metals in these substrates. A hypothesis deserved of further research. Hyperaccumulation of nickel was observed for both species on the Anaconda Nickel Ltd. SAP substrate only. Appreciable cobalt accumulation (≈90 mg/kg) was observed on the SAP substrate for both species and on the Boddington Gold Mine B5 substrate for *B. coddii*. Phytomining scenarios were determined for both species grown on the SAP substrate. *A. bertolonii* could produce 13 kg of nickel and 0.8 kg of cobalt per hectare with a value of US$ 163. *B. coddii* could produce 23.8 kg of nickel and 2.1 kg of cobalt per hectare at a value of US$ 319. These levels of production could be improved by fertilisation and/or substrate acidification.

A preliminary investigation into induced gold accumulation from laterite substrates by *I. Intermedia*, *A. longiflora*, *Brassica juncea* and *Linum usitatissimum* was made using the acid biased chelator ammonium thiocyanate. It was found that an acidified amendment of ammonium thiocyanate greatly improved the

phytoaccumulation of gold from the lateritic substrates. An amendment of 2M HCl produced appreciable gold mobility and phytoaccumulation and indicates that gold solubility is the primary control on plant uptake. Analysis of various plant tissues indicated that *Acacia longiflora* stored significant gold in its roots compared to foliar components. All plant-substrate combinations indicated a trend towards increasing acidification and gold phytoaccumulation. No plant-substrate-treatment combination produced an economically viable phytomining scenario.

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The work is dedicated in memory of my grandmother, Elsie.

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Chapter One - The Science of Phytoextraction

1.1 Introduction

As early as 1583 the Florentine scientist Caesalpino observed an unusual stunted serpentine flora among the 'black rocks' (ultramafic rocks) in the Upper Tiber Valley of Tuscany (Italy) and recorded the presence of a ubiquitous 'alyson' (*Alyssum bertolonii*).

Metalliferous regions often support characteristic plant assemblages of species tolerant to high concentrations of otherwise phytotoxic elements. There are many established and distinct plant communities currently identified including the serpentine flora (growing on Ni, Cr, Mn, Mg, and Co rich soils), the seleniferous flora (growing on Se rich soils), the calamine flora (growing on Zn- and Cd-rich soils), the uraniferous flora (growing on U rich soils) and the cobalt/copper floras of Zaïre.

There are three basic categories of plants that grow on metalliferous soils (Figure 1). Metal excluders effectively prevent metals from entering the plant's aerial tissues when growing in a broad range of concentrations of specific metals in the soil. Metal accumulators actively accumulate metals in their aboveground tissues and can be further divided into two groups, *indicators* and *hyperaccumulators*. Metal levels in the tissues of indicator species generally reflect the metal levels in the soil. In contrast hyperaccumulators are capable of concentrating metals in their foliage far in excess of levels in the soil. These plants, by their very nature, are rare oddities within the plant kingdom and were termed *hyperaccumulators* by Brooks *et al.*, in 1977.

Baumann (1885) recorded the first hyperaccumulator of any metal when he reported over 1% zinc in the dried leaves of *Thlaspi calaminare* (a small herbaceous biennial plant) and *Viola calaminaria* growing over a well known zinc deposit near Aachen, Germany. Some 60 years later Minguzzi and Vergnano (1948) reported that *Alyssum bertolonii* had a nickel concentration of 1%, on a dry weight basis. In 1961,

Doksopulo discovered a second nickel hyperaccumulator, *Alyssum murale*. In the early 1970's, Severne and Brooks (1972) discovered a third nickel hyperaccumulator, *Hybanthus floribundus*, in Western Australia.

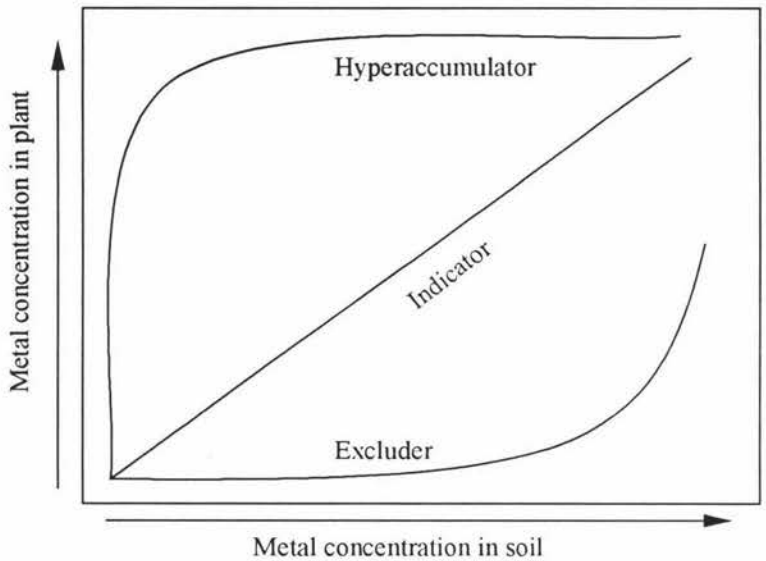

Figure 1. The possible uptake responses of plants to heavy metals in soils. Source Robinson 1997.

This species was also discovered independently by Cole in 1973. Shortly afterwards, Jaffré and Schmid (1974) reported hyperaccumulation of nickel in *Geissois pruinosa, Homalium guillaini* and *Hybanthus austrocaledonicus*. Brooks *et al.* (1977a) analysed over 2000 herbarium specimens of the genera *Homalium* and *Hybanthus* and detected five other hyperaccumulators of nickel, as well as confirming all previously known taxa with this capability. Much early work into biogeochemical prospecting (Malyuga 1964, Brooks 1983, Kovalevsky 1987) was carried out by Russian scientists (e.g. Aferov *et al.*, 1968, Khotamov *et al.*, 1966, Doksopulo, 1961) using indicator plants. Biogeochemical prospecting is the strategy of using concentrations of trace elements in plant material to infer mineralisation in the substrate.

Following this pioneering work, over 300 hyperaccumulators of nickel have been identified, largely as the result of chemical tests on herbarium specimens. At present there are also about 26 species of known cobaltophytes but none for gold.

1.2 Hyperaccumulators

Minguzzi and Vergnano (1948) reported nickel hyperaccumulation in *Alyssum bertolonii* from the Impruneta region near Florence, Italy. They found 7900 mg/kg nickel on a dry weight basis. This was extraordinary not only because it was over two orders of magnitude higher than other species growing on the same or similar soils, but also because the metal concentration in the plant was actually greater than that of the soils (4900 mg/kg).

Table 1. Normal elemental concentrations in plants and the lower limits for hyperaccumulation. (After Reeves et al., 1995 and Anderson et al., 1998). Concentrations in mg/kg.

Element	Normal Range (mg/kg)	Lower limit for hyperaccumulation
Nickel	0.2 – 100	1000
Cobalt	0.05 – 50	1000
Gold	0.001-0.010	1

All plants remove metals from soils to some degree. Some of these metals are essential to plant growth and longevity and are actively taken up. Uptake of other metals may occur incidentally or inadvertently, and at higher concentrations may have a detrimental effect on plant growth. Brooks (1983) classified plant-available elements as essential and nonessential, based on whether or not the plant could complete it's life cycle with or without them being present. Plant responses to essential elements can be divided further into three categories according to increasing elemental concentrations: (1) deficient, (2) optimal, and (3) toxic. Nonessential elements are tolerated by plants at low concentration and may be toxic at higher concentrations.

The ability of plants to phytoextract large quantities of metals appears to contradict the above classification. Hyperaccumulating plants take up exceptional concentrations of metals with respect to other species, far more than that required for growth and usually in excess of substrate concentrations. This unusual uptake could in part have been attributed to a high concentration of metal in the substrate; e.g. the very high zinc concentrations in *Thlaspi calaminare*.

So, what actually defines a 'hyperaccumulator'? In the case of nickel, this is relatively easy because of the order of magnitude difference in nickel levels between "normal" plants and hyperaccumulators, relative to substrate concentrations. A level of 1000 mg/kg metal on a dry weight basis was established to differentiate hyperaccumulators from other species. In the case of cobalt however, no such clear separation exists.

Brooks *et al.* (1980) analysed 400 species from mineralised areas of the Shaban Copper Arc (Zaïre) for cobalt and copper and reported a hyperaccumulation discontinuity at 1000 mg/kg for both metals (plotted as cumulative frequency curves). This indicated that the concentrations in the samples did not belong to a single log normal distribution but rather represented 2 distinct types of cobalt accumulation within the species. Type A cobaltophytes tend towards Brook's classification as indicator species while Type B cobaltophytes tend towards hyperaccumulator status. For the purpose of this study both nickel and cobalt hyperaccumulating plants are classified as those metallophytes containing <1000 mg/kg nickel and/or cobalt on a dry-weight basis.

The geographical distribution of nickel hyperaccumulators throughout the world is limited to regions that have not previously been glaciated. In descending order the taxa are: Cuba (128), Southern Europe and Anatolia (92), New Caledonia (56), Southeast Asia (12), Brazil (11), Southern Africa (8), North America (6), Australia (2) and Dominican Republic (1). The presence of these plants only in nonglaciated regions is likely a reflection of the time required for a plant to evolve towards hyperaccumulation upon an undisturbed developing metalliferous substrate. Presumably plant evolution, and adaptation to the hostile environment of nickel-rich soils, is a process taking considerably longer than the 10,000 years that have elapsed since the last glaciation. The development of metal-rich soils and laterites occurs over mineralised basement rocks and typically require time intervals of 5-10 million years to develop. The distribution of hyperaccumulators may also, in part, reflect the botanical isolation of mineralised terrains over the last 10-30 million years and also the relative solubilities of different heavy metals within the usual plant growth ranges of trace element concentrations and pH levels.

The geographical distribution of cobalt hyperaccumulators throughout the world is currently limited to the Shaban Copper Arc in Central Africa. This singular known occurrence of a cobalt (and copper) hyperaccumulator community may in part reflect the rarity of non-glaciated cobalt surface deposits.

1.3 Phytoextraction

With the high concentrations of some trace elements encountered in herbarium plant samples, it wasn't long before scientists began to investigate the use of these unusual plants in such diverse fields as archaeology, mine dump rehabilitation, pollution control and extraction of metals from substrate media. The last decade has seen the interest in hyperaccumulating plants (and also bacteria and fungi) intensify with the science of phytoextraction spawning two ideologically different, yet inextricably linked, disciplines of phytoremediation and phytomining.

Phytoremediation is the process of decontaminating heavy metal polluted soils using the process of phytoextraction. Remediating contaminated soils is a costly exercise and in some cases may simply not be possible due to the sheer volumes of material involved. Phytoremediation has the potential to offer a low cost and environmentally friendly method of pollution control and reversal while also improving the physical properties of the soil and providing atmospheric amelioration through photosynthesis.

So far the majority of phytoremediation work has focused on cadmium, lead and zinc (McGrath *et al.*, 1993, Brown *et al.*, 1994; Huang and Cunningham, 1996). However, nickel contamination is a problem in many soils associated with smelters, areas where effluent has been used as a soil amendment or in agricultural practices where heavy metals effect plant growth and yield.

This third point may have particular relevance in Third World regions as a means of ameliorating metalliferous soils used in agriculture, while returning a dividend from metal production and thus still supporting the local inhabitants while the process of phytoextraction takes place in the absence of crop production. The aforementioned species (*Alyssum bertolonii*, *A. murale*, *Thlaspi calaminare*, *Viola*

calaminaria, Hybanthus floribundus, H. austrocaledonicus, Geissois pruinosa, and Homalium guillaini) may therefore have a role in remediating such soils.

Phytomining is the commercial phytoextraction of metals. Although this has never been tested industrially, the concept is becoming more familiar within the minerals industry. The process could in theory be used, in addition to modern mining practices, to elevate metal production levels and subsequent revenues of existing mining enterprises. It may also have applications in areas currently considered uneconomic for modern mine practices and as being an economically sustainable environmental practice.

Chaney (1983) first suggested using the nickel hyperaccumulator *Strepthanthus polygaloides* to farm a crop of nickel. Nicks and Chambers (1995) demonstrated that by cultivating a crop of *S. polygaloides* on an ultramafic soil and then burning the harvested material, up to 100 kg nickel/ha could be recovered from the resulting ash (termed *bio-ore*) which had a nickel concentration of over 15%. The net economic yield of the operation at the time would have been $385/ha, excluding the costs of processing. An important factor in this calculation is that it assumes 25% of the energy derived from combustion could be utilized to produce electricity. This yield is similar to the net return on a crop of wheat grown over the same area. The process was called *phytomining*.

Since this initial study, two other species, *Alyssum bertolonii* and *Berkheya coddii* have shown promise as commercial extractors of nickel (Robinson *et al*, 1997a). Work carried out on *Berkheya coddii* established that a yield of 110 kg of nickel per hectare with a value at the time of US$ 792 could be achieved. Later work in the same year (Robinson *et al.* 1997b) recommended that addition of sulphur or acid mine tailings had the potential to enhance metal uptake. This resulted in a metal value increase of 4.1 on the previous scenario. Both economic models assume a return on 25% of the electricity.

The concept of phytoextraction is to:

1. grow and harvest a plant species on a soil containing modest concentrations of some heavy metal(s),
   1a. addition of metal chelators prior to harvest where necessary,
2. reduce the biomass to bio-ore,
   2a. possible generation and sale of electricity,
3. smelt and sell, or store the residual ash.

Commercial extraction of nickel, cobalt and gold is performed upon ores that have high concentrations of these metals, typically sulphide deposits for nickel and cobalt. To be a viable business venture, such operations require a localised high-grade ore body (3% nickel in the Kambalda orefield, Western Australia). These types of ore bodies are becoming exhausted, particularly those at near-surface positions. Often much larger occurrences of low-grade near-surface mineralisation accompany (halo) these high-grade zones and are currently not economically viable to exploit using conventional mining methods.

Most nickel and cobalt ore bodies are associated with ultramafic (*serpentine*) lithologies, which usually support a characteristic flora. Botanists know this distinctive stunted vegetation as serpentine flora growing in soils derived from ultramafic rocks. In a serpentine-derived regolith, nickel is usually present in concentrations of between 1000 and 7000 mg/kg and cobalt from 0.1 to 0.3 mg/kg. These values are clearly well below the cut-off grade of 3% (30,000 mg/kg) nickel and 0.2% (2000 mg/kg) cobalt required for modern nickel sulphide mining operations.

1.4 Heavy Metal Bioavailability

Central to the premise of phytoextraction is the metalliferous potential of the soils or substrates on which the hyperaccumulator plants are to be grown. Ernst (1996) and Robinson *et al.* (1997a, b) have shown that the metal concentration in a plant is proportional to the plant available metal concentration in the soil, and that the

majority (perhaps 80%) of the metal in the plant is sequestered as water soluble compounds. It has been noted that a high total concentration of the target metal in the substrate does not necessarily infer a high concentration of its soluble form and therefore bioavailability. Predictions have been made of the expected metal concentration in plants by measuring the 'soluble' metal content of the substrate using the ammonium acetate extraction method (Robinson *et al*, 1998).

The acidity of the substrate must also be considered when attempting to determine the plant-available metal fraction and potential for phytoextraction. All heavy metals require a specific range (stability field) of pH conditions in order to become water-soluble and subsequently plant available. Fortuitously the pH stability fields for nickel and cobalt fall within normal plant growth limits. This may partially explain the relative abundance of nickel hyperaccumulators to other element metallophytes. The situation for gold however, is considerably different because gold solubility favours strongly acid conditions. Robinson *et al.* (1999a) concluded that higher rates of nickel and cobalt phytoextraction in *Berkheya coddii* are associated with acidic substrates. This was determined by using substrate amendments of acid mine tailings and elemental sulphur to modify the acidity. The effect of low pH on nickel hyperaccumulation is constrained by plant tolerance to acidity, and high solubility of other heavy metals.

Robinson *et al.* (1999a) also investigated the use of other substrate amendments to promote nickel hyperaccumulation. The addition of calcium and magnesium, as carbonates to artificial potting mix, produced an increase to substrate pH that in turn decreased the bioavailability of nickel. This experiment highlights the need to determine other trace element abundances and growth parameters when evaluating a substrate for phytoextraction. Particularly when attempting to estimate the hyperaccumulation potential of a substrate for any type of commercial operation.

The metal chelating chemicals NTA, DTPA and EDTA were also investigated to improve hyperaccumulation in *Berkheya coddii*. EDTA was found to induce lead accumulation in *Brassica juncea* by Blaylock *et al.* (1997). The work by Robinson *et al.* (1999a) expanded on this by including two other closely related heavy metal chelators and found that all chelators produced a decrease in nickel

hyperaccumulation. These authors concluded that, although the bioavailable nickel and cobalt fractions increased with the addition of each chelator, competition with the plant's own nickel-binding agents resulted in a net decrease in nickel hyperaccumulation. Cobalt accumulation was ambiguously unaffected by the presence of these chelators (Robinson *et al.* 1999a).

There is an important difference between the total concentration of a metal in a soil, and the fraction that interacts with the biota, i.e. the plant available fraction. The total concentration in the soil may give no indication of the soils' potential to be used in a phytoextraction operation. The bioavailable nickel fraction generated by heavy metal chelation has an important affect on hyperaccumulation too. Clearly NTA, DTPA and EDTA are not suitable for improving nickel accumulation in *Berkheya coddii*.

The amount of nickel and cobalt available for plant uptake in a substrate is dependent upon the weathering rates of the nickel- and cobalt- bearing assemblages, as well as pH and rhizospheric interactions. Obviously a greater weathering rate would release a larger amount of nickel and cobalt into the soil solution. Laterites are by definition extremely weathered materials; it would follow then that the nickel-cobalt laterites supplied in this study should have suitable bioavailable metal fractions for hyperaccumulation to occur.

The rate of chemical exchange between mineral and soluble phases of the metals should also be high due to the relatively weak chemical bounds associated with laterite geochemistry. This may play an important role in chelator selection and loading necessary for induced phytoextraction.

From the perspective of phytoextraction, the total concentration of a metal in a soil can be divided into three fractions.

1. Plant available; the metal fraction that is readily available for uptake by an organism at any given time,

2. Potentially plant available; the organo-exchangeable metal fraction that becomes phytoextractable by equilibrium exchange once the available fraction has become depleted,
3. Unavailable; the metal fraction that is bound in an organic or silicate form, or present as a very insoluble salt that cannot be phytoextracted.

Whether metal solubility occurs naturally, or is induced using a chelate, all nutrients entering a plant are translocated in a soluble form. Due to the large variety of compounds associated with heavy metal transport in different plant species, it is obvious that the bioavailability of heavy metals is species dependent. One only has to look to the numbers of hyperaccumulators of easily soluble metals like nickel or copper relative to those for harder metals such as chromium or gold to appreciate the complex nature of plant metal accumulation. The soil-root interactions clearly play an important role in phytoextraction in several ways.

1. The root membrane acts as a 'gateway' through which only genetically predetermined metals and compounds may pass in a soluble form.
2. The root membrane may secrete metal specific ligands, which act as transport complexes within the soil solution and across the root membrane.
3. The roots may secrete substrate altering compounds that act upon the mineral phase to release elements into the soil solution.

Chelator induced phytoextraction of heavy metals also appears to be species dependant. This is evident from Blaylock *et al.* (1997) working on EDTA induced lead accumulation in *Brassica juncea* contrasting with the nickel work of Robinson *et al.* (1997a) on *Berkheya coddii* using NTA, DTPA, EDTA as chelators. Recent data from Anderson *et al.* (1998, 1999) on induced gold accumulation in several species, particularly *Brassica juncea,* using ammonium thiocyanate and ammonium thiosulphate chelation identifies the first cross-species heavy metal chelators. This has yet to be tested on other heavy metals, but emphasizes the complex biogeochemistry associated with gold accumulation in plants

1.5 Laterite Formation

In Australia, nickel-bearing ores and their host rocks mainly formed as parts of ancient volcanic lava flows. The Western Australian deposits were formed in the Archaean, around 2.7 billion years ago, when ultramafic lavas were erupted along major volcanic arcs. These lavas carried nickel sulphides that eventually fractionated out as they cooled and slowed. The sulphides collected within depressions at the base of the flows, forming long, ribbon-like ore bodies. The nickel grade of these sulphide orebodies typically ranges from 1-4% nickel (Anaconda Nickel Ltd., 1998).

Laterite genesis is, by definition, the process of extreme *in situ* biological and chemical weathering (and possibly erosion or deposition) within a long-exposed landscape. The extreme weathering of minerals assemblages containing pentlandite $(Fe, Ni)_9S_8$, pyrrhotite ($Fe_{n-1}S_n$), pyrite (FeS), and chalcopyrite ($CuFeS_2$) in mafic and ultramafic igneous rocks produce garnierite ($(Ni,Mg)SiO_3.nH_20$) and nickeliferous clays (particularly limonite) which constitute the ore mineralogies in most laterite deposits. These highly leached soils form in regions of tropical climate such as New Caledonia and Cuba today. A tropical climate with high temperatures and abundant rainfall will weather nickel-bearing mafic and ultramafic rocks (iron and magnesium rich) resulting in laterite (and red soil) formation. Garnierite ($(Ni,Mg)SiO_3.nH_20$) and nickeliferous limonite dominate the ore mineralogy of Australian laterites.

The process of lateritisation generates a very distinctive soil profile that in places may extend to 120m depth. Undisturbed laterites often preserve the original rock structures (e.g. faults, folds, and mineralogical structures) as an overprint in the profile (Butt, 1996).

The laterite profile at Anaconda Nickel Ltd's Murrin Murrin site has a typical morphology, described below (Anaconda Nickel Ltd., 1999).

1. A haematite-kaolinite-rich pisolitic duricrust cemented by an outer coating of goethite.

2. Underlying the duricrust is a deep mottled zone of predominantly quartz and transitional clay minerals. The mottled zone typically has large vertical mottles developing into a quartz-kaolinite transition zone of the upper saprolite.
3. Saprolites are often marked by silcrete and cemented arenose horizons indicating that weathering and redistribution of elements has been an on going process over a long period of geologic time.
4. Saprolite profiles either grade smoothly into fresh unaltered bedrock or more typically end abruptly in fresh, unaltered bedrock. Because of the age and marked chemical changes within the saprolite it is often difficult to say whether this indicates an unconformity within the profile or simply the maximum depth of weathering.

Approximately 65% of the worlds nickel deposits are hosted within laterite profiles. Of this, 38% can be found in countries of the Pacific Rim. The distribution of contemporary laterites in Western Australia led early workers to speculate that laterites formed only sporadically and in response to ancient local conditions. Many studies in Australia have assumed that laterites formed under seasonally dry, humid tropical conditions resulting in intense weathering processes during the Tertiary. It is now generally agreed that laterite formation occurred as a uniform blanket over large areas of the landscape, and that the present occurrences of laterites are erosional island remnants of fossil laterites. It is also generally agreed that the level of chemical weathering exhibited by laterite would require exposure of the land surface over a period of 5-10 million years with a periodically active hydrogeological system. Outside of the humid tropics, laterite occurrences are considered indicators of climate change and/or continental drift.

Any discussion of laterite geochemistry would not be complete without reference to *supergene* enrichment. The process of supergene enrichment occurs where metal rich brines are allowed to percolate through and precipitate metals within the ore body as weathering progresses. In laterite terrains supergene enrichment often results as metalliferous overprinting and may also produce a distinctive lateral mineralisation within the profile in high contrast to the original 'balloon' shaped laterite orebody, usually developed above basement rock mineralisation. The vertical position of the supergene orebody within the laterite

profile has been argued by many geologists to coincide with a paleo water table or pre-existing organic horizon where rock-water interactions result in metal deposition (Gray *et al.*, 1992). Secondary enrichment of gold is well known in Australian gold deposits. Many 5-20 oz nuggets have been found using metal detectors in laterites of the Coolgardie district of Western Australia. The author has also recovered several small nuggets in the same fashion from laterites in the Meekatharra-Wiluna district of Western Australia while undertaking geological exploration.

The genesis of nickel/cobalt laterites is associated with the weathering and redistribution of elements hosted by sulphide complexes in humid, oxidising environments (. Many elements hosted by sulphides (cadmium, cobalt, copper, nickel and zinc) are commonly strongly leached deep in the profile, although a proportion is retained in iron oxides derived from the sulphides. Weathering of ferromagnesian minerals (pyroxene, olivine, amphibole, biotite) results in the formation of iron oxides that partially retain minor and trace elements, such as nickel, cobalt, copper and manganese, and progressively lose manganese and silicon, except where retained in smectite (manganese, silicone).

Contemporary lateritisation is no longer occurring in Western Australia. The low rainfall and water table is promoting a high flux of very saline groundwaters. This would suggest that the dominant complexes involved in gold mobility in Western Australia today would be the low pH chloride compounds, that gold mobility would be restricted to the current level of the water table and that supergene enrichment may still be occurring today. Supergene enrichment could therefore be argued as an arid environment phenomenon unrelated to lateritisation.

1.6 Gold Phytotechnology

Gold mineralisation occur in association with many varied geologic settings and scales (e.g. volcanogenic massive sulphide deposits, hydrothermal systems, alluvial systems and under intense weathering) at concentrations ranging from less than 1 mg/kg to many kg per ton. Modern mining methods require gold concentrations of $\geq$0.7 g/t to support long term sustainability. This gold

concentration implies a large resource where the scale of mining allows for a lower-than-customary average resource grade. The usual 'cut off' grade for a gold resource in Australia is currently around 1 g/t. Gold recoveries from these ores are currently in the vicinity of 92-96%. Considering the vast waste dumps and low-grade stockpiles dotted across the 'outback' associated with a long history of gold mining and poor historical gold recovery rates it is believed that considerable gold remains within these materials.

Recovery of gold using hyperaccumulating plants and chelating agents is a relatively recent development in the growing technology of phytoextraction. Due to the poor solubility of gold in aqueous solutions and its inherent chemical stability, no known plants will naturally accumulate gold to levels sufficient to accomplish phytoextraction successfully.

For phytoextraction to occur, the target metal must be mobile in the growth media. Most research conducted on gold mobility over the past 50 years has been based on experiments conducted under laboratory conditions. These data suggest that three chemical species are important to gold mobility in plants. Each has a solubility domain dependent upon depth, pH and Eh. The three species are:

1. Organic acids associated with the decomposition of organic material within the surface layers of the weathering profile.
2. Transient free thiosulphate created through the weathering of mineral sulphides.
3. Halogen species, in particular the Cl^- ions derived from movement of often very saline waters through the weathering profile in arid environments.

The presence of gold in plants was reported nearly two century ago (Malte-Brun, 1824). Quantitative analysis performed by Harrison (1908) on tree samples from an auriferous region in British Guyana established that gold concentrations in the ash of wood ash and bark were small, ranging from 0.06 mg/kg in bark to 0.6 mg/kg in wood from the interior of the tree. Later investigators (Aripova *et al.*, 1966, Razin *et al.*, 1963, Warren *et al.*, 1950) reported gold concentrations in many species of plant from various mineralized regions around the world. Although the concentrations given in some reports are dubious at best, the fact that plants can

absorb gold from the soil solution is well established. The amounts found in plants are usually much smaller than 1 mg/kg and are of the order of 1 to 10 µg/kg consequentially, research into gold in plants has been sporadic. The relative concentrations of gold in seeds, seed pods, leaves, upper twigs, stems, and roots, as determined by activation analysis, were studied by Khatamov *et al.* (1966). They found the maximum content in the leaves and suggested that only leaves need to be analysed in biogeochemical projecting for gold. However quantitative analysis of a species potential within the domain of phytomining requires that all above-ground plant material be analysed.

Gold is not known to be essential to plant growth, and therefore may be classed as a non-essential element (Brooks, 1983). Gold may also be considered phytotoxic because of its affinity for amino, imino and sulphydryl groups in plants (Girling and Peterson, 1978).

Aferov *et al.* (1968) concluded from studies of rocks, plants and waters on the Darasum gold deposit, that a gold-ion complex is the most probable form of gold migration in contemporary waters and inferred that the gold in plants had been absorbed in this form. Cyanide is known to render gold soluble and would provide a suitable transport complex for plant accumulation (Lungwitz, 1900, Lakin *et al.*, 1974). Early work carried out by Shacklette *et al.* (1970) on the nature of the gold complex entering the roots of *Impatiens holstii* and *I. balsamina* determined that a cyanide-soluble gold complex gave the greatest mobilisation of gold across the root membrane. Also of note in this work was the report that a gold- S_2O_3 (thiosulphate) solution of 2.6 mg/L concentration at pH 6.2 had transport potential. Shacklette *et al* (1970) concluded that within the widespread distribution of higher plants (and other organisms) that contain cyanogenic substances, the solubilisation of native gold by hydrogen cyanide and the absorption of cyanide compounds containing gold by various plant species were likely to occur in natural environments. These authors did not consider the role of thiosulphate to be of any real significance in gold mobility.

1.7 Industrial support to my research

This research acknowledges the support of Anaconda Nickel Ltd. and Boddington Gold Mine, both situated in Western Australia.

The largest and most important nickel laterite miner to emerge from Australia is Anaconda Nickel Limited. Anaconda's Murrin Murrin Project is a world class nickel cobalt laterite resource located 60 km north of Leonora in the NorthEast Goldfields of Western Australia. The development of this resource is a fine example of visionary thinking, state of the art technology and scale in every sense of the word. Anaconda operates the Murrin Murrin Project (among other nickel interests in Australia) as a joint Venture between Anaconda Nickel Limited, holding 60% and Glencore International, holding 40%. The operation has been treating laterite ore and producing metal since early 1999. An extensive amount of resource drilling to date has delineated over 220 million tonnes of dry nickel cobalt laterite resources contained within 14 separate lodes along a 32 km basement structure. The resource has a grade of 1.04% nickel and 0.08% cobalt (2.288 million tonnes nickel and 176,000 tonnes cobalt metal contained). The Murrin Murrin orebodies are all surficial and cover an area of some 25 square kilometres of flat, open terrain. The grade profile and the shallow nature (to an approximate depth of 70 m) of the orebodies allow for the mining of high-grade ore during the first 10 to 15 years of the mine's operation, a critical factor in the success of the project.

Project development successfully scheduled the commissioning of a 45,000 tonnes per annum nickel and 3,000 tonnes per annum cobalt production plant at the end of 1998. The Stage II expansion to 115,000 tonnes per annum nickel and 9,000 tonnes per annum cobalt is scheduled for the first half of this year. This rate of production (excluding offsite resource) gives the Murrin Murrin Project a mine life of around 20 years. Total development costs for both stages is estimated at a capital cost in the vicinity of AU$2 billion with operating costs, after cobalt credits, estimated at around US$1.10/kg.

The preliminary acid leach testwork indicated that metal recovery rates of around 92-93% nickel and 90-91% cobalt could be achieved. This equates to 2.116 million tonnes nickel and 159,280 tonnes cobalt that is currently recoverable and a

residual metal in waste volume of 171,600 tonnes nickel and 16,720 tonnes cobalt. Both metals are produced and sold as high-grade, finished products.

The development of the High Pressure Acid Leach (HPAL) extraction technology significantly improved the viability of treating limonitic laterite ores, in particular autoclave technology from the gold industry and solvent extraction-electrowinning from the copper sector have revolutionised the way in which the mineral industry views laterite terrains.

The Boddington Gold Mine was the largest laterite gold mining operation in the Southern Hemisphere and is situated about 120 kilometres southeast of Perth on the Darling Plateau, Western Australia. The mine is owned by Acacia Resources Limited (33.33%), Normandy Boddington Pty Limited (44.44%) and Newcrest Operations Limited (22.22%), and is managed on their behalf by Worsley Alumina Pty Limited.

The Darling Plateau has developed on Archaean crystalline rocks of the Yulgan Block (Craton). These are principally granites and granitic gneisses with minor mafic rocks. Belts of metamorphosed volcanic rocks, such as the Saddleback Greenstone Belt within which the minesite is situated, now represent those ancient mafic units. Proterozoic dolerite dykes (Churchwood and Dimmock, 1989) later intruded this belt of metamorphosed Archaean granites, granitic gneisses and minor mafic units. The resulting weathering profiles have been subjected to lateritisation, which has formed a deep blanket of iron and aluminium sesquioxides and kaolinitic clays over them.

The operation has been treating laterite and oxide gold ores since 1987, working to treat the most profitable areas earliest, to favour rapid debt repayment and in more recent times to counter a low world bullion price. There have been several changes of scale and cut-off grade as a result of steady improvements in mining and milling efficiency over time. Two adjacent treatment plants (a Direct Leaching operation and Basement Extraction Plant), separately treat softer and harder ore types respectively, for a combined throughput of 8.7 million tonnes of ore a year. Current oxide reserves and stockpiles are sufficient for about two more years of operation using present mining methods, and ways of extending the oxide life by hydraulic mining methods are being trialled.

Recent resource drilling has defined Australia's largest undeveloped gold resource of some 440 tonnes (15.5 million ounces) and still growing. This hard rock resource, previously unknown, resides beneath the existing laterite operation. Resource consent, approval and mine design procedures are now underway to develop this deposit.

1.8 Research Outline

The broad aim of this study was to investigate the viability of the phytoextracting of nickel, cobalt and gold using the species in Table 2 and may be subdivided as follows:

1. To further define nickel and cobalt phytoextraction parameters for several known hyperaccumulators.

2. To further define the effects of several chelating compounds upon gold hyperaccumulation for several known hyperaccumulators as well as several other species deemed relevant to this study

3. To test auriferous material from Western Australia for its hyperaccumulation potential.

4. To produce nickel and cobalt hyperaccumulation curves *for Alyssum bertolonii*.

5. To produce a cobalt hyperaccumulation curves for *Berkheya coddii*.

An additional aim to the research was to relate these findings to the estimation of the viability of commercially treating low-grade and waste lateritic materials from two leading mining companies in Western Australia.

Table 2. Species investigated in this study

Name	Natural Range	Climate	Growth Habit	Propagation	Metal Accumulated	Metal conc.
Alyssum bertolonii	Central Italy	Mediterranean	Perennial shrub	Seed	Ni, Co	<3% Ni
Berkheya coddii	Central Africa	Warm	Perennial herbaceous	Seed	Ni, Co, induced Au	< 3% Ni , 50 Co mg/kg
Iberis intermedia	Southern and Central Europe	Cool to warm	Annual herb	Seed	Induced Tl	Up to 4% Tl
Linum usitatissimum	Central Europe	Cool to warm	Annual herb	Seed	Induced Au	<50 mg/kg
Acacia longiflora	Australia	Warm	Perennial shrub	Seedling	Induced Au	Unknown
Brassica juncea	Global	Temperate	Annual herb	Seed	Induced Au	<57 mg/kg

1.9 Materials and Methods.

All experiments were conducted in glasshouses at the Plant Growth Unit, Massey University, New Zealand. The climate was controlled over the growth period in order to counteract low average temperatures and short daylight periods consistent with the winter season. In Palmerston North mean maximum and minimum temperatures for July (winter) are 12.1° C and 4.0° C. For January (summer) the values are 22.3 ° C and 13.1° C. In the winter a rare ground frost could be as low as -5° C. Consequently glasshouse temperatures were controlled in a range from 15° C at night to 25° C during the day and in addition the use of growth lamps increased the daylight by 7 hours.

All artificial nickel and cobalt pot trials were conducted using a potting mix of 50% peat and 50% pumice with additions of lime, dolomite and "Osmocote", at rates recommended by the manufacturer.

All artificial gold pot trials were conducted using clean ground silica sand with additions of lime, dolomite and "Osmocote", a slow release fertiliser, at rates recommended by the manufacturer.

1.9.1 Artificial Nickel and Cobalt Substrates.

The nickel-bearing substrate amendments (Ni) were prepared by addition of 44.66 g of finely ground nickel sulphate powder to 1 kg of clean dry sand to produce a 10,000 mg/kg Ni content preparation. This preparation was blended with 9 parts of

potting mix to produce the first pot trial substrate containing 1000 mg/kg, of which a proportion sufficient for plant trials was retained. The remainder of this amendment was sequentially diluted with potting mix at a rate of 1:2 and subsampled to produce single-element substrate amendments with concentrations of 333, 111, 37, 12, and 4 mg/kg nickel.

Cobalt amendments (Co), using 44.8 gm of finely ground cobalt sulphate powder, were prepared using the same method outlined above.

The nickel and cobalt (NiCo) mixed substrate was produced by blending 1 part of 1000 mg/kg nickel substrate with 1 part of 1000 mg/kg cobalt substrate resulting in a 500 mg/kg mixed Ni/Co substrate. Blending and subsequent dilution using potting mix, of this initial 500 mg/kg mixture, produced 333, 111, 37, 12 and 4 mg/kg mixed Ni/Co substrates

Six-week-old seedlings of *Berkheya coddii* and *Alyssum bertolonii* were transplanted into 100 gm pots for this trial. For each of the 5 different amendments, 8 pots of both species were planted as well as 8 control pots of each species in unamended potting mix.

The plants were then left inside the Plant Growth Unit on an inclined bench and regularly rotated (to randomise local lighting and heating inside the glasshouse) for a growth period of 10 to 12 weeks.

1.9.2 Artificial Gold Trials.

The gold-bearing substrate was produced by slowly dripping 150 mL of 1000 mg/kg gold chloride solution into 1 kg of clean ground silica sand. This preparation was then oven dried at 105° C and reground to normalise grain size and blended with 29 kg of river sand and Osmocote to produce a 5 mg/kg artificial gold substrate.

Six-week-old seedlings of *Berkheya coddii* and 6 month-old-specimens of *Iberis intermedia* were transplanted into 100 gm pots for this trial. For each of the chelate treatments, 8 pots of each species were planted as well as 8 control pots in unamended sand. The pots were left for a growth period of 10 weeks on an inclined bench and periodically rotated (to randomise local lighting and heating inside the glasshouse) before the chelate amendments were applied for this experiment.

1.9.3 pH Determination.

All of the pH determinations were carried out using a 10 gm sample and 25 mL of deionised water. The treatments were stirred vigorously using a homogeniser and left to stand overnight. The pH was recorded without prior stirring.

1.9.4 Biomass Analysis

All biomass samples were air-dried and the dry weights recorded. The dried samples were then placed into boro-silicate test tubes and ashed overnight at 540° C or until the sample was fully combusted in a muffle furnace. One gram of the resulting ash was accurately weighed into a tube and digested in near boiling 2M HCl, agitated and analysed by flame atomic absorption spectroscopy (FAAS).

1.9.5 Ore Quality Determination.

The Anaconda Nickel Limited and Boddington Gold Mine laterite samples were oven dried at 80° C overnight and crushed to <5 mm in a jaw crusher. Subsamples were crushed to powder by hand for total metal, solvent extraction and chelator potential experiments. All metal determinations were performed by Atomic Adsorption Spectroscopy (AAS). High concentrations were determined by FAAS and low concentrations were determined by GFAAS.

1.9.6 Total metal determination.

A 1 g sample of the hand-ground ore was digested over a water bath in 10 mL of a 1:1 mixture of hydrofluoric and nitric acids until a volume of 2-3 mL was achieved. Additional acid was used where necessary. The sample was then transferred to a hotplate and made up to 10 mL of hot 2M hydrochloric acid for analysis. Nickel and cobalt concentrations were then determined by FAAS.

Gold determination was performed by using a methylisobutylketone (MIBK extraction, after Brooks and Naidu (1985). 1.5 mL of MIBK and 1 mL of HCl were placed into a 5 mL subsample of the 10 mL acid digestion. This solution was agitated for 5 minutes and the MIBK pipetted off for analysis using GRAAS.

1.9.7 Bioavailable metal determination.

Total bioavailable metal was determined by end-over-end agitation of a 1 g sample of finely ground ore in 10 mL of 1M ammonium acetate buffered to neutral pH. The solution was then vacuum filtered using 42 grade filter paper prior to analysis using both FAAS and GFAAS. The gold samples were MIBK extracted as outline above in the section on total metal determination.

1.9.8 Chelator potential metal determination.

A 1 g sample of the hand-ground ore was agitated end-over-end with 10 mL of 1% solutions of ammonium thiosulphate and ammonium thiocyanate for 24 hrs. The solutions were vacuum filtered using 42-grade paper prior to analysis. The nickel and cobalt analyses were determined directly using FAAS. The gold analyses were determined using MIBK extracts as outlined above in the section on total metal determination and analysed using GFAAS.

1.9.9 Artificial Induced Gold Phytoextraction Trials.

The artificial gold substrate phytoextraction trails were carried out using 100 g pots. The plants used were *Berkheya coddii* and *Iberis intermedia* seedlings. The plants were then left inside the Plant Growth Unit on an inclined bench and periodically rotated (to randomise local lighting and heating inside the glasshouse) for a growth period of 10 weeks prior to applying the chelator amendments. The chelator amendment concentrations were 0.25, 0.5 and 1% solutions of either ammonium thiocyanate or ammonium thiosulphate. The plants were then left for another 2 weeks before harvesting and analysed, as outlined above in the section on Biomass Analysis.

1.9.10 Laterite Induced Gold Phytoextraction Trial.

The laterite ore trails were carried out using 100 g pots and 500 g pots. The 100 g pots were used to trial *Berkheya coddii*, *Alyssum bertolonii*, *Brassica juncea* and *Linum usitatissimum* seedlings. The 500 g pots were used to trial the *Acacia longiflora* because this species was obtained in larger root trainer pots from a commercial nursery. To promote rapid plant growth and root development, the laterite ores were blended with one third (by weight) of finely sieved pumice.

The plants were then left inside the Plant Growth Unit on an inclined bench and periodically rotated (to randomise local lighting and heating inside the glasshouse) for a growth period of 10 weeks prior to applying the chelator amendments. the Pretreatment experiment used substrate amendments of 10 mL 2M HCl (T1), a 1% solution of ammonium thiocyanate (T2) and a combination of both a 1% solution of ammonium thiocyanate and 10 mL 2M HCl (T3). The full trial experiment used amendments of a 1% ammonium thiocyanate solution (T1), a 1% solution of ammonium thiocyanate with 10 mL 2M HCl (T2) and with 20 mL 2M HCl (T3).

Chapter Two - Nickel and Cobalt Phytoextraction using Artificial Substrates

2.1 Introduction

Alyssum species contain by far the greatest number of individual nickel hyperaccumulating plants in any one genus. Some 48 members of section Odontarrhena (formerly described as a separate genus) have this hyperaccumulating ability. All of the plants are confined to a belt of ultramafic (serpentine) rocks stretching along Southern Europe from Portugal to Eastern Turkey. Anatolia is the centre of maximum multiplicity and diversity within the genus. The degree of endemism is also very high as some taxa are confined to outcrops of only a few hectares in area (Brooks *et al.* 1979). It has been proposed by Brooks *et al.* (1979) that the greater the nickel concentration in *Alyssum* species, the narrower their distribution range. For example, *A. troodii* (1.71% Ni) is confined to a small area in Cyprus, whereas *A. alpestre* (0.45% Ni) is found throughout the Eastern Mediterranean. There appears to be a relationship between high concentrations of nickel on the one hand, and a high degree of diversity, proliferation, and endemism on the other.

Hyperaccumulation of nickel appears to be a strategy whereby genera such as *Alyssum* have been able to evolve a physiological tolerance to phytotoxic nickel-rich soils, and avoid competition from other species by flourishing in environments so hostile, that often *Alyssum* is the only coloniser of the area (Brooks, 1998).

Another genus with a large number of hyperaccumulators of nickel is *Thlaspi*; a genus that seems to occupy the same ecological niches in Southern Europe as does *Alyssum* (Brooks, 1998). Unlike the latter however, *Thlaspi* is much more widespread in Central and Western Europe, and has poor representation in Anatolia. Reeves and Brooks (1983b) found 12 species of *Thlaspi* with hyperaccumulator status.

New Caledonia, along with Cuba, has perhaps the world's greatest concentration and diversity of nickel hyperaccumulator plants. About 50 hyperaccumulators from 14 genera and eight different families have been reported from this isolated Pacific island. These nickel plants belong to the following genera and families: *Agatea* (Violaceae), *Argophyllum* (Escalloniaceae), *Casearia* (Flacourtiaceae), *Hybanthus* (Violaceae), *Cleidion* (Euphorbiaceae), *Geissois* (Flacourtiaceae), *Oncontheca* (Oconthecaceae), *Homalium* (Flacourtiaceae), *Lasiochlamys* (Flacourtiaceae), *Pancheria* (Cunoniaceae), *Phyllanthus* (Euphorbiaceae), *Psychotria* (Rubiaceae), *Sebertia* (Sapotaceae), and *Xylosma* (Flacourtiaceae) (Brooks, 1998). One of the most striking hyperaccumulators is *Sebertia acuminata* which possesses a vivid blue-green sap with over 11% nickel on a wet-weight basis (Jaffré *et al.*, 1976), see Figure 2.

Until recently, there had been no record of nickel-hyperaccumulating plants from South America. However, Brooks *et al.* (1990) have identified 12 hyperaccumulators from ultramafic soils in Goiás State, Brazil. Among them was *Jatropha* sp. (Euphorbiaceae) with a creamy latex containing 1.35% nickel on a dry weight basis. This plant appears to have a similar nickel storage mechanism to *Sebertia acuminata* from New Caledonia. Reeves *et al.* (1997) discovered a further 128 nickel plants in Cuba, bringing the total number of known nickel-hyperaccumulating plants to well over 300.

The first hyperaccumulator of cobalt to be discovered was *Crotalaria cobalticola* by Duvigneaud (1959), who reported 345 mg/kg cobalt in this taxon. Later, Brooks *et al.* (1980) reported higher values of 970-3010 mg/kg in this plant. *Crotalaria cobalticola* (Figure 3) is only one of many hyperaccumulators of cobalt in this genus (Brooks *et al.* (1977b).

All known cobalt hyperaccumulators are found in the Shaban Copper Arc in Zaïre, Central Africa. Most cobalt hyperaccumulators are confined to the Lamiaceae and Crassulaceae families. The discovery (Duvigneaud and Denaeyer de Smet, 1963) of cobalt and copper hyperaccumulators added *Haumaniastrum robertii* (10200 mg/kg cobalt) and its near relative *H. katangense* (2240 mg/kg cobalt), the 'copper flowers', to this group of plants.

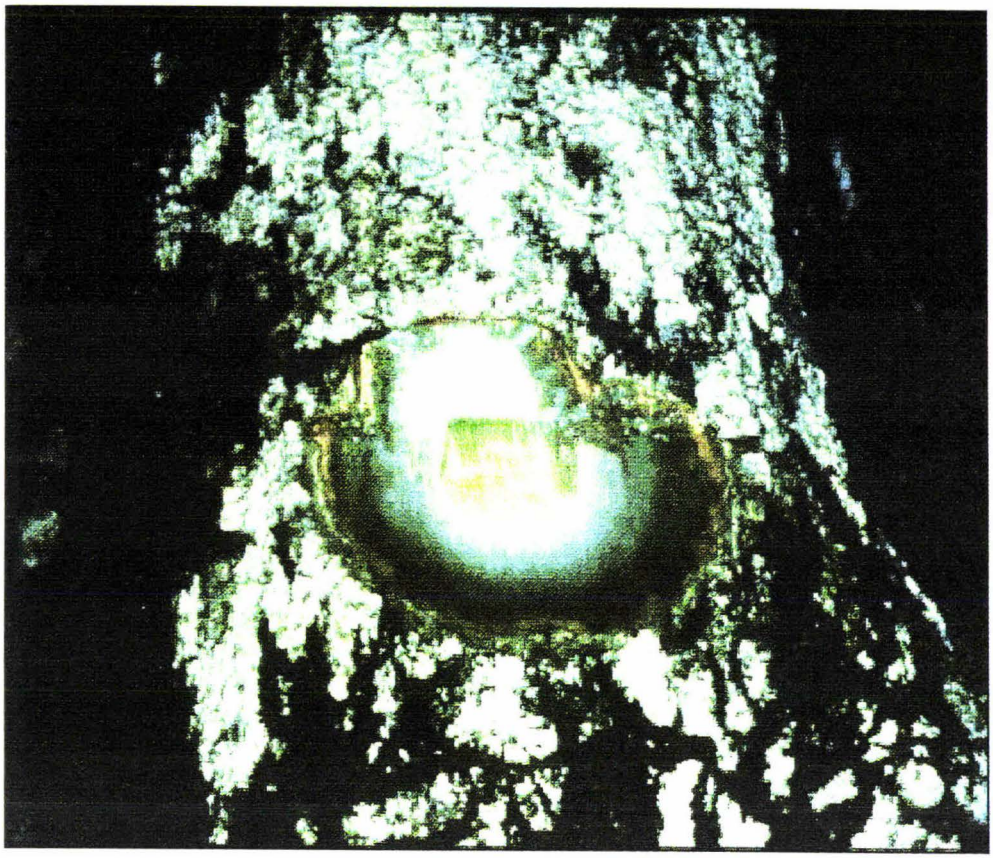

Figure 2. Trunk of *Sebertia acuminata*. Bark has been removed to reveal this species' vivid blue-green high-nickel-content sap. Photo by T. Jaffré.

On mineralised soils, normal plant concentrations of cobalt are between 0.05 and 5 mg/kg. Since this element is not normally considered essential to plant growth, its concentration in plants tends to be externally controlled and reflects the cobalt content of the substrate (Brooks, 1983). Phytotoxicity of cobalt in non-accumulating plants usually results in chlorosis, stunting and reduced root growth and possibly necrosis (Homer, 1991).

Cobalt hyperaccumulators are far more rare than hyperaccumulators of nickel, or for that matter, many other metals.

Currently there are 26 known hyperaccumulators of cobalt, of these 17 species are cobalt-specific. *Haumaniastrum robertii* is by far the most unusual cobaltophyte, (up to 10200 mg/kg) and is confined to the western limb of the Shaban Copper Arc. What makes this plant so unusual is that it also accumulates up to 0.2% copper in dry material. It should be noted that the closely allied *H. katangense* is

confined to the eastern limb of the Shaban Copper Arc, and although it is tolerant of cupriferous substrates, it does not generally take up significant quantities of this element, except in several localised areas.

Figure 3. The cobalt hyperaccumulator *Crotalaria cobalticola* growing over copper/cobalt-rich soils in Zaïre. Photo by R R Brooks.

A geobotanical association and evolutionary path with cobalt mineralisation may explain the lack of cobalt hyperaccumulators outside the Shaban Copper Arc (Brooks, 1998). Whereas most surface expressions of cobalt mineralisation cover only a few hectares, the Shaban Copper Arc contains more than 100 mineralised areas ranging in size up to several km^2 dispersed over 22000 km^2. This has afforded a relatively large area for the evolution of endemic taxa capable of accumulating, or tolerating, high levels of heavy metals in the soil. The multiplicity and diversity of these endemic species has been further assisted by evolution during several geological periods with diverse climates. Diversity of soil type inducing a diverse flora, has served to enlarge the number of endemic taxa in this large metallogenic province (Brooks *et al.* 1980).

2.2 Aims of the experiment

The work carried out by previous researchers here at Massey University and overseas has indicated that both *Alyssum bertolonii* and *Berkheya coddii* have the potential to become valuable species for a commercial phytoextraction operation. Nickel hyperaccumulation is well known in both species, but little research into cobalt has been carried out on either species. A review of cobalt and copper accumulating species by Brooks *et al.* (1980) reported that virtually all known cobaltophytes were indigenous to Central Africa.

I was interested in the cobalt uptake of these species and the relationships between nickel and cobalt hyperaccumulation on mixed substrates as nickeliferous laterites often carry appreciable cobalt mineralisation. It is often the early recovery of cobalt that proves whether an operation is economically feasible or not. The nickel concentration of the laterite determines whether it is classified as ore or waste, with little preliminary attention being given to secondary cobalt mineralisation. Once an inferred nickel resource of a mineable size is delineated to the point where resource definition and mine design procedures are required, the economic impact of cobalt recovery is calculated to optimise dept repayments incurred during mine development.

This study focuses on the potential of both plants to nickel and cobalt accumulation from either a single element or from a 1:1 nickel-cobalt mixed substrate. The accumulation responses observed are then used to evaluate the suitability of both species for polymetallic phytoextraction in the field.

The aims of these experiments were;

To determine the quantitative characteristics of nickel and cobalt hyperaccumulation of *Alyssum bertolonii* and *Berkheya coddii* using artificial substrates amended with nickel and cobalt sulphates.

To identify any interactions between nickel and cobalt accumulation under single and mixed element conditions.

To produce a nickel hyperaccumulation curve for *Alyssum bertolonii* and cobalt accumulation curves for both species.

To determine the effects of increasing substrate metal concentrations on biomass production.

2.3 Phytoextraction of Nickel and Cobalt by *Berkheya coddii* from Artificial Metalliferous Substrates

Table 3 shows the plant accumulation response to different concentrations of substrate metal in single element and mixed substrates.

Substrate concentrations of 500 mg/kg were not trialled for the nickel only, and cobalt only, pot trials, because the loose logarithmic scale of substrate concentrations was deemed sufficient to investigate the plant's hyperaccumulation potential with increasing nickel and cobalt concentrations.

The 1000 mg/kg treatment was not trialled for the mixed (nickel and cobalt) substrate because cobalt concentrations in mineralised laterites would not support a similar level of ammonium-acetate-extractability. Ammonium acetate extraction can be used to determine the bioavailable metal fraction in a substrate. The concentration of ammonium acetate extractable cobalt determined in Chapter Three for auriferous laterites from Western Australia was considerably less than for a 500 mg/kg artificial substrate extraction. The interactions, if any, between nickel and cobalt hyperaccumulation would therefore best be determined under the 500 mg/kg metal level for correlation to a natural setting.

Table 3. Average nickel and cobalt accumulation (n=8), including standard error of the mean. *Berkheya coddii* grown on artificial substrates.

Substrate	Concentration in plant (mg/kg dry matter)						
(mg/kg)	4	12	37	111	333	500	1000
Ni-only	17 ± 7	57 ± 36	231 ± 216	658 ± 335	1674 ± 676	NT	3569 ± 1267
Co-only	20 ± 16	66 ± 23	475 ± 192	925 ± 217	1779 ± 746	NT	3034 ± 539
Ni-mix	46 ± 50	49 ± 20	83 ± 21	250 ± 101	319 ± 49	2284± 1828	NT
Co-mix	11 ± 9	65 ± 23	344 ± 53	820 ± 296	1843 ± 1002	1393 ± 1103	NT

NT = not tested.

The nickel-only accumulation response is consistent with previous published glasshouse data for these substrate concentrations.

The cobalt-only response is what should be expected from a hyperaccumulating plant and the trend of these data is similar to that of the nickel-only response data. The capacity for cobalt accumulation in *Berkheya coddii* from a single element substrate appears to be greater than its ability to hyperaccumulate nickel up to concentration to 333 mg/kg.

Nickel accumulation from the nickel-cobalt mixed substrate indicates a decrease in net accumulation of this metal in the presence of cobalt at concentrations below 333 mg/kg.

The cobalt accumulation for the same mixed substrate indicates a uniform accumulation up to a substrate level of approximately 350 mg/kg. Above this level the net accumulation of cobalt decreases. This may indicate a hyperaccumulation maximum for cobalt and/or a phytotoxic effect on the plant of such high levels of cobalt in the substrate. The plants grown on high cobalt content substrates (< 333 mg/kg) were noted to be stunted, yellowed and brittle to the touch at harvest time indicating plant toxicity at these concentrations. This may indicate an interference relationship between nickel and cobalt at high substrate concentrations. This interference relationship occurs where cobalt is either preferentially accumulated to the detriment of nickel uptake or perhaps is a result of cobalt phytotoxicity interfering with the nickel uptake pathways in *Berkheya coddii*.

2.3.1 Nickel accumulation in *Berkheya coddii* vs ammonium acetate extractable nickel from a nickel-only substrate

The nickel-only hyperaccumulation curve (Figure 4) indicates a steady increase in plant nickel concentrations with increasing substrate nickel contents.

Flattening of the hyperaccumulation curve towards higher concentrations may indicate the approach of a nickel hyperaccumulation rate maximum or indication of phytotoxicity. The hyperaccumulation maximum occurs where the level of accumulation nears the maximum allowable for metal uptake by the species. Any further increase in substrate metal concentration will not increase the level of

accumulation in the plant and may result in reduced plant growth from metal toxicity.

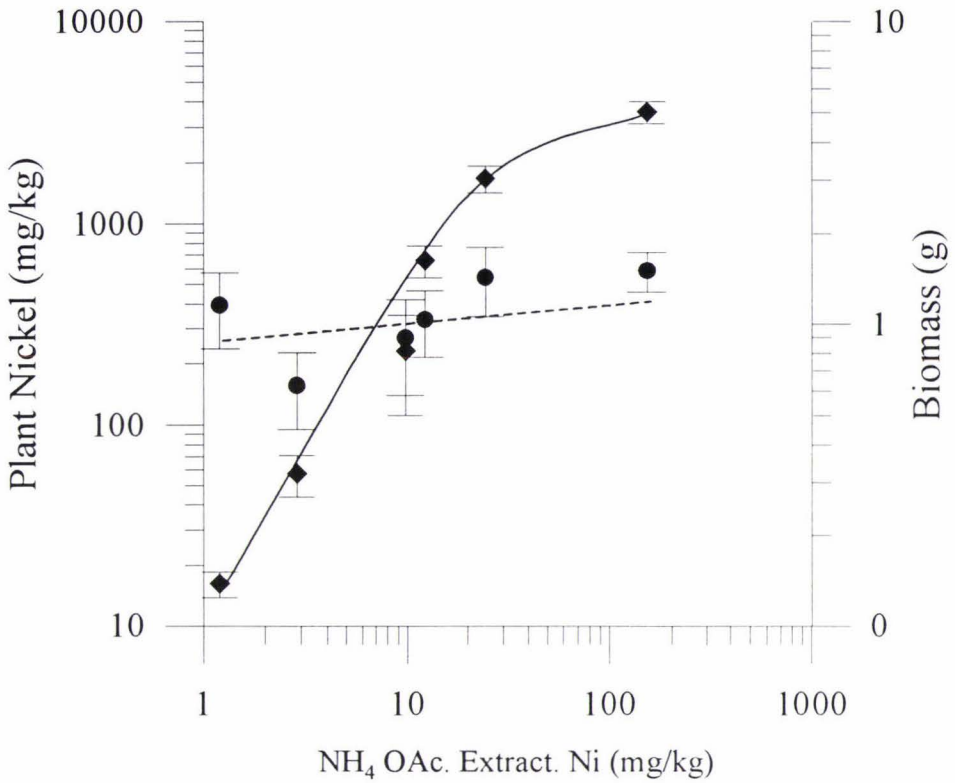

Figure 4. Nickel accumulation (solid line, diamond symbol) and biomass (dashed line, circle symbol) production for *Berkheya coddii* (n=8) vs ammonium acetate extractable nickel from an artificial nickel-only substrate

These data indicate that an ammonium-acetate-extractable substrate concentration of approximately 10 mg/kg nickel is necessary for hyperaccumulation of nickel to 1000 mg/kg. Therefore if the minimum plant-nickel concentration for a viable phytoextraction operation was 1000 mg/kg, the substrate must have a minimum ammonium-acetate-extractable nickel concentration of 10 mg/kg. This represents a nickel accumulation coefficient in *Berkheya coddii* of 100 for a nickel only substrate.

2.3.2 The relationship between a nickel-only substrate and biomass production in *Berkheya coddii*

Biomass production is relatively unaffected by increasing substrate nickel concentrations over the experimental range investigated. In fact, the slight increase in biomass production to a substrate concentration of 300 mg/kg may indicate a

physiological preference for nickel levels in this range. However, no phytotoxic response to substrate nickel concentrations similar to those used in this experiment have been reported in previous research. All plants appeared healthy and vigorous at harvest time with no noticeable symptoms associated with metal toxicity.

2.3.3 Cobalt accumulation in *Berkheya coddii* vs ammonium-acetate-extractable cobalt from a cobalt-only substrate

The cobalt-only hyperaccumulation curve above (Figure 5) identifies a steady increase in plant cobalt concentrations with increasing level in the substrate. Cobalt accumulation appears to be constant over the experimental range indicating that *Berkheya coddii* will readily accumulate this metal. The trend of the curve shows no evidence of a hyperaccumulation maximum, however experimentation at higher substrate concentrations should be carried out to determine this parameter.

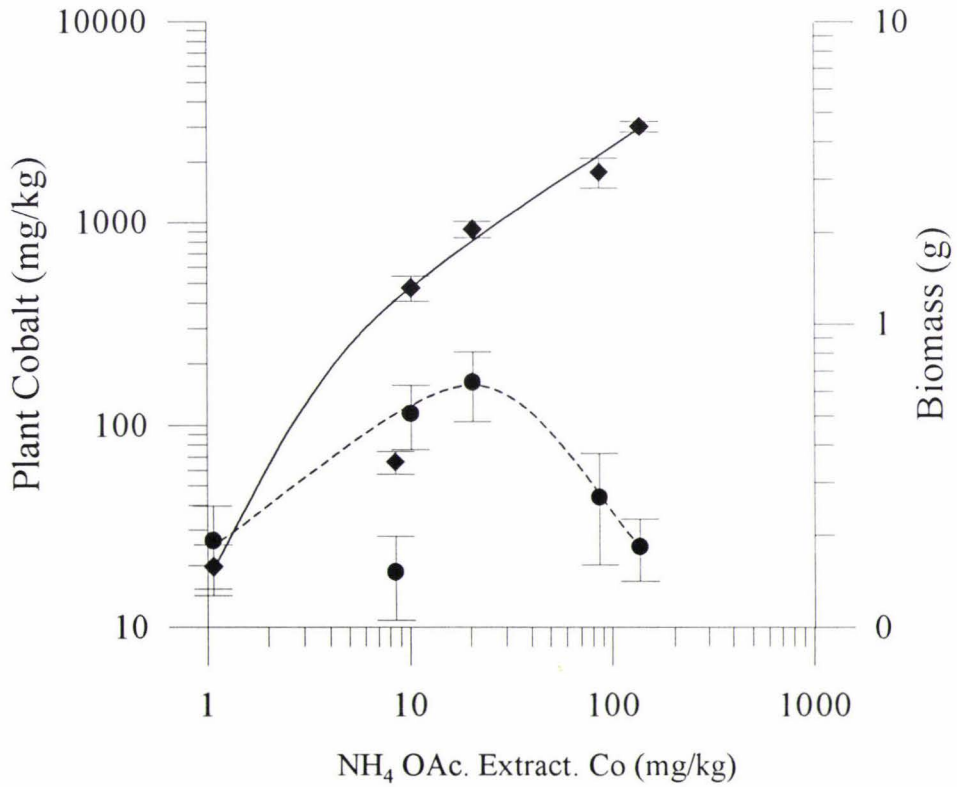

Figure 5. Cobalt accumulation (solid line, diamond symbol) and biomass production (dashed line, circle symbol) vs ammonium-acetate-extractable cobalt in a cobalt-only substrate.

The data indicate that a substrate concentration of approximately 20 mg/kg of ammonium acetate extractable cobalt is required before *Berkheya coddii* will accumulate the 1000 mg/kg cobalt that defines a cobalt hyperaccumulator. This represents a cobalt accumulation coefficient in *Berkheya coddii* of 50 for a cobalt-only substrate.

2.3.4 Biomass production of *Berkheya coddii* on cobalt-only substrate

Biomass production appears to favour substrate cobalt concentrations below 30 mg/kg. Above this level biomass production decrease considerably indicating adverse plant growth at these higher substrate cobalt concentrations.

It should be noted that at harvest time, a number of plants exhibited growth deformities that can only be attributed to cobalt phytotoxicity. These plants showed pronounced yellowing of plant and leaf stems and leaf axial regions, frequently accompanied on foliage by white spotting. The plants themselves were stunted with short inter-nodal stems and brittle foliage that broke when grasped firmly, lacking the characteristic suppleness of a healthy specimen. The root systems of these plants were shallow and poorly developed by comparison with a healthy nickel-only plant. This would suggest that a substrate concentration of 30-40 mg/kg cobalt would begin to have a detrimental effect of plant growth without necessarily affecting the concentration of plant cobalt. If this is true, then accumulation of cobalt in *Berkheya coddii* must be an involuntary process related to cobalt bioavailability in the substrate rather than a plant-initiated response to, or need for, cobalt. The phytotoxic effect of cobalt in a mixed substrate (see section 1.3.5) appears to occur at a lower substrate cobalt concentration than in this single element substrate.

2.3.5 Nickel accumulation in *Berkheya coddii* vs ammonium-acetate-extractable nickel from a mixed nickel-cobalt substrate

The nickel and cobalt hyperaccumulation curves below (Figure 6 and 7) indicate that both metals are accumulated to concentrations above the hyperaccumulation threshold of 1000 mg/kg.

The nickel hyperaccumulation curve indicates a steady increase in plant metal uptake relative to substrate concentrations.

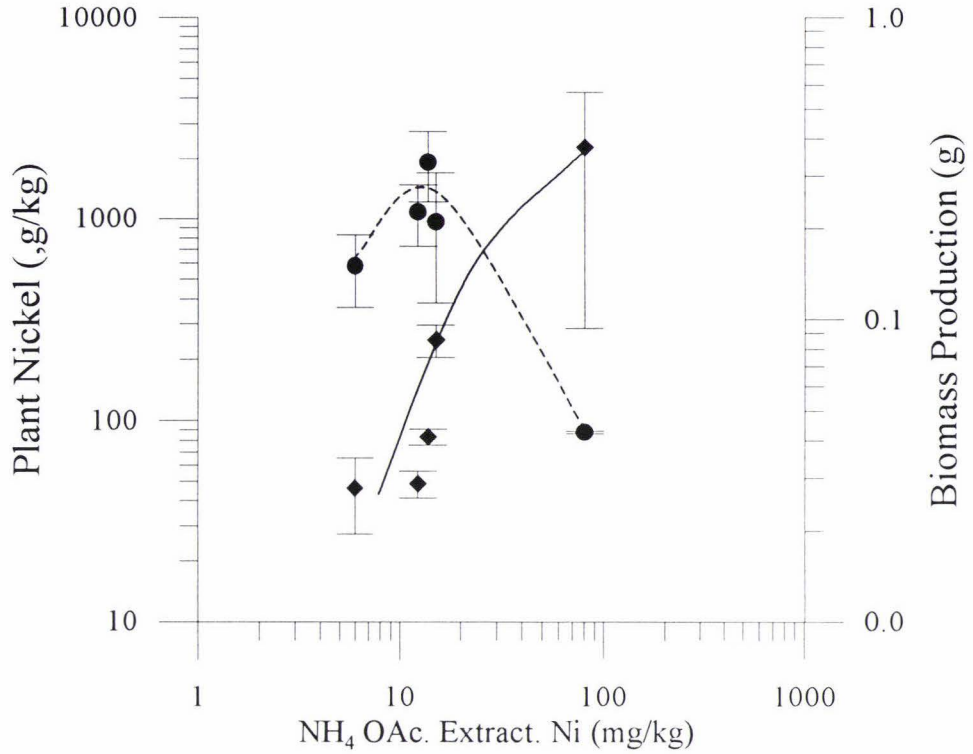

Figure 6. Nickel accumulation response (solid line, diamond symbol) and biomass production (dashed line, circle symbol) vs ammonium-acetate-extractable nickel from a nickel-cobalt mixed substrate.

Nickel accumulation is less efficient here compared to the accumulation by *Berkheya coddii* on a nickel-only substrate. This mixed substrate requires a 70 mg/kg substrate metal concentration to produce a 1000 mg/kg nickel-in-plant response compared to the 10 mg/kg substrate metal required in the nickel-only trial. This is a drop in the accumulation coefficient from 100 to 14. This can only be attributed to the presence of cobalt at equal concentrations. Clearly cobalt is reducing nickel accumulation by either being accumulated itself and affecting the net metal accumulation by the plant or by limiting nickel accumulation via substitution of nickel for cobalt as the cation of preference.

The cobalt hyperaccumulation curve indicates a rapid uptake of cobalt as a function of substrate concentrations to an approximate substrate concentration of 20 mg/kg. Above this value, cobalt uptake decreases rapidly to a constant level indicating that cobalt phytotoxicity or metal saturation is occurring. This may suggest a hyperaccumulation uptake maximum by *Berkheya coddii* where

bioavailable cobalt surpasses the plant's ability to absorb it. Above this concentration the plant appears to either: A) exclude any further increase in cobalt uptake as the substrate concentration is raised or B) the plant's hyperaccumulation system may break down thereby inhibiting or decreasing cobalt uptake. The data also suggests that this may allow for increased nickel uptake however, to confirm this would require further investigation at higher substrate concentrations. The required substrate metal concentrations for *Berkheya coddii* to achieve 1000 mg/kg cobalt is approximately 45 mg/kg, a biological adsorption coefficient (b.a.c.) of 22 compared with 50 observed in the cobalt-only trial.

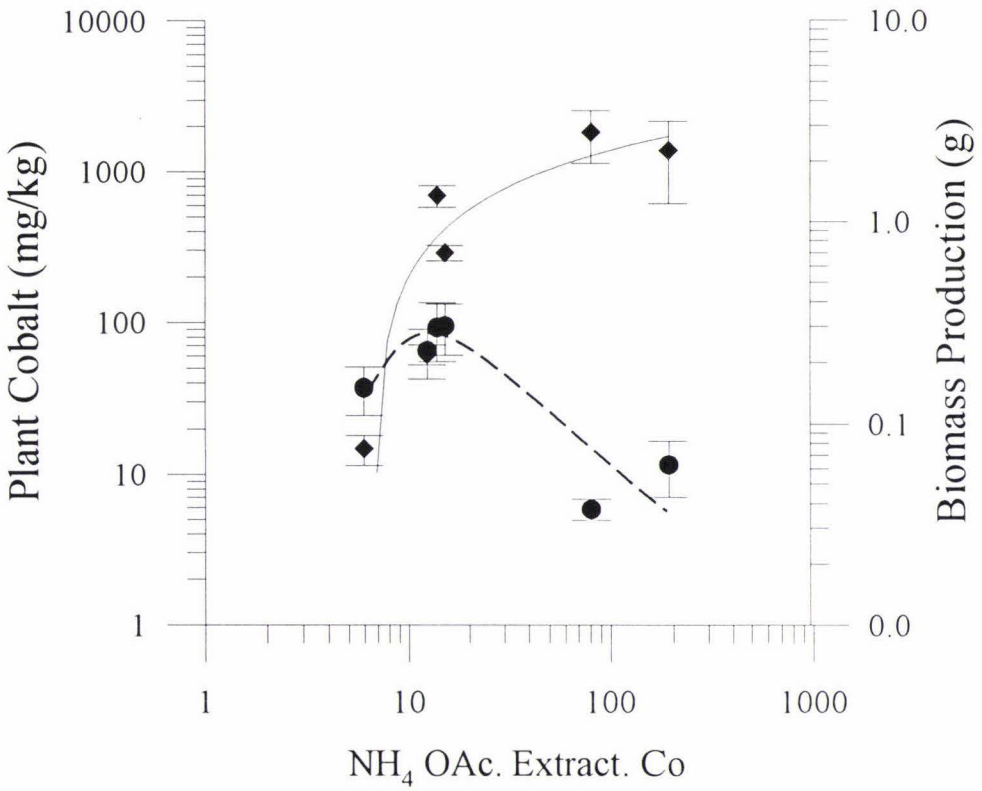

Figure 7. Cobalt accumulation (solid line, diamond symbol) and biomass production (dashed line, circle symbol) vs ammonium-acetate-extractable cobalt from a nickel-cobalt mixed substrate.

A comparison of accumulation results from these plant trials at the 10 mg/kg substrate concentration highlights the impact of cobalt on nickel accumulation and biomass production. If we assume that the nickel-only trial represents an optimal accumulation scenario for *Berkheya coddii* under glasshouse conditions. The nickel-only trial produced accumulation of 1000 mg/kg (b.a.c.=100) and a biomass production of 1 g per plant at the 10 mg/kg substrate concentration. The cobalt-only trial produced accumulation of 800 mg/kg (b.a.c.=80) and biomass production of 0.5

g per plant. The mixed substrate trial produced accumulations of 150 mg/kg (b.a.c.=15) nickel and 200 mg/kg (b.a.c.=20) cobalt with a biomass production 0.3 g per plant. The impact of equal parts of substrate nickel and cobalt on metal accumulation and biomass production in considerable. The cobalt-only data indicate an accumulation efficiency of 0.8 and a biomass production of 50% relative to a nickel-only trial. The mixed substrate data show accumulation efficiencies of 15 and 20 for nickel and cobalt respectively and a biomass production of 0.3 compared to the nickel-only trial.

The general nickel accumulation response under these conditions is poor by comparison with the nickel-only analyses. The plant/soil quotient of accumulated nickel for the two plant trials increases with increasing substrate concentration. At a substrate concentration of 37 mg/mg, the nickel-only response was 231 mg/kg and the nickel-mixed response was 83 mg/kg, a difference of a factor of 2.7. The substrate concentration of 111 mg/kg produced accumulations of 658 mg/kg in the nickel-only trail and 250 mg/kg in the nickel-mixed trail, a difference by a factor of 5.6. At a substrate concentration of 333 mg/kg, the nickel-only accumulation was 1674 mg/kg compared with 319 mg/kg in the mixed substrate, a difference of a factor of 5.2.

The above demonstrates that cobalt has a negative interaction upon biomass production and nickel accumulation in *Berkheya coddii* over the substrate concentration range of 0-333 mg/kg. The data also indicates a lesser negative interaction between substrate nickel concentrations and cobalt accumulation. No corresponding effect on biomass production was observed.

A decrease in the net difference in accumulation quotients at high concentrations may indicate a return to nickel-dominant hyperaccumulation and cobalt toxicity at these substrate concentrations. The 1000 mg/kg single element substrates indicate that nickel accumulation exceeds cobalt accumulation for the first time in this trial. Nickel and cobalt accumulations from the mixed substrate show nickel accumulation exceeding cobalt accumulation at a substrate concentration of 500 mg/kg. This is first incidence of nickel accumulation dominance for high concentration substrates for the mixed substrate trial. This 'envelope of cobalt dominance' (where cobalt negatively impacts on nickel accumulation) at high

substrate concentrations would need to be proved by additional experimentation in the substrate range 300-2000 mg/kg nickel and cobalt. However, considering the unlikelihood of ever developing a phytoextraction operation on this level of ammonium-acetate-extractable heavy metal contamination in the wild (because this level of metal concentration supports modern mining methods) it has little relevance in evaluation of substrates for a phytoremediation or phytomining operation.

2.3.6 Biomass production of *Berkheya coddii* on nickel-cobalt mixed substrate

Biomass production appears to favour substrate metal concentrations up to 20 mg/kg. Above approximately 30 mg/kg the biomass production decreases indicating cobalt phytotoxicity. The average herbage weight recorded at the grown maximum of 30 mg/kg substrate metal was 0.4 g for a 12 week old plant. Observations made at harvest time, record yellowing of leaves and stems similar to that seen in the cobalt-only trial, yet not as pronounced. These plants were also stunted with brittle foliage and poorly developed root systems similar to the cobalt-only trial. Phytotoxicity at substrate concentrations greater than 110 mg/kg nickel and cobalt resulted in death for half the sample population. The remaining plant weights averaged of <0.1 g. This indicates that cobalt is the dominant factor in plant growth control on a mixed substrate because the nickel-only trial did not appear toxic over the experimental range. This compares favourably with the results of the cobalt-only trial and indicates that a substrate concentration of more than 30 mg/kg cobalt will negatively impact on biomass produce for *Berkheya coddii*. The cobalt-only trial had a growth maximum of 0.7 g per plant at 30 mg/kg substrate cobalt decreasing to 0.2 g per plant above 100 mg/kg substrate cobalt.

Biomass production is affected at ammonium-acetate-extractable substrate cobalt concentrations above 30 mg/kg. The presence of nickel in the mixed substrate decreases the impact of cobalt phytotoxicity on average herbage production in *Berkheya coddii*. However, mean plant weights of biomass production maximums for the three trials show a slight decrease in the presence of cobalt relative to the nickel-only trial.

The ability of *Berkheya coddii* to hyperaccumulate nickel and cobalt from an equal-parts mixed substrate plant growth is seriously affected. Both metals were accumulated to the 1000 mg/kg hyperaccumulation threshold but only at substrate concentrations above biomass production maxima. An average plant weight for nickel and cobalt hyperaccumulation in the mixed trial was little more than 0.1 g per plant. Compare this with 1 g per plant in the nickel-only trial and 0.7 g per plant (the biomass production maximum) in the cobalt only trial. Clearly the plants are struggling to survive in these high concentrations of ammonium-acetate-extractable cobalt. A comparison of accumulation coefficients for *Berkheya coddii* in single and mixed element substrates indicate that the mixed environment exerts a degree of preference or competition in metal uptake reflected by higher single element substrate accumulation levels. Both accumulation coefficients are considerably lower than for single element substrates. In a pure environment with only nickel available for plant uptake the accumulation coefficient was 100 and that of cobalt was 66. This level of accumulation dropped in a mixed medium to 14 for nickel and 22 for cobalt. This negative interaction between nickel and cobalt in mixed media has a considerable impact when determining the potential of *Berkheya coddii* for a phytoextraction programme where the bioavailable cobalt concentrations in the substrate is, at or above, 30 mg/kg in the presence of appreciable bioavailable nickel.

2.4 Phytoextraction of Nickel and Cobalt by *Alyssum bertolonii* from Artificial Metalliferous Substrates

Table 4 shows the average accumulation of nickel and cobalt in *Alyssum bertolonii* for the substrate metal concentrations trialled in this experiment.

Table 4. Average plant hyperaccumulation response to nickel and cobalt.

	Concentration in plant (mg/kg dry matter)						
Substrate	4	12	37	111	333	500	1000
Ni-only	40 ± 35	177 ± 120	735 ± 301	2904 ± 693	5180 ± 945	NT	5809 ± 893
Co-only	31 ± 13	157 ± 116	522 ± 285	1178 ± 548	1328 ± 966	NT	3295 ± 822
Ni-mix	36 ± 12	100 ± 48	117 ± 43	124 ± 81	411 ± 44	534 ± 149	NT
Co-mix	42 ± 18	135 ± 68	693 ± 244	712 ± 508	2116 ± 307	2097 ± 296	NT

NT = not trialed.

The plants' nickel hyperaccumulation trend is consistent with previously published data (Robinson et al., 1998). Accumulation of cobalt in a single element

substrate indicates the characteristic exponential trend associated with nickel hyperaccumulation, albeit at a dramatically lower level. The plants' response when grown on a mixed substrate indicates a preferential uptake of cobalt in the presence of nickel. Cobalt uptake appears to be augmented in the presence of nickel above a substrate concentration of 37 mg/kg. Below 37 mg/kg cobalt accumulation follows a similar accumulation trend as that of cobalt only medium.

Statistical analysis (Table 5) identified a very highly significant difference in nickel accumulation between the single element and mixed metal substrate. There was no significant difference in cobalt accumulation between the cobalt-only and nickel-cobalt mixed substrates. This illustrates well the elemental interference relationship for accumulation of nickel and cobalt by Alyssum bertolonii, where nickel accumulation suffers in the presence of cobalt.

Table 5. ANOVA statistics for *Alyssum bertolonii* hyperaccumulation response under artificial conditions. S** = very highly significant, NS = not significant.

Target Metal	Substrate	Metal in plant (mean)	P Value
Ni	Ni only Ni mix	2163 137	0.000002 S**
Co	Co only Co mix	690 629	0.6790 NS

2.4.1 Nickel uptake by *Alyssum bertolonii* and ammonium-acetate-extractable nickel in a nickel-only substrate

The nickel-only hyperaccumulation curve below (Figure 8) indicates a uniform rate of accumulation at lower substrate concentrations. At higher substrate metal concentrations, hyperaccumulation decrease and may reflect the approach of a hyperaccumulation maximum.

Levelling off of the accumulation curve above a substrate concentration of 30 mg/kg probably reflects the approach of metal saturation with respect to the plant ability to take up nickel. Above this substrate nickel concentration, the plant appears to inhibit further increases in nickel accumulation possibly indicating a maximum rate of nickel accumulation for the species. The data indicates that *Alyssum bertolonii* requires a nickel substrate concentration of approximately 4 mg/kg to reach the defining hyperaccumulation threshold of 1000 mg/kg. This is a hyperaccumulation coefficient of 250 for a nickel-only substrate.

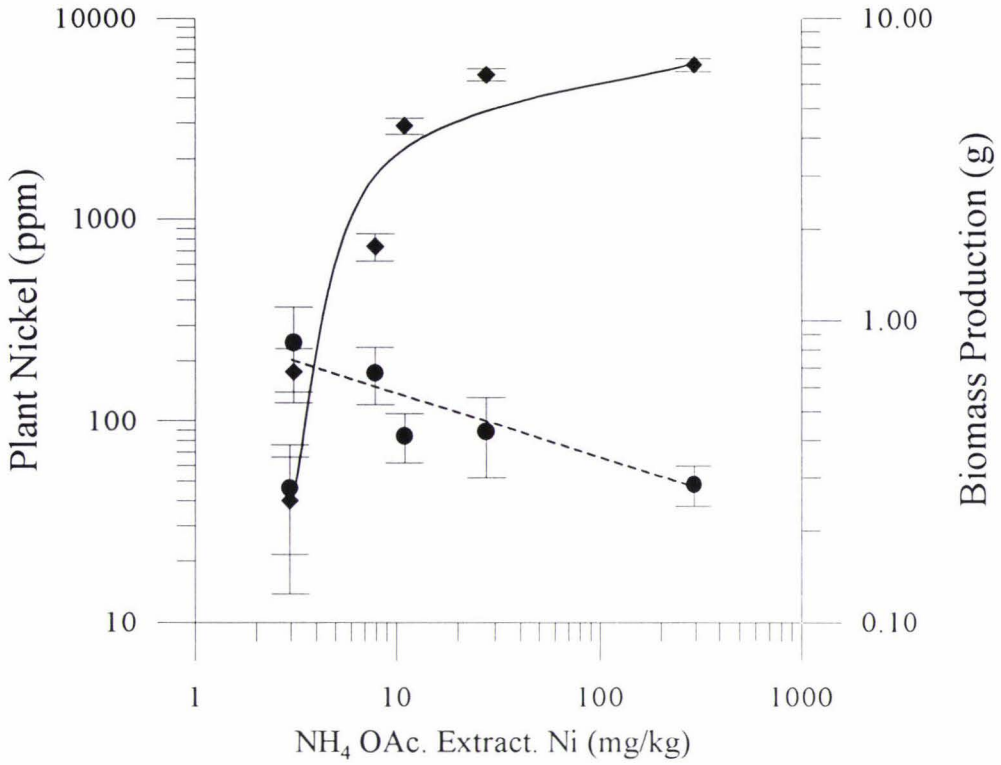

Figure 8. Nickel uptake (solid line, diamond symbol) by *Alyssum bertolonii* and biomass production (dashed line, circle symbol) vs ammonium-acetate-extractable nickel in a nickel-only substrate.

2.4.2 The relationship between a nickel-only substrate and biomass production in *Alyssum bertolonii*

No marked effect of increasing substrate concentrations on biomass production was observed in *Alyssum bertolonii*. The biomass production appears to favour substrate nickel concentrations of less than 30 mg/kg. Consistent with previous research, the range of substrate nickel concentrations used did not result in any noticeable phytotoxicity at harvest time. A substrate concentration of 4 mg/kg produced accumulation to 1000 mg/kg with a resultant biomass production of 0.7 g per plant. A substrate concentration of 300 mg/kg produced accumulation to 5800 mg/kg, however only yielded 0.2 g of biomass per plant. The effect of increasing substrate nickel content appears to simply stunt plant growth in *Alyssum bertolonii* and not affect general plant health. This relates to the level of substrate nickel tolerance that *Alyssum bertolonii* has evolved to withstand. Stunting of normal plant growth is associated with the onset of trace element phytotoxicity. A more

pronounced effect would result in more noticeable symptoms, such as leaf yellowing and spotting.

2.4.3 Cobalt accumulation by *Alyssum bertolonii* and ammonium-acetate-extractable cobalt from a cobalt-only substrate

The cobalt hyperaccumulation (Figure 9) indicates a relatively uniform increase in plant metal uptake to an ammonium-acetate-extractable substrate level of approximately 60 mg/kg. Above this level, accumulation appeared to decrease over the range of 60-250 mg/kg.

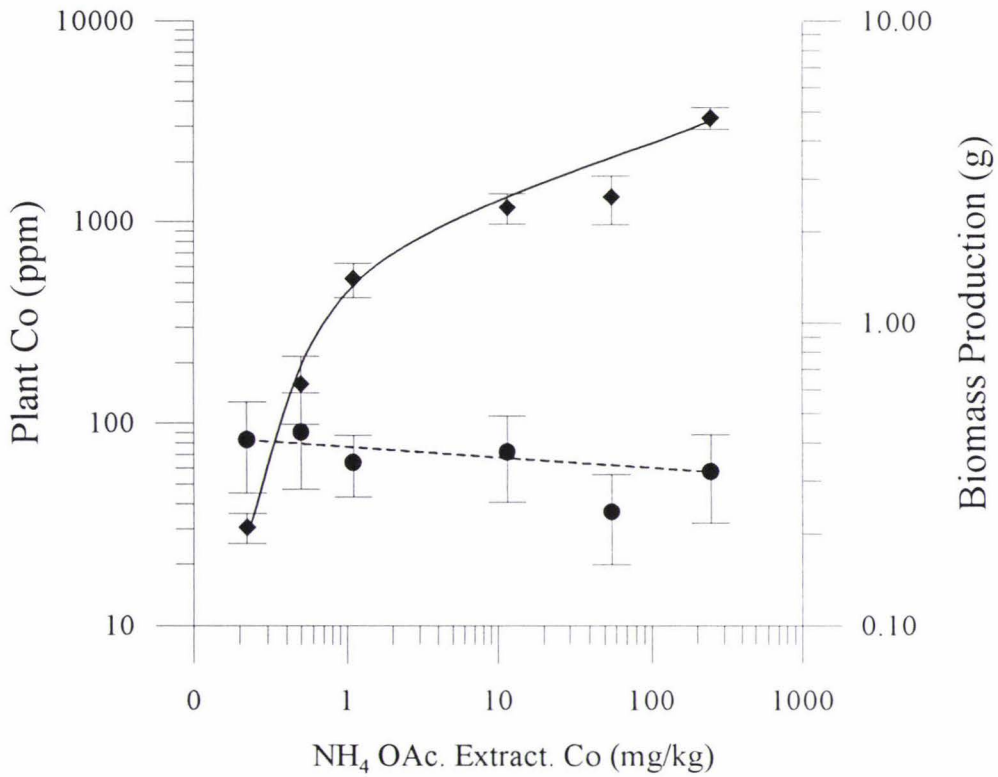

Figure 9. Cobalt accumulation (diamond symbol, solid line) and biomass production (circle symbol, dashed line) vs ammonium-acetate-extractable cobalt from a cobalt-only substrate.

Uptake of cobalt above an ammonium-acetate-extractable substrate concentration of 250 mg/kg increased rapidly at a rate similar to initial accumulation rates at lower substrate concentration. This may represent a change in uptake behaviour, however further work is needed to determine this. A substrate concentration of 4 mg/kg cobalt produced accumulation to 1000 mg/kg, a hyperaccumulation coefficient of 250 for a cobalt-only substrate. For correlation between trials, the 10 mg/kg substrate concentration produced cobalt

hyperaccumulation of 1200 mg/kg (an accumulation coefficient of 120) and biomass production of 0.4 g per plant.

2.4.4 The relationship between substrate cobalt content and biomass production in *Alyssum bertolonii*

The biomass production remained constant over the range of metal concentrations investigated in this experiment, indicating that the growth of *Alyssum bertolonii* is unaffected by changing substrate cobalt concentrations. A decrease in leaf size and yellowing in the nickel-only trial was noted above a substrate concentration of 111 mg/kg at harvest time. Biomass production at hyperaccumulation (1000 mg/kg) was 0.4 g per plant. Biomass production observed in the nickel-only trial at hyperaccumulation was 0.7 g per plant. Plant growth is clearly inhibited by the presence of cobalt and/or absence of nickel in the substrate. Plant yields at high substrate concentrations were similar between trials, indicating the onset of phytotoxicity at substrate concentrations above 250 mg/kg. At substrate concentrations above 333 mg/kg, plant mortality rose from zero to 25-40%. Interestingly, the degree of cobalt-accumulation was relatively unaffected by increasing substrate cobalt.

2.4.5 Nickel accumulation in *Alyssum bertolonii* and ammonium-acetate-extractable nickel for a mixed nickel-cobalt substrate

The nickel accumulation curve above indicates a relatively slower rate of nickel accumulation when compared to the hyperaccumulation curve for a nickel-only substrate and represents the effect of cobalt in the system. At a nickel-only substrate concentration of 10 mg/kg nickel hyperaccumulation was 2000 mg/kg. The nickel accumulation under mixed conditions for a 10 mg/kg substrate was 120 mg/kg. In the presence of equal parts nickel and cobalt the accumulation coefficient for nickel uptake by *Alyssum bertolonii* drops from 250 to 12. No substrate metal concentration produced hyperaccumulation of nickel for this mixed substrate trial.

Initial nickel accumulation levels were similar to a nickel-only substrate up to a concentration of approximately 5 mg/kg . Above 5 mg/kg, the rate of accumulation in a mixed substrate decreased through the range 5-300 mg/kg relative to the nickel-only trial. This indicates a suppression of nickel uptake in the presence of cobalt at

these concentrations and a preference for cobalt by the transport ligands operating within the plant's metal uptake pathway.

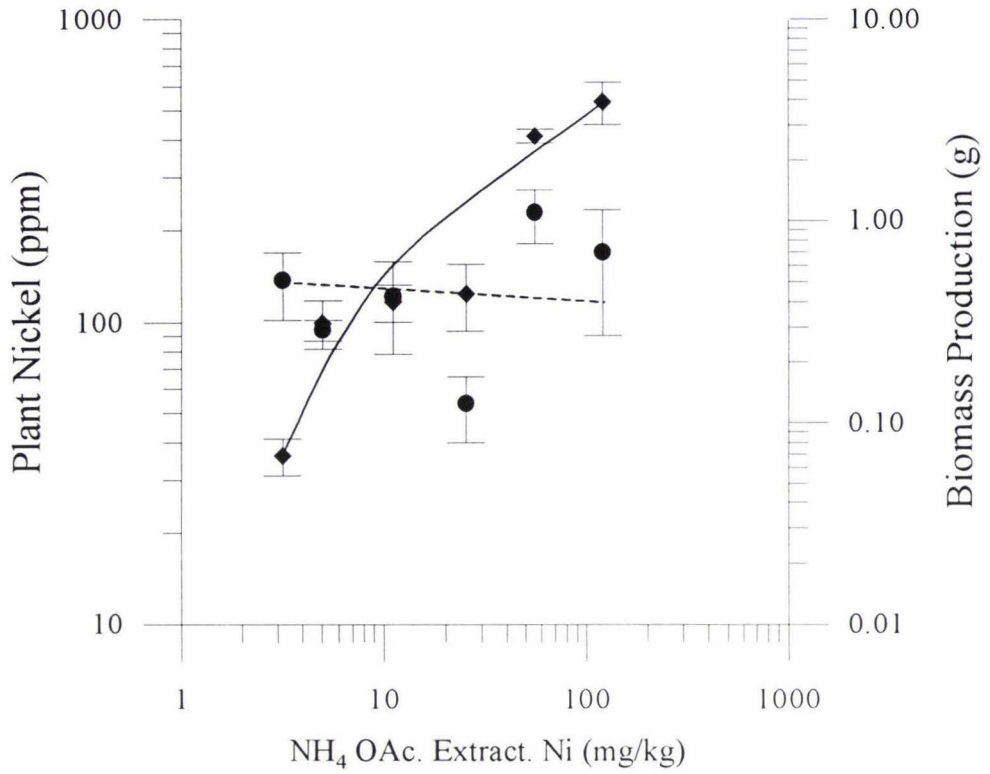

Figure 10. Nickel hyperaccumulation (solid line, diamond symbol) and biomass production (circle symbol, dashed line) vs ammonium-acetate-extractable nickel for a nickel-cobalt mixed substrate.

2.4.6 Cobalt accumulation in *Alyssum bertolonii* and ammonium-acetate-extractable cobalt for a mixed nickel-cobalt substrate

The figure above illustrates the relationship between ammonium-acetate-extractable cobalt from a mixed nickel-cobalt substrate and cobalt hyperaccumulation in *Alyssum bertolonii*. The data indicate a relatively constant rate of cobalt accumulation with increasing substrate cobalt concentration. This is a similar behaviour to the cobalt-only trend. A substrate concentration of 40 mg/kg cobalt produced accumulation to the 1000 mg/kg hyperaccumulation threshold. This represents an hyperaccumulation coefficient of 25 for cobalt from a mixed substrate.

A substrate concentration of 10 mg/kg metal produced accumulation to 700 mg/kg cobalt, 120 mg/kg nickel and a biomass production of 0.7 g for a twelve-week-old plant, indicating a preferential accumulation quotient for cobalt over nickel of 5.8.

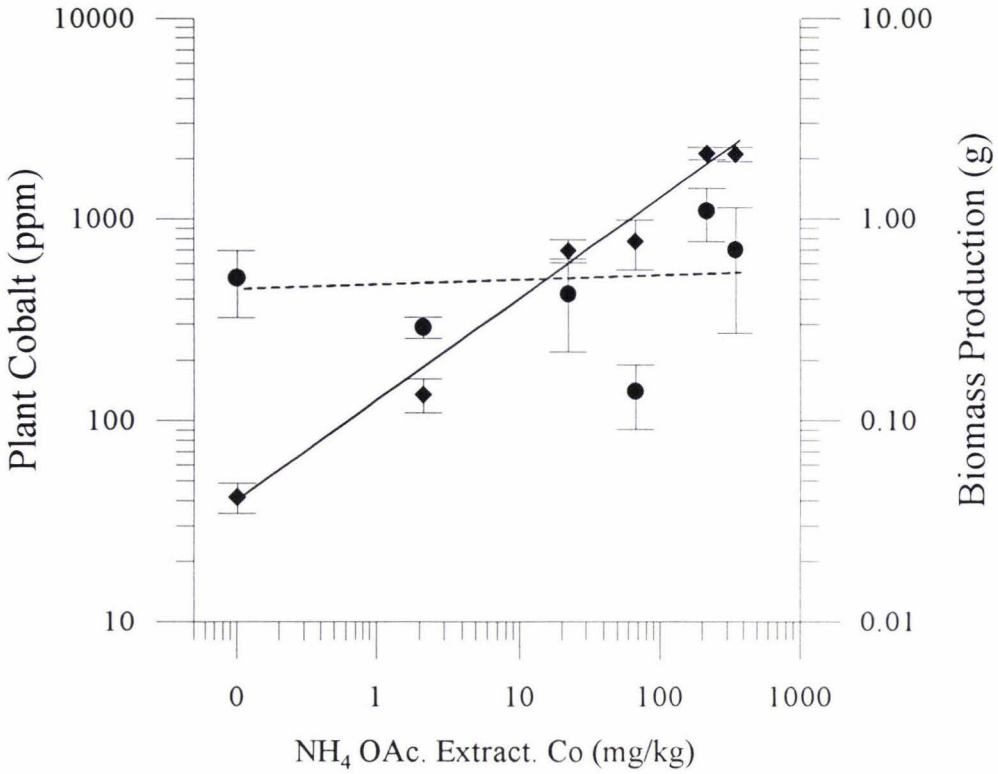

Figure 11. Cobalt hyperaccumulation (solid line, diamond symbol) and biomass production (dashed line, circle symbol) vs ammonium-acetate-extractable cobalt from a nickel-cobalt mixed substrate.

2.4.7 The relationship between substrate cobalt and biomass production in *Alyssum bertolonii*

Over the experimental range, biomass production indicates constant levels of production, of 0.47 g per plant. Cobalt-only biomass production was 0.35 g per plant at a 10 mg/kg substrate concentration. Biomass production for the nickel-only substrates at a bioavailable metal concentration of 10 mg/kg was 0.57 g per plant. Clearly the effect of cobalt in a substrate was to greatly affect the biomass production of *Alyssum bertolonii* at this substrate concentration of ammonium-acetate-extractability. The effect of cobalt also appears to increase with increasing substrate concentration of cobalt. The pronounced drop in biomass production on a mixed substrate shows a similar negative interaction between nickel and cobalt to that of *Berkheya coddii* resulting in phytotoxicity and reduced levels of accumulation.

2.5 Conclusions

Berkheya coddii and *Alyssum bertolonii* accumulated nickel from single-element substrates relative to ammonium-acetate-substrate-extractable nickel at levels similar to previous glasshouse research (Robinson *et al.*, 1997a and 1997b). Hyperaccumulation coefficients in excess of 100 occur for both species in the absence of cobalt.

Accumulation levels at lower concentrations were similar. However, nickel-in-plant concentrations at higher substrate metal levels were considerably lower than those reported here. Substrate cobalt concentrations reported by Robinson *et al.* (1997b) are below the concentrations identified here and deserve re-evaluation of the data in light of cobalt phytotoxicity. No indication of metal toxicity was apparent during this experiment.

The hyperaccumulation potential for ammonium acetate extractable nickel reported in *Alyssum bertolonii* by Robinson (1997a) is considerably higher than observed during this project. This results from other trace element deficiencies in the artificial substrate used, affecting plant health and accumulation potential compared to a serpentine soil from Italy or quite possibly relates to the maturity of plants sampled in Robinson's report. An accumulation coefficient of 900 can be calculated from Robinson (1997) at a substrate concentration of 10 mg/kg ammonium-acetate-extractable nickel compared to only 200 observed in this experiment. There can be no doubt, however, of the potential of *Alyssum bertolonii* to phytoextract nickel from low concentration substrates.

Cobalt accumulation from single element substrates reached hyperaccumulating levels over the experimental range for both species. The cobalt hyperaccumulation coefficient for *Berkheya coddii* was 80 and that of *Alyssum bertolonii* was 250 relative to substrate metal concentrations of 10 mg/kg. The abilities of both species to accumulate cobalt are considerably different. *Berkheya coddii* exhibited pronounced cobalt phytotoxicity above an ammonium-acetate-extractable substrate cobalt level of 30 mg/kg represented by a reduction in biomass

production, stunting and yellowing of foliage and finally necrosis. Cobalt hyperaccumulation (1000 mg/kg) occurred at a substrate concentration approximating optimum biomass production with respect to cobalt phytotoxicity.

Cobalt hyperaccumulation in *Alyssum bertolonii* occurred in an ammonium-acetate-extractable substrate concentration of 4 mg/kg. No discernible phytotoxic effect in *Alyssum bertolonii* became apparent until biomass production rates were compared with the nickel-only substrate, assumed to represent an optimal growth condition. Cobalt lowered biomass production by 50% in an ammonium-acetate-extractable cobalt-only substrate concentration of 10 mg/kg. A small degree of stunting and leaf yellowing was reported at high substrate concentrations. However, the degree of cobalt phytotoxicity displayed was much smaller than that of *Berkheya coddii*.

Accumulation of nickel and cobalt from mixed substrates indicated a general preference for cobalt accumulation and reduction in overall nickel accumulation relative to single element substrates. The accumulation coefficients of both metals for both species decreased appreciably for the mixed substrate.

Hyperaccumulation (>1000 mg/kg) of nickel by *Berkheya coddii* from a mixed substrate increased the substrate concentration required of 10 mg/kg in the nickel-only substrate to 70 mg/kg. Average biomass production for a 10 mg/kg substrate decreased by 90% in the mixed substrate. The accumulation coefficient for nickel hyperaccumulation decreased by 85% in the mixed substrate. The accumulation coefficient for cobalt hyperaccumulation decreased by 90% in the mixed substrate This is possibly associated different chemical bond strengths between nickel and cobalt attached to organo-metallic complexes exerting a thermodynamic preference for absorption across the root membrane.

Biomass production by *Berkheya coddii* from both cobalt-bearing substrates indicated the onset of cobalt phytotoxicity at substrate concentrations in excess of 30 mg/kg. In a cobalt-only substrate, hyperaccumulation was achieved at substrate concentrations above 15 mg/kg. Cobalt hyperaccumulation from a mixed substrate increased to a substrate concentration of 45 mg/kg and occurred within the established cobalt phytotoxicity field above. A maximum biomass production level

of 0.65 g per plant, coinciding with hyperaccumulation, in *Berkheya coddii* was achieved in a cobalt-only substrate concentration of 15 mg/kg. Maximum biomass production of 0.3 g per plant yielded 300 mg/kg cobalt at a mixed substrate concentration of 15 mg/kg. Obviously a substrate concentration of >15 mg/kg cobalt seriously affects plant growth and hence biomass production. Hyperaccumulation in a cobalt-only substrate will occur, but any further increase in substrate concentration will decrease plant growth. The relative level of hyperaccumulation does not change in these plants with increasing substrate concentration, only biomass production and subsequent metal yield. Plant mortality became considerable above substrate concentrations of approximately 150 mg/kg cobalt.

The effect of cobalt on general plant growth and accumulation potential in *Berkheya coddii* is an important find. If we assume that the nickel-only trial is a 'best case scenario' for plant growth and nickel hyperaccumulation the following points can be made:

Nickel phytotoxicity does not occur in *Berkheya coddii* at substrate concentrations below 1000 mg/kg nickel.

Berkheya coddii is capable of hyperaccumulating cobalt, however in the presence of equal parts of nickel hyperaccumulation levels occur at ammonium-acetate-extractable substrate concentrations inside the envelope of cobalt phytotoxicity.

Nickel accumulation decreases significantly in the presence of equal parts of cobalt.

Bioavailable substrate cobalt concentrations above 30 mg/kg had a negative effect on plant growth without necessarily affecting the hyperaccumulation potential of either species.

The effect of cobalt on general plant growth and accumulation potential in *Alyssum bertolonii* is considerably different from that of *Berkheya coddii*, probably reflecting different evolutionary pathways to heavy metal accumulation. If we assume that the nickel-only trial is a 'best case scenario' for plant growth and nickel hyperaccumulation in *Alyssum bertolonii* the following points can be made:

Nickel phytotoxicity in *Alyssum bertolonii* does not occur in the ammonium-acetate-extractable substrate concentrations used in this trial.

Alyssum bertolonii readily hyperaccumulates cobalt in the absence of bioavailable nickel, to the detriment of biomass production.

Nickel accumulation is greatly suppressed in the presence of equal parts of cobalt.

Hyperaccumulation of cobalt in the presence of equal parts of nickel, increased the required substrate concentration tenfold over cobalt-only substrates.

The absence of nickel, and presence of cobalt, decreases biomass production in *Alyssum bertolonii*, possibly indicating nickel essentiality in plant growth or cobalt phytotoxicity.

2.6 Further research arising from this investigation

The relationship between different ratios of substrate cobalt and nickel to accumulation potential and phytotoxicity should be investigated for both species. The 1:1 ratio used in the mixed metal experiment indicated an interference relationship in accumulation with a general reduction in net nickel accumulation relative to cobalt. Artificial substrates with nickel-cobalt ratios of 0.5:1, 2:1, 5:1 and 10:1 should be trialled so that a correlation with naturally occurring nickel-cobalt mineralisations could be made.

Chapter Three - Induced Gold Accumulation using an Artificial Gold Substrate

3.1 Introduction

The ability of plants to absorb small quantities of gold has been known since 1824. The concentrations of gold observed in plant dry matter has typically been <10 μg/kg (ppb). Shacklette *et al.* (1970) reported that cyanide-gold complexes were responsible for gold transport across the root membrane of *Impatiens holstii* in hydroponic experiments This identified the need to investigate cyanide complexes in substrate gold mobility and plant gold accumulation. Work carried out by Anderson *et al.* (1998) using the chelating agent ammonium thiocyanate showed that *Brassica juncea* could be "induced" to hyperaccumulate gold (above their defined hyperaccumulation level of >1 mg/kg dry weight).

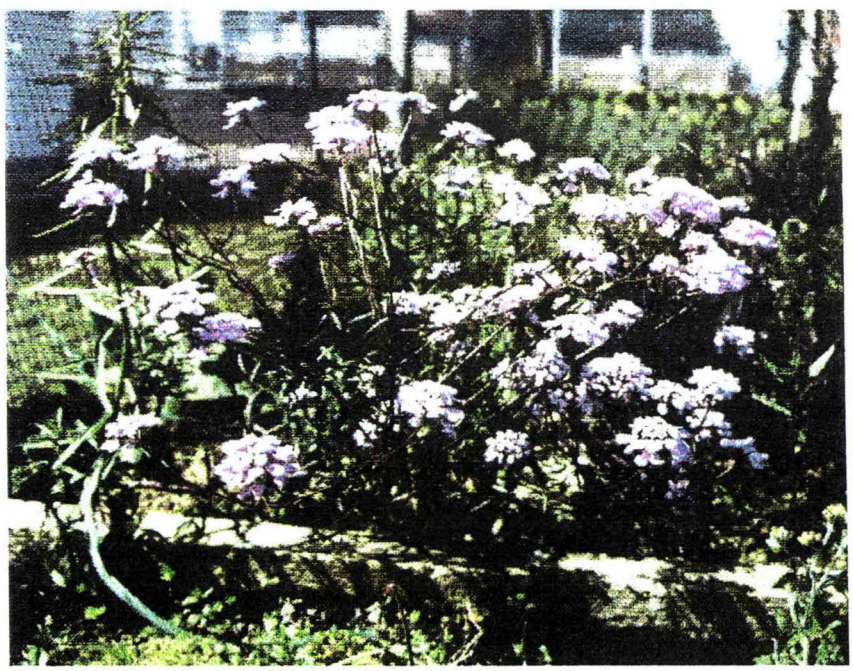

Figure 12. *Iberis intermedia* growing in an outdoor plot at the Plant Growth Unit, Massey University, Palmerston North, New Zealand. Note *Alyssum bertolonii* in the background and *Thlaspi caerulescens* in the foreground. Photo by author.

This experiment assessed two known hyperaccumulators (*Berkheya coddii* and *Iberis intermedia*) responses to induced gold accumulation using ammonium

thiocyanate and ammonium thiosulphate. *Berkheya coddii* in a known nickel and cobalt hyperaccumulator and *Iberis intermedia* is a known thallium accumulator.

3.2 The source of free thiosulphate

Goldharber (1983) reported that metastable sulphur oxyanions accumulate as intermediate species in the oxidation pathway of pyrite over the pH range of 6 to 9. Other mineral sulphides, such as chalcopyrite, are then released by sulphur oxyanions under slightly different oxidation conditions. Goldharber went further by saying that these metastable species showed a systematic pH dependence, with the more oxidised assemblages found at lower pH levels.

The transient nature of $S_2O_3^{2-}$ under weakly acidic conditions was shown by Lyons and Nickless (1968), who believed that thiosulphate was readily oxidised to tetrathionate by weak oxidising agents according to equation 1:

$$2S_2O_3^{2-} + 2H^+ + \tfrac{1}{2}O_2 \rightarrow S_4O_6^{2-} + H_2O \qquad \text{equation 1}$$

Under more acid conditions, Davis (1958) observed disproportionation to elemental sulphur and sulphite according to equation 2:

$$S_2O_3^{2-} + H^+ \rightarrow HSO_4^- + S^\circ \qquad \text{equation 2}$$

Goldharber inferred that the thiosulphate species was metastable under alkaline conditions as an intermediate in the sulphur oxidation pathway, along which further oxidation was inhibited. However, Rolla and Chakrabarti (1982) showed that thiosulphate is eventually oxidised to sulphate by dissolved oxygen in an alkaline medium according to equation 3.

$$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+ \qquad \text{equation 3}$$

3.3 Laterite Gold Mobility

Based on the above $S_2O_3^{2-}$ pathways and stability fields of the above three oxyanionic sulphur species, it appears that gold mobility as a $S_2O_3^{2-}$ complex would be unlikely because of highly acid conditions within laterising fluids. Oxidation of ferrous iron (ferrolysis) within the lateritic profile is responsible for acidification according to Webster (1986). One could then expect these acid conditions to favour gold mobility in the form of a Cl^- ion and/or humic acid complex in organic-rich profiles.

If during lateritisation sufficient carbonate were present, then weathering pyrite would be buffered leading to the release of thiosulphate in a neutral to moderately alkaline environment. Mann (1984) calculated that 400 – 800 g of $CaCO_3$ would be sufficient for every 240 g FeS_2 to maintain a pH at which $S_2O_3^{2-}$ (equation 1) would remain in solution, supporting the existence of supergene thiosulphate gold deposition within a lateritic profile. Lintern *et al.* (1996) illustrated the relationship of gold values between the soil and substrate and overlying vegetation for three goldfields in southern Western Australia, which are characterised as having high levels of pedogenic carbonate.

The formation of pedogenic carbonate is likely to be associated with processes post-lateritisation where initial weathering of the profile occurred. Redistribution of gold during arid climatic conditions by the above carbonate buffered thiosulphate regime would suggest this process was ongoing during both the lateritisation and redistribution phases of ore genesis and laterite formation.

Humic acids have often been implicated in the lateritic movement of gold (Baker, 1973, Baker, 1978, Mann, 1984). Wood (1995) went further by attributing the potential mobility of gold, as well as Pt, Pd, U, V, to individual organic acid components of humic macromolecules. Baker (1995) reported, in a limited study on the effect of 500 mg/L humic substance solutions, that significant levels of gold (and a range of other economic metals) were detected after a 50-day extraction. There has been, however, no publication of conclusive evidence to support or refute the uptake of gold-humic or gold-organic acid compounds by plants. This suggests that further

investigations into the biochemical pathways of gold organic acid should be carried out.

3.4 The Biochemical Pathway of Gold

There is no general agreement in the literature on the forms of gold that are absorbed by plants. It is generally agreed however, that heavy metals absorbed by plants are in a soluble form (Robinson, 1997). The physical and chemical state of the gold that moves into plants and through the biosphere has been a subject of much speculation but little experimentation. Lungwitz (1900) at first proposed that under natural conditions gold is solubilise as a cyanide complex. He later rejected this hypothesis because no trace of cyanide had been reported in association with organic matter. Gold usually exists as the insoluble metal in a natural environment, and yet if plants do absorb this element it must be in a soluble form.

Cyanide complexes have attracted much attention as solubilising agents for gold, due to the natural presence of these compounds in plants. Natural cyanides exist due to the hydrolysis of syngenetic glycosides (Lakin *et al.* 1974), and thiocyanates can be created through similar biochemical pathways. The dissolution of gold by cyanide and thiocyanate can be written as:

$$4Au + 8CN^- + O_2 + 2H_2O = 4[Au(CN)_2]^- + 4OH^-$$

$$2Au + 8[CNS]^- + O_2 + 2H_2O = 2[Au(CNS)_4]^- + 4OH^-$$

Boyle (1979) reported the various gold cyanide and thiocyanate complexes to be stable in mildly acid, neutral and alkaline conditions suitable for normal plant growth. This may be true for gold cyanide complexes, however, recent work by Anderson (2000), indicates that acidic conditions are necessary in forming stable gold thiocyanate complexes, whereas gold-thiosulphate complexes favour mildly alkaline conditions.

3.5 Gold thiosulphate uptake constraints

A common anomaly within the laterite profiles of Western Australia is the appearance of a near-surface zone of secondary gold enrichment. There appears to be little relationship between this secondary zone and the primary zone of gold enrichment located up to 20m below (Gray *et al.* 1992). Lintern *et al.*, (1996) postulated that gold enters plant roots at depth, is transported within the plant and eventually is returned to the surface horizon in the form of native gold held within deciduous leaf tissues. In this way, gold is cycled from depth within the regolith, and over tens of thousands of years of leaf fall, this results in a near-surface zone of enrichment. This appears possible since deep-seated tree roots will preferentially seek out zones of weakness within the laterite (such as shear zones) which are commonly sites of primary ore deposition.

If thiosulphate is, in part, responsible for gold mobility and uptake, a narrow range of pH conditions must prevail in order for the species to exist within the profile.

Some plants are capable of regulating local acidity within the substrate by exuding acids from their root hairs. If acid exudation, into the local environment, changes the pH by as little as one unit it would cause decomposition of the $S_2O_3^{2-}$ complex resulting in precipitation of elemental sulphur and subsequent deactivation of gold. Kulhavy and Cervena (1991) showed that such a pH change is realistic for at least some plant species. The process of acid extrusion may also explain the relative lack of gold accumulators within the plant kingdom since the mobile gold complexes would breakdown at or near the root membrane thereby precipitating the gold. It would follow then that an exploration strategy of targeting pre-existing root zones as potential areas of gold enrichment may be of benefit. It may also explain the high fineness of some laterite gold deposits found in Western Australia.

3.6 Cyanogenesis and Chelation

Plants that produce cyanide compounds as defences against predators were

recognised early. Finnemore and Gledhill (1928) found the first glycosides that are poisonous to wild as well as domesticated animals. These plants were termed cyanogenic, 'having the ability to produce hydrocyanic acid and thiocyanates as a physiochemical response to predation'. The strength of a plant's cyanogenic reaction also appears to be seasonally dependent with higher levels of cyanide compounds present in cyanogenic tissues in spring and summer compared to the cooler seasons. Since the cyanoglycoside content of plants varies with the season, increased spring plant gold concentrations may be related to the action of greater amounts of free soil cyanide or increased microbial activity (Girling and Peterson, 1978). This factor may shed more light on interpreting current gold phytoaccumulation data, however only if the sampling dates have been recorded for seasonal correlation.

Cyanogenesis is one of the few instances where it has been possible to prove plant chemical defence against herbivores (Jones, 1998). This defence mechanism is by no means perfect, a few specialised soil organisms and insects have evolved cyanide-detoxifying metabolisms and others exhibit adaptive feeding techniques to remove the cyanide compounds before ingestion. It is interesting to note that of the 318 species that Jones (1998) screened for cyanide compounds, 68 tested positive. This means that statistically significant proportions of human food plants are cyanogenic. Man appears to have chosen suitable food plants not only on the plant's ability to sustain him, but also on the plant's ability to defend itself. Conn (1969) reported that "approximately 1000 species representing 90 families and at least 250 genera have been found to be cyanogenic".

Kingsbury (1964) isolated hydrocyanic acid and thiocyanates from plants, which had caused poisoning of livestock. Both of these compounds are formed from glycosides and both were found to be solvents of gold. Glycosides yield hydrocyanic acid when hydrolysed by enzymatic action. Several dozen cyanogenic species have been studied in detail, in some instances because of their economic significance, and 11 cyanogenic glycosides have since been identified. In a report on cyanogenic plants of Western Australia Aplin (1976) detected traces of thiocyanate in leaves, stems and roots in the family Brassicaceae specifically *Brassica oleracea* L.

In a study on the resistance of cultivated flax (*Linum usitatissimum* L.) to the fungus *Fusarium lini*, Reynolds (1931) reported that varietal resistance to the fungus depended on the amount of the glycoside linamarin in the plant tissue. Timonin (1941) demonstrated that the linamarin in flax is hydrolysed to form hydrogen cyanide and that in laboratory experiments 25-37 mg of this compound was excreted or diffused from the root system of a single flax plant into the surrounding medium. He stated further that "it can be assumed that the same toxic matter (hydrogen cyanide) is secreted by the resistant variety under natural (field) conditions.

Flax (*Linum*) plants contain two major cyanogenic monoglucosides linamarin and lotaustralin, aglgycones that are all derived from L-valine and L-isoleucine, respectively. These two compounds, without apparent exception, always occur together in different species, although not necessarily in the same ratio. The co-occurrence of these two glucosides in linum flax is ascribed to the existence on a single set of biosynthetic enzymes (Niedz;wied;z;-Siegien;, 1998).

There are no known species of natural gold hyperaccumulator. However, while working on induced phytoaccumulation of heavy metals using a chemical amendment of thiocyanate, Anderson *et al.* (1998) demonstrated that *Brassica juncea* and other species could be induced to accumulate gold in mean concentrations ranging from 9.3 to 19.3 mg/kg. This is the first evidence that a plant could be induced to accumulate gold.

Girling and Peterson (1978) investigating the uptake, transport, and storage of gold observed several important processes.

1. Many plants which contained higher-than-normal gold concentrations were also cyanogenic,
2. Cyanogenic plants play an important role in gold solubilisation
3. Addition of calcium ions stimulated gold uptake, confirming that gold intake is at least partially metabolic.
4. Transport of gold compounds within the plant is partially controlled by the rate of transpiration.

5. Gold cyanide in the leaves is stored in an aqueous form, presumably in the leaf vacuole
6. Gold chloride is predominantly insoluble and bound to the cell wall.
7. Distribution of localised gold in the leaves was in the leaf tips and in small discrete areas around the edge of each leaf lobe that may represent deposition of metallic gold.
8. Of all the cell components analysed, the mitochondria contained the most gold.
9. Gold cyanide was mainly soluble in the shoot fraction and less soluble in the root. Gold chloride was nearly insoluble in the root, but significantly soluble in the shoot.

The above would suggest that the roots produce soluble (transportable) gold compounds and cell walls store gold in the chloride (insoluble) form as well as the established aqueous cyanide complexes. High mitochondria gold levels indicate that gold has a high rate of flux through the mitochondria irrespective of the gold transport complex. Girling and Peterson confirmed earlier work by Shacklette *et al.* (1970) because they both indicated the importance of gold cyanide complexes in gold phytoaccumulation.

3.7 Aims of this experiment

The aim of this experiment was to determine the potential of ammonium-thiosulphate and ammonium-thiocyanate-chelating agents for induced phytoaccumulation of gold by two species of known heavy metal hyperaccumulators. *Berkheya coddii* is a South African hyperaccumulator of nickel and cobalt. *Iberis intermedia* is a thallium hyperaccumulator from France. These two species' ability to hyperaccumulate heavy metals offered an existing biochemical pathway that was hoped to be exploited using chelators to induce gold accumulation. Both species have a biomass suitably high to support a phytoextraction operation, as well an ability to accumulate high levels (>1000 mg/kg) of heavy metals.

The following experiment was conducted using the methods and materials outlined in the Methods Section of Chapter One.

The below table (Table 6) shows the average induced gold accumulation in mg/kg (ppm), and standard deviation of the response, for *Berkheya coddii* and *Iberis intermedia* at incremental concentration loadings of ammonium thiocyanate and ammonium thiosulphate.

Table 6. Induced gold accumulation (n=5) using ammonium thiocyanate (NH_4SCN) and ammonium thiosulphate ($(NH_4)_2S_2O_3$). All concentrations (mean and standard deviation) in mg/kg.

Species	Cont.	0.25% NH_4 SCN	0.5% NH_4 SCN	1% NH_4 SCN	0.25% $(NH_4)_2$ S_2O_3	0.5% $(NH_4)_2$ S_2O_3	1% $(NH_4)_2$ S_2O_3
B. coddii	>0.005	>0.005	0.06 ± 0.02	0.1 ± 0.05	>0.005	0.6 ± 0.5	9.4 ± 8.2
I. intermedia	>0.005	>0.005	0.15 ± 0.05	0.3 ± 0.005	9.3 ± 0.9	12.9 ± 6.4	48.8 ± 23

3.8 Results and Discussion

The analyses indicate an overall increase in the level of accumulation with increasing concentrations of both chelators and both species. The general response of both chelators, under natural pH conditions, to native gold accumulation, indicates that ammonium thiosulphate is the more efficient chelator (Table 6).

Data for *Berkheya coddii* response indicate that this species has little potential for phytoaccumulating an appreciable concentration of ammonium-thiocyanate-chelated gold or ammonium-thiosulphate-chelated gold at low concentrations. However, a 1% solution of ammonium thiosulphate induced gold accumulation in *Berkheya coddii* of 9.4 mg/kg indicating that higher concentrations of this chelator could improve gold uptake. It would be wise to investigate this trends given the plants natural ability to hyperaccumulate nickel and cobalt.

The *Iberis intermedia* response data indicates that this plant could be induced to accumulate gold with for ammonium thiocyanate. This may highlight the multiplicity of biochemical pathways and root membrane interactions in different species associated with metal uptake in plants.

The data indicate that *Iberis intermedia* is a particularly good accumulator of gold using ammonium thiosulphate chelation. The mean level of 48.8 mg/kg is by far

the best result from this experiment and is derived from analysis values ranging from 35 to 83 mg/kg gold.

It is recommended that further work be carried out on induced hyperaccumulation of gold in both species using both chelators. This work should include modifying substrate pH to promote chelation, increased rates of chelator application at greater concentrations (e.g. 0.1-5% by wt.) and pot trials using auriferous soils as substrates. Experimentation should also be carried out to determine the differences in gold complex transport between species and the interactions between other mobile elements in the substrate and the chelating compounds.

3.9 Induced Accumulation of Gold by *Berkheya coddii* using Ammonium Thiocyanate as a Chelator

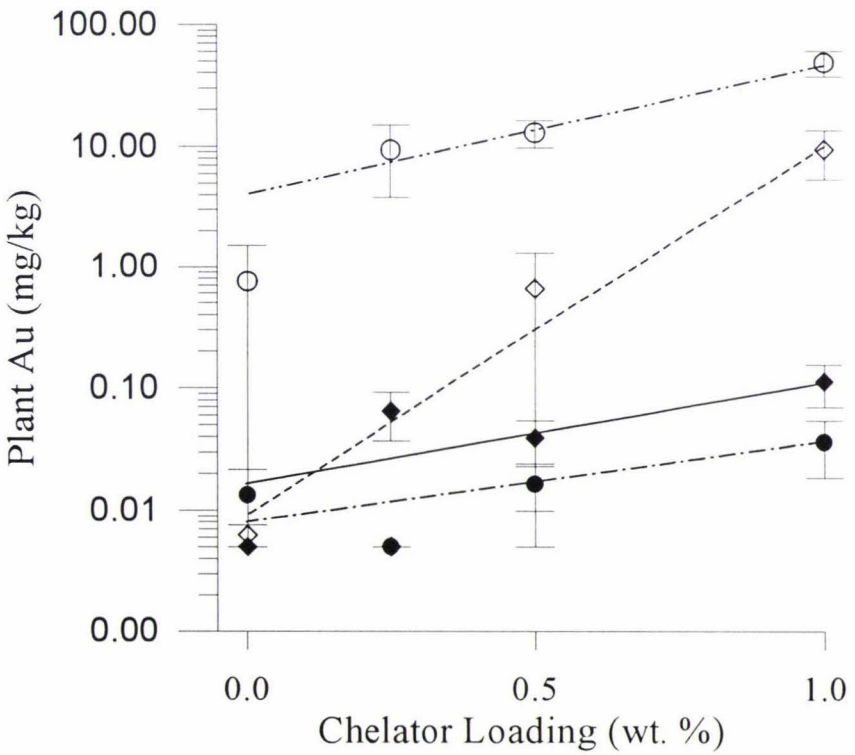

Figure 13. Chelator induced gold accumulation for *Berkheya coddii* (solid diamond = SCN, open diamond = S_2O_3) and *Iberis intermedia* (solid circle = SCN and open circle = S_2O_3).

The graph above (Figure 13) shows the induced gold accumulation response of *Berkheya coddii* using different concentrations of ammonium thiocyanate. The

plant response indicates a positive correlation between gold accumulation and increasing chelator concentration at natural pH levels.

It is recommended that further work be carried out on *Berkheya coddii* using higher concentrations of ammonium thiocyanate to induce gold accumulation.

3.10 Induced Accumulation of Gold by *Berkheya coddii* using Ammonium Thiosulphate as a Chelator

The graph above (Figure 13) shows the induced gold hyperaccumulation response of *Berkheya coddii* under different concentration loadings of ammonium thiosulphate. The plant's response indicates a positive correlation between gold accumulated and increasing chelator concentration.

The data indicate that a loading of approximately 0.5% ammonium thiosulphate is necessary to induced gold accumulation of ≈1 mg/kg. The lower-than-expected average result for the 0.25% treatment is the result all samples returning zero analyses.

3.11 Induced Accumulation of Gold by *Iberis intermedia* using Ammonium Thiocyanate as a Chelator

The graph above (Figure 13) shows the induced gold accumulation response of *Iberis intermedia* under different concentration loadings of ammonium thiocyanate.

The plant response indicates a positive correlation between gold accumulated and increasing chelator concentration. The lower-than-expected average result for the 0.25% treatment is the result of all samples at this loading returning zero analyses.

The data indicates that a chelator loading of 0.5% ammonium thiocyanate is necessary to induce gold accumulation to ≈1 mg/kg necessary for hyperaccumulation to occur.

The graph may also indicate an induced hyperaccumulation threshold (or maximum) occurring at a loading of approximately 1% solution of ammonium

thiocyanate. A hyperaccumulation threshold occurs where the plants ability to accumulate greater concentrations of the target element is impeded by chelation induced phytotoxicity, complexing efficiency dictated by the concentration of the amendment, or by the plants ability to absorb and store a greater concentration of the target element. However, further experimentation should be carried out to confirm this conclusion of an upper accumulation limit. This apparent limit may be a result of the gold being made bioavailable in the substrate by chelation or the chelators binding potential for gold at this substrate concentration and pH. All plants died as a result of ammonium thiocyanate treatment. These data have important implications in terms of the cost of an induced gold phytoextraction programme. This threshold would indicate an upper limit of chelator loading and therefore an upper cost level for a phytoextraction programme.

The apparent lack of plant response to a 0.25% amendment of either chelator, many relate to a concentration gradient for both compounds required before appreciable gold is absorbed or may relate to a physiological response in the roots to exclude gold uptake, possibly by acidic exudation.

3.12 Induced Accumulation of Gold by *Iberis intermedia* using Ammonium Thiosulphate as a Chelator

The graph below (Figure 13) shows the induced gold hyperaccumulation response of *Iberis intermedia* under different concentration loadings of ammonium thiosulphate.

The plant response indicates a strong, positive, correlation between gold accumulated by the plants and increasing chelator concentration. Ammonium thiosulphate treatment resulted in total plant mortality 10-12 days after application.

The data indicate that a relatively small amount of ammonium thiosulphate in needed to induce *Iberis intermedia* to accumulate gold. The lowest concentration loading of 0.25% ammonium thiosulphate appears to induce significant uptake of gold, to a level of ≈10 mg/kg. This level of plant gold indicates that *Iberis intermedia* is a good accumulator of gold as described by Anderson *et al.*, 1998.

Clearly, further experimentation is necessary using chelator solution strengths in the range of 0.05% to 0.25% ammonium thiosulphate to optimise the plant's uptake response. This would allow for determination of the most efficient loading necessary to induce a substantial gold-in-plant concentration of 1 mg/kg. This graph may also indicates the approach of an induced hyperaccumulation threshold (or maximum) occurring at a loading near the 1% solution of ammonium thiosulphate. A higher chelator concentration may increase the soluble gold fraction, however the plant may not survive long enough to achieve a higher gold level of gold uptake.

3.13 Conclusions

The response of *Iberis intermedia* to ammonium thiocyanate gold chelation was poor by comparison with the response of *Berkheya coddii* over the same range of treatments, but the data support the premise that *Iberis intermedia* can be induced to accumulate gold to levels sufficient to deem this species an induced hyperaccumulator. The data indicate that a chelator loading of 1% ammonium thiocyanate is necessary to induce gold hyperaccumulation (>1 mg/kg gold).

The ammonium thiosulphate data show that a relatively small quantity of ammonium thiosulphate (0.25%) is necessary to induce gold hyperaccumulation in *Iberis intermedia*. This loading appears to induce significant uptake of gold to a level of ≈9 mg/kg. The 1% ammonium thiosulphate treatment induced gold hyperaccumulation to an average level of 49 mg/kg. The highest gold-in-plant concentration for the 1% chelator loading was 83 mg/kg making this response the best data point of the experiment.

It is recommended that further work be carried out on *Berkheya coddii* using higher concentrations of ammonium thiocyanate to induce gold accumulation so that a better understanding of this species' inducible gold uptake can be ascertained.

Other concentrations of chelator loadings should also be investigated to determine the maximum level of induced gold hyperaccumulation for this species so

that a true picture of *Iberis intermedia* gold accumulation potential can be ascertained.

Chapter Four - Nickel and Cobalt Phytoextraction from Laterite Substrates

4.1 Introduction

By far the largest body of research into the hyperaccumulation of heavy metals has been carried out under glasshouse conditions. These experiments have invariably used an artificial substrate amended with the target metal. This controlled environment reduced the need for irrigation by maintaining a high level of soil moisture. Accumulation of heavy metals occurs in an aqueous environment, which leads us to conclude that the rate of plant uptake is controlled by transpiration (Robinson 1997). Glasshouse results published to date have never recorded levels of hyperaccumulation similar to those found in the wild, although a high degree of consistency exists between experimental data (Robinson, 1997, Anderson, 2000).

The decision to use heavy-metal-bearing lateritic materials as substrates in this experiment was made in order to better understand the hyperaccumulating potential of *Alyssum bertolonii* and *Berkheya coddii* when grown on a naturally occurring nickel- and cobalt-bearing substrate so that I could reference this back to findings reported in Chapter Two.

In the mining industry, metal-bearing materials which are sub economic (i.e. below a predetermined cut-off grade) are discarded as waste and are therefore not exploited. Lateritic nickel-cobalt anomalies cover vast areas of Western Australia. For the most, part the level of mineralisation is below the feasibility concentrations necessary for modern mining practices. This experiment was hoped explore the implications of phytomining for nickel and cobalt in lateritic terrains. This experiment also investigated the use of chelators in determining the bioavailability of heavy metals, and to allow for the estimation of a laterite's metal potential for a phytoextraction programme. Controls on the hyperaccumulation potential of these species are also discussed.

4.2 Objectives of this experiment

1. To investigate nickel and cobalt accumulation by *Berkheya coddii* and *Alyssum bertolonii* grown on lateritic substrates supplied by Anaconda Nickel Ltd. and Boddington Gold Mine Pty Ltd., both from Western Australia.

2. To investigate the use of chelating agents (ammonium thiocyanate and ammonium thiosulphate) in addition to ammonium acetate and deionised water as a determinant for the bioavailable metal fraction of lateritic substrates.

4.2.1 Heavy-Metal Bioavailability

The use of an ammonium acetate extraction to determine the bioavailable metal fraction in a substrate was proposed by Robinson (1997). He reported that the total metal concentration had little bearing on the bioavailable metal fraction and hyperaccumulation potential of a soil. This work determined that a 1 M solution of ammonium acetate could reproducibly bioavailable metal, including nickel, from a typical serpentine soil. The ammonium-acetate-extractable fraction can therefore be used as a 'baseline' to help correlate the accumulation potentials between different substrates. Determination of the bioavailable cobalt fraction using ammonium-acetate extractions has not previously been reported.

4.2.2 Heavy Metal Chelation

Robinson et al. (1999a) investigated the use of substrate amendments to enhance nickel and cobalt hyperaccumulation in *Berkheya coddii*. They reported that the chelating agents NTA, DTPA and EDTA produced a significant decrease in nickel uptake and had no effect on cobalt hyperaccumulation. Paradoxically, Blaylock *et al.* (1997) reported an amendment of EDTA to a lead-bearing soil increased the bioavailability of lead resulting in *Brassica juncea* accumulating up to 1.5% lead in its aerial tissues, by dry weight.

The above responses are interesting in that the bioavailability of these metals was increased in all substrates. Clearly, chemical complexing of the metals either decreases their bioavailability or the chelators themselves produce a plant uptake-inhibiting response in the rhizosphere. Robinson *et al.* (1999a) also reported that the addition of acid mine tailings and elemental sulphur as substrate pH modifiers produced a significant increase in nickel and cobalt uptake and that addition of calcium and magnesium significantly decreased plant uptake of both metals.

Gold chelation using ammonium thiocyanate appears to operate best in acid substrates, while ammonium thiosulphate appears to favour alkaline conditions (Anderson *et al* 1999). These chelators have not before been used to investigate enhancement of natural hyperaccumulation of nickel and cobalt by known hyperaccumulating species. However, Anderson *et al.* (1998) used ammonium thiocyanate to induce gold accumulation in *Brassica juncea* to a level of 57 mg/kg dry weight.

Ammonium thiocyanate and ammonium thiosulphate were used in this experiment because of they are both known gold chelators and are relatively inexpensive by comparison with EDTA and DTPA.

The expense of using chelators to induce accumulation will have a significant impact on the feasibility of a phytoextraction operation. These chemicals are considered less damaging to the environment than EDTA and DTPA as they biodegrade in the soil relatively quickly after application.

4.2.3 Substrate Preparation and Final Metal Concentrations

The duration of the plant trial was approximately 12 weeks, utilising the rapid plant growth obtained under favourable glasshouse conditions. In order maximise plant growth and hence accumulation response of the plants, the laterites were blended with pumice to decrease substrate density. It was felt that the high clay content samples, once wetted, would become too heavy to support the level of growth required for this trial. The blend was a two parts of sample to one part of sieved pumice by weight, with additions of appropriate fertilisers. Table 7 shows the final blended substrate metal concentrations for the laterites used in this experiment.

Table 7. Final substrate metal concentrations (mg/kg) of laterite growth medium.

Substrate	pH	Nickel	Cobalt	Gold	Magnesium	Calcium
B5	4.9	637	874	0.34	1,884	1.3
M4	6.4	448	917	1.08	104	4.9
Z1	5.3	222	846	0.14	136	3.7
FZ	7.7	7,921	1,079	0.45	1,745	93
SAP	8.5	18,252	1,443	0.13	64,820	679

Of note are the unusually high nickel and magnesium contents of the SAP and FZ material. This may affect the nickel accumulation potential of *Alyssum bertolonii* as reported by Robinson *et al.* (2000).

4.3 The Boddington Consignment

4.3.1 Introduction

Boddington Gold Mine Pty Ltd. supplied oxidic lateritic gold-bearing material from their Western Australian mine site. This material represents the upper weathered zone of a much deeper hard rock resource, only recently identified. For a full site description refer to Chapter One – Industrial Contributors.

The material has been named in accordance with the deposit where the material originated, i.e. B5 open pit on the Boddington Gold Mine.

4.3.2 Laterite Descriptions

The B5 material is a high clay content substrate originating midway down the laterite profile, in the upper saprolite to lower mottled zone. This material is grey-salmon pink in colour with rare ferruginous pisoliths to 6 mm in size.

The M4 material is from the upper laterite profile. This highly ferruginous laterite capstone is composed of iron-cemented pisoliths and quartz grains. This material contains 1.6 mg/kg gold and represents a low-grade gold ore by modern standards.

The Z1 material originates from the upper mottled zone in the laterite profile. Fine-grained and iron-rich it contained rare ferruginous pisoliths, quartz grains and highly weathered rock fragments.

4.3.3 Ore Quality

The table below (Table 8) shows the total concentration of nickel, cobalt, magnesium, calcium and gold (in mg/kg) including the pH levels of the materials.

Boddington Gold Mine was unable to provide assay information for correlation with the analyses below because these materials constitute sub economic mineralisation and are therefore of no current value to the mining operation.

Table 8. Ore quality of Boddington Gold Mine samples. Concentrations are in mg/kg (ppm), n=5.

Ore Type	pH	Tot. Ni	Tot. Co	Tot. Mg	Tot. Ca	Tot. Au
B5	4.9	955	1311	2826	1.9	0.51
M4	6.4	672	1276	156	7.3	1.62
Z1	5.3	333	1269	204	5.5	0.21

The pH levels of these materials are within those required for normal reproductive plant growth. However, the B5 material is considered to be at the lower limit of substrate acidity tolerated by most plants. The slightly acid condition of B5 and Z1 may have a positive effect on the water solubility and therefore bioavailability of the metals in this material.

The total nickel and cobalt concentrations of all the samples are well within the range of concentrations found under stands of indigenous hyperaccumulators, and in previous publications, indicating that these materials have sufficient total metal concentrations to proceed with the experiments.

Concentrations of magnesium and calcium are relatively low, reflecting the slight acidity of the laterites. The concentrations of both elements indicate that no effect on nickel and cobalt hyperaccumulation should occur for *Berkheya coddii* and *Alyssum bertolonii*. It can therefore by assumed that the observed accumulation responses of both plants are under favourable substrate conditions reflecting the true potential of the substrate for hyperaccumulation in plants.

The gold concentration of the above materials is typically sub economic to low-grade by modern mining standards. The M4 material is the 'higher-grade'

material from this consignment of laterite, with an average gold concentration of 1.6 mg/kg (g/t in mining notation).

4.3.4 Nickel Bioavailability as Determined by Solvent Extractions

The table below (Table 9) shows the concentrations of nickel removed (in mg/kg) by end-over-end solution extractions using reverse osmosis (RO) water, 1 M ammonium acetate and 1% solutions of ammonium thiocyanate and ammonium thiosulphate at neutral pH. The use of ammonium thiocyanate and ammonium thiosulphate was a comparative exercise in solvent extraction. Both these compounds are known chelators of gold and it was hoped their ability to extract nickel could also be used to enhance and/or determine the plant-available nickel fraction.

Table 9. Nickel extractability (n=4) as determined by end-over-end solution extractions using (RO) water, 1 M ammonium acetate and 1% ammonium thiocyanate and ammonium thiosulphate. Concentrations are in mg/kg (ppm). Standard Errors is approx. ±15%.

Ore Type	pH	RO H_2O	NH_4 Acetate	1.0% SCN	1.0% S_2O_3
B5	4.9	0.23	0.07	0.49	<0.005
M4	6.4	1.47	1.77	1.89	0.15
Z1	5.3	0.56	0.10	0.32	<0.005

The reverse osmosis water extraction of M4, removed a comparable amount of nickel to the ammonium acetate extraction and ammonium thiocyanate, indicating that the bioavailable nickel occurs in a water-soluble form. These data are similar to those reported by Robinson *et al.* (1997a) for nickel uptake using *A. bertolonii* in Italy.

Ammonium-acetate-extractable (bioavailable) nickel analysis of 1.77 mg/kg indicates that the M4 laterite has potential for nickel phytoextraction. The nickel accumulation curve reported by Robinson (1997) indicates that this level of substrate nickel extractability should produce nickel hyperaccumulation in *Alyssum bertolonii* of approximately 3800 mg/kg. The remaining two materials appear to have little bioavailable nickel.

The ammonium-thiocyanate extraction is more efficient at removing nickel from the substrates compared to ammonium thiosulphate, a factor probably explained by the slightly acid conditions of the materials favouring ammonium-thiocyanate

extraction. A slightly higher nickel concentration in all ammonium-thiocyanate extractions compared to the ammonium-acetate extractions may indicate that ammonium thiocyanate is extracting closer to the total (100%) bioavailable nickel fraction compared to ammonium acetates reported 80% extraction rate (Robinson, 1997). However, to quantify this conclusion would require further experimentation.

The ammonium-thiosulphate treatments returned analyses below detection limits of µg/kg (ppb). The low analysis for M4 material is probably related to chemical complexation of other element in preference to nickel, a fact exemplified by the high RO water and ammonium-acetate extractable nickel concentrations determined here.

In comparing the nickel extraction data for RO water and ammonium-acetate extractions at neutral pH, it is evident that an RO water extraction removes a similar amount of nickel to an ammonium acetate extraction. I conclude that an RO-water extraction would provide adequate information on the bioavailable nickel fraction of these laterites. This would lower the cost of sample analysis for a phytoextraction exploration program by removing a chemical cost from laboratory expenses.

Table 10 below shows the concentrations of nickel removed by the extraction solutions expressed as a percentage of the total nickel of the substrate.

Table 10. Total and extractable nickel from the Boddington Gold Mine samples (n=4) expressed as a percentage of the total metal concentration, in mg/kg (ppm). Standard Errors is approx. ±17%.

Ore Type	Tot. Ni	NH_4 Acet.	1% SCN	1% S_2O_3
B5	955	0.01%	0.05%	T
M4	672	0.3%	0.3%	0.02%
Z1	333	0.03%	0.1%	T

T = Trace analyses or below detection limits.

At a pH of 4.9, a 1% solution of ammonium thiocyanate removed 0.05% of the total nickel present in the B5 material. The same concentration solution removed 0.3% of the total nickel present in M4 material at pH of 6.4 and 0.1% of the total nickel from the Z1 material at pH of 5.3.

A 1% solution of ammonium thiosulphate removed only a trace of the total nickel present in the B5 and Z1 materials at pH 4.9 and 5.3 respectively. The same solution removed 0.02% of the total nickel from the M4 material at a pH level of 6.4

The ammonium-acetate extractions removed similar, if not slightly lower, amounts of nickel from the samples compared to the ammonium thiocyanate extractions. There is no correlation between ammonium-thiosulphate and ammonium-acetate extractions. This indicates that ammonium thiocyanate is two orders of magnitude more effective than ammonium thiosulphate at mobilising nickel within the laterite samples. The next logical step would be to determine the effect of ammonium thiocyanate on the accumulation potentials of known hyperaccumulators. The data also indicate that an ammonium acetate extraction for nickel is just as effective and less expensive than ammonium thiocyanate for determining the plant available nickel fraction from these lateritic materials.

4.3.5 Cobalt Bioavailability as Determined by Solvent Extractions

The table below (Table 11) shows the concentrations of cobalt removed (in mg/kg) by the end-over-end solution extractions. Little work has been carried out on determining the bioavailability of cobalt in a substrate. This experiment is designed to identify a suitable method of determining the plant-available cobalt fraction.

The reverse osmosis water extractions closely correlate with ammonium acetate extractions for the Z1 material only. The below-detection values reported for ammonium acetate extractions of B5 and M4 materials indicate their low cobalt phytoextraction potential. The concentrations of aqueous extractable cobalt are too low for phytoextraction to reach hyperaccumulation levels (1000 mg/kg). Ammonium-acetate-extractable cobalt of Z1 (1.6 mg/kg) suggests this laterite that may be suitable for an accumulation trial.

Table 11. Cobalt extractability (n=4) as determined by end-over-end solution extractions using (RO) water, ammonium acetate, ammonium thiocyanate and ammonium thiosulphate. Concentrations are in mg/kg (ppm). Standard Errors is approx. ±22%.

Ore Type	Tot. Co	RO H_2O	NH_4 Acet.	1.0% SCN	1.0% S_2O_3
B5	1311	0.26	<0.005	0.06	0.6
M4	1276	0.12	<0.005	0.08	0.39
Z1	1269	1.61	1.56	1.61	0.52

Both the ammonium thiocyanate and ammonium thiosulphate are capable of mobilising cobalt in the lateritic materials. The similar levels of ammonium-thiosulphate-extractable cobalt between substrates may indicate that this chelator is better suited to determining the bioavailable cobalt concentration in these laterites. It

may be possible to model plant-available cobalt using ammonium thiosulphate, however this is beyond the scope of this project.

The extractability trends of RO-water-extractable cobalt compared well with both the ammonium-acetate and ammonium thiocyanate extractions. It can be assumed then that a simple extraction using RO water is sufficient to determine the bioavailable cobalt fraction in the laterite samples.

4.3.6 Gold Bioavailability as Determined by Solvent Extractions

Table 12 shows the concentrations of gold removed (in mg/kg) by end-over-end solution extractions, as described for the nickel and cobalt extractions above. The objective of this exercise was to determine the efficiencies of ammonium thiocyanate and ammonium thiosulphate at solubilising gold in these materials for an inducing hyperaccumulation pot trial. This experiment also investigates a chelator based plant available gold indicator. All solutions in this experiment are buffered to neutral pH levels.

The table indicates the expected results for RO water and ammonium acetate gold extractability, as these solutions have no chemical reactivity with gold. These analyses were included to determine whether any fine clay or colloidal fractions hosted gold. Considering the low total gold concentrations of these materials, the likelihood of detecting any extractable gold was small at best.

Table 12. Gold extractability (n=4) as determined by end-over-end solution extractions using (RO) water, ammonium acetate, ammonium thiocyanate and ammonium thiosulphate. Concentrations are in mg/kg (ppm). Standard Errors is approx. ±40%.

Ore Type	Tot. Au	RO H_2O	NH_4 Acet.	1% SCN	1% S_2O_3
B5	0.5	<0.001	<0.001	0.1	0.1
M4	1.6	<0.001	<0.001	0.001	0.01
Z1	0.2	<0.001	<0.001	0.01	0.02

The data indicate that both chelators are capable of mobilising gold in the laterite samples. No clear separation exists between chelator efficiencies for ammonium thiocyanate and ammonium thiosulphate. The acidic preference for gold chelation by ammonium thiocyanate would suggest that a higher level of chelation would occur compared to ammonium thiosulphate. However, ammonium

thiosulphate extracted the same or more gold from the laterites than ammonium thiocyanate. This highlights the similar nature of gold chelation by these two compounds at near neutral pH levels.

Msuya *et al.* (2000) reported that ammonium thiocyanate selectively removes gold from a mixed heavy metal-bearing material. This chemical preference to gold chelation was also reported to increase with decreasing pH. Ammonium thiosulphate was reported to be less efficient and less selective at removing gold from a mixed heavy metal-bearing medium compared to ammonium thiocyanate. The effect of changing pH on ammonium thiosulphate extractability has a similar trend to that of ammonium thiocyanate.

The pH of the laterite samples can be seen exerting an effect on the relative chelator potential and gold mobility between substrates. A 1% solution of ammonium thiocyanate removed 17.6% of the total gold present in the B5 material at a pH of 4.9, 3.4% of the total gold present in the Z1 material at pH of 5.3 and 0.6% of the gold present in the M4 material at a pH of 6.4. A 1% solution of ammonium thiosulphate removed 28.2% of the total gold in the B5 material, 8.7% of the total gold from the Z1 material and 0.6% of the total gold from M4. Clearly, substrate acidity is determining the chelator available gold fraction.

These data (and those presented below for the Anaconda Consignment) may also be indicating the relative ligand stability of a range of metals in these materials. Msuya *et al.* (2000) demonstrated that ammonium thiocyanate selectively removed gold from mixed heavy metal-bearing mine tailings. Whereas ammonium thiosulphate, to a much lesser degree, selectively removed gold from the same tailings material it also removed considerable amounts of other heavy metals. The elemental preference for and stability of the metal-ligand complex using either of these chelator compounds should be quantitatively determined over a range of pH conditions for the substrate in question to better understand the controls on their extractabilities.

I therefore recommend that further work on the heavy metal complexing potential's of these chelators be carried out so that a better understanding of their heavy metal mobilising abilities in mixed metal substrates can be made and related to

our present understanding of hyperaccumulating plants and induced accumulation of heavy metals.

4.4 The Anaconda Consignment

4.4.1 Introduction and Laterite Descriptions

Anaconda Nickel Ltd. supplied dry nickel-cobalt lateritic material from their Murrin Murrin Project mine site in Western Australia. This material represents a 2.25 billion-tonne resource of dry nickel-cobalt laterite. These samples retain their original ore codes from Murrin Murrin as identification.

The FZ material represents a rock-code relating directly to its high iron and low nickel concentration. This highly ferruginous material represents an upper laterite profile unit of iron-cemented ferruginous pisoliths, rare quartz grains and rock fragments.

The SAP rock-code designates a low-grade nickel-cobalt mining unit containing appreciable magnesium. This material is saprolitic, originating from the lower laterite profile and is composed of basement rock minerals and secondary transitional clays.

4.4.2 Ore Quality

Table 13 shows the total concentration of nickel, cobalt, magnesium, calcium and gold in mg/kg as determined in the Methods Section of Chapter One.

Anaconda Nickel Ltd were unable to provide a detailed ore reports of metal concentrations for correlation with the analyses below because these materials constitute sub economic mineralisation and are therefore of no current value to the mining operation. However, Anaconda Nickel were able to state that the FZ material was sub economic at present and treated as waste or stockpiled as low-grade ore. The SAP material, occurring at the base of the current mining profile, is too high in magnesium content to be fed through the mill (metal extraction facility) and is consequentially left *in situ* or stockpiled where necessary.

Table 13. Ore quality for nickeliferous material supplied by Anaconda Nickel Mine. Concentrations are in mg/kg (ppm), n=4. Standard Errors is approx. ±18%.

Ore Type	pH	Tot. Ni	Tot. Co	Tot. Mg	Tot. Ca	Tot. Au
FZ	7.7	11,882	1619	2618	139	0.68
SAP	8.5	27,378	2165	97,230	1019	0.20

The FZ material has typically low-grade nickel and cobalt concentrations of 1.19% and 0.16% respectively. However these total substrate concentrations are far in excess of those necessary for a hyperaccumulation plant. At pH 7.7, the substrate will support normal plant growth. However, this slightly alkaline condition may lower the hyperaccumulation potential and bioavailability of nickel and cobalt in *Berkheya coddii*, as reported by Robinson *et al* (1999a). The concentration of magnesium in the SAP material should also have a detrimental effect on nickel accumulation potential of *Berkheya coddii*.

The SAP material contained 2.7% nickel and 0.2% cobalt, considerable mineralisation for a material currently outside the ore reserve. The pH (8.5) of this material is more alkaline than FZ, reflecting the high concentration of magnesium. This level of magnesium, and consequent alkaline pH, will have a negative effect on the bioavailability of the metals and the hyperaccumulation potential of *Berkheya coddii*.

Future laboratory or field investigations of both laterites should be carried out using amendments of pH modifiers, such as elemental sulphur or locally available acid mine tailings, to lower the pH and increase the bioavailable metal fraction.

4.4.3 Nickel Bioavailability as Determined by Solvent Extraction

Table 14 shows the analyses of extractable nickel (in mg/kg) removed using extractants of RO water, ammonium acetate, ammonium thiocyanate and ammonium thiosulphate, performed on the Anaconda Nickel Ltd. Laterite samples.

Table 14. Nickel extractability (n=4) as determined by end-over-end solution extractions. Concentrations are in mg/kg (ppm). Standard Errors is approx. ±15%.

Ore Type	pH	RO H_2O	NH_4 Acet.	1% SCN	1% S_2O_3
FZ	7.7	1.97	1.68	1.43	<0.005
SAP	8.5	1.37	1.56	1.46	0.36

Concentrations of nickel extracted using RO water, ammonium acetate and ammonium thiocyanate compared favourably, indicating that nickel is present in a readily water-soluble (bioavailable) form. At a substrate pH of 7.7, a 1 M ammonium-acetate extractant removed 1.7 mg/kg of nickel from the FZ material indicating a high bioavailable nickel coefficient. This is a similar amount to the nickel removed by RO water and ammonium thiocyanate. The amount of nickel removed by ammonium thiosulphate is negligible. At a substrate pH of 8.5, a 1 M solution of ammonium-acetate extractant removed 1.6 mg/kg of nickel from the SAP material. This is a similar amount to that removed by RO water and ammonium thiocyanate extractions. Again the amount of nickel removed by ammonium thiosulphate is negligible.

The above data indicate that ammonium acetate and the ammonium thiocyanate treatments have similar extraction efficiencies at this total nickel concentration and pH level. Interestingly, a similar level of nickel removed by RO water would suggest that nickel occurs as relatively water-soluble salts and carbonates. Perhaps a drop in head-grade really does reflect recent precipitation on slightly alkaline nickel ores.

The response of ammonium thiosulphate is puzzling because it is believed that this chelator performs best in alkaline conditions. The data presented here may indicate that ammonium thiosulphate has an upper, pH dependent, chelation limit. However, I suspect this anomalous response to ammonium thiosulphate chelation, at high pH, is a function of the nickel-bearing complex occluding the metal from binding to ammonium thiosulphate, or that other metals are complexing more strongly with ammonium thiosulphate. This hypothesis would require total element analysis using ICP measurements.

The ammonium-acetate-extractable nickel fraction expressed as a percentage of the total nickel concentration of these materials, when compared with that observed in the Boddington Gold materials confirms that the total metal concentration has little bearing on the bioavailable metal fraction in a substrate.

The predictive ability of ammonium-acetate extraction in determining the bioavailable metal fraction of these laterites is consistent with Robinson (1997). The RO water extractions produced similar results to ammonium acetate extractions for most laterite samples. This leads me to conclude that an RO water extraction would be sufficient to determine the bioavailable nickel fraction in these laterites.

4.4.4 Cobalt Bioavailability as Determined by Solvent Extractions

Table 15 shows the concentrations of cobalt removed (in mg/kg) by end-over-end solution extraction using RO water, ammonium acetate, ammonium thiocyanate and ammonium thiosulphate buffered to neutral pH.

Table 15. Cobalt extractability (n=4) as determined by end-over-end solution extractions. Concentrations in mg/kg (ppm). Standard Errors is approx. ±15%.

Ore Type	pH	RO H_2O	NH_4 Acet.	1% SCN	1% S_2O_3
FZ	7.7	0.09	0.06	0.1	0.71
SAP	8.5	1.04	<0.005	0.07	0.6

At a substrate pH of 7.7, RO water, ammonium acetate and ammonium thiocyanate removed similar amounts of cobalt from the FZ material. The amount of cobalt removed by ammonium thiosulphate was higher compared to ammonium thiocyanate indicating that ammonium thiosulphate is better suited to complexing cobalt from this material. This extraction relates to the chemical reactivity of the cobalt host mineralogy in association with ammonium thiosulphate.

At a substrate pH of 8.5, a 1 M solution of ammonium acetate was unable to remove a detectable amount of cobalt from the SAP material. The amount of cobalt removed by ammonium thiosulphate is higher than that removed by ammonium thiocyanate indicating that ammonium thiosulphate is more efficient at chelating cobalt in this material. The unusually high RO water extraction indicates that cobalt in present in a soluble form.

4.4.5 Gold Bioavailability as Determined by Solvent Extractions

Table 16 shows the concentrations of gold removed (in μg/kg) by end-over-end solution extraction using reverse osmosis (RO) water, ammonium acetate at a 1 mol concentration, ammonium thiocyanate at sequential concentrations and ammonium thiosulphate at similar sequential concentrations.

Table 16. Gold extractability (n=4) as determined by end-over-end solution extractions using deionised water, ammonium acetate, ammonium thiocyanate and ammonium thiosulphate. Concentrations are in mg/L (ppm). Standard Errors is approx. ±42%.

Ore Type	Tot. Au	RO H_2O	NH_4 Acet.	1% SCN	1% S_2O_3
FZ	0.7	<0.001	<0.001	<0.001	0.011
SAP	0.2	<0.001	0.001	<0.001	<0.001

The ammonium thiocyanate gold extractability from both materials (FZ and SAP) was undetectable using the analytical methods described in the Methods Section of Chapter One.

The FZ extraction data indicate that ammonium thiocyanate was unable to mobilise gold from this laterite material containing 0.7 mg/kg gold in an end-over-end extraction. Ammonium thiosulphate removed a detectable, but very small concentration of gold in the FZ material. This reflects the low total gold concentration and alkaline pH dependence of ammonium-thiosulphate gold chelation.

4.4.6 Lateritic Chelator-available Gold

Both ammonium thiocyanate and ammonium thiosulphate were capable of mobilising gold from the laterites. Under acidic substrate conditions, represented by the Boddington Gold Mine samples, both compounds were capable of removing measurable gold concentrations. In the more alkaline substrate conditions of the Anaconda Nickel samples, only ammonium thiosulphate was capable of removing a detectable gold concentration. The absence of ammonium thiosulphate extractable gold from the SAP material reflects the low total gold concentration of this material. This confirms previous research reporting pH dependence on gold chelation for ammonium thiosulphate. The efficiencies to gold chelation of these compounds indicate that mildly acid conditions are favoured by both. The chelator efficiencies of both compounds decrease considerably in alkaline substrate conditions. At a pH of 4.9 and gold concentration of 0.5 mg/kg, ammonium thiosulphate extracted 0.1 mg/kg of gold from the Boddington Gold Mine B5 laterite. At a pH of 7.7 and similar gold concentration of 0.7 mg/kg the same chelator removed only 0.01 mg/kg gold from the Anaconda Nickel Ltd. FZ laterite. This represents an order of magnitude decrease in chelator efficiency over the pH range 4.5-7.7. Ammonium thiocyanate was unable to remove any detectable gold from the alkaline Anaconda Nickel laterites.

4.5 Nickel and Cobalt Accumulation by *Alyssum bertolonii* grown on Lateritic Substrates

The objective of this experiment was to investigate the species' potential for nickel and cobalt hyperaccumulation from naturally occurring sub economic nickel-cobalt mineralisation. This material is currently being removed to access higher-grade mineralisation at the Murrin Murrin Project nickel and cobalt mine in Western Australia, owned and operated by Anaconda Nickel Ltd.

It was hypothesised that *Alyssum bertolonii* could provide a method of further metal recovery from existing Murrin Murrin operation. It was hoped to determine if sub economic mineralisations and waste laterite (containing nickel and cobalt) could be successfully treated using a known nickel-hyperaccumulating plant to extract both metals.

4.5.1 Nickel Accumulation in *Alyssum bertolonii* grown on Lateritic Substrates

Nickel accumulation on the auriferous materials (B5, M4 and Z1) supplied by Boddington Gold Mine show poor accumulation (Figure 14) potential. This reflects the low bioavailable metal fractions for B5 and Z1, but does not explain the poor response from the M4 material. The M4 material had an ammonium-acetate-extractable nickel level similar to both Anaconda Nickel samples but accumulated only one third the nickel of FZ and less than a tenth that of SAP. The response by *Alyssum bertolonii* when grown upon the nickeliferous laterites supplied by Anaconda Nickel, indicates a better potential to accumulate nickel than the Boddington Gold materials.

Similar levels of bioavailable nickel, as determined by ammonium acetate extraction, exist between both FZ and SAP laterites, and the Boddington B5 laterite. The artificial nickel-cobalt mixed substrate experiment (Chapter Two, Section 2.4, Figures 8 & 10) using *Alyssum bertolonii* indicated that at similar substrate-nickel ammonium acetate extractabilities (≈2 mg/kg), this plant would accumulate ≈20 mg/kg nickel. The SAP laterite appears to be the only material from which *Alyssum bertolonii* is able to hyperaccumulate nickel to the threshold concentration of >1000 mg/kg without requiring substrate modification.

The hyperaccumulation responses from *Alyssum bertolonii* grown on the laterites is clearly different to the artificial substrate results. The laterite response suggests the presence of hyperaccumulation-inhibiting elements, such as calcium or magnesium, occur in the laterites. Analyses show that the SAP laterite contains appreciable magnesium (9.7%) which is known to inhibit nickel uptake in *Berkheya coddii* (Robinson *et al.*, 1999a). *Alyssum bertolonii* appears unaffected by a similar level of substrate magnesium. I can only conclude that other controls on nickel accumulation from a laterite substrate with this level of ammonium acetate extractable nickel exist outside the known effects of pH, magnesium and calcium. The relative concentration of substrate iron may represent a logical place to begin searching for the accumulation control indicated here. Although identification of further nickel accumulation controls is beyond the scope of this project, the Boddington Gold Mine laterites and the Anaconda Nickel FZ laterite are visibly iron-rich. Other elements may also be present that are influencing the accumulation potentials observed in this investigation.

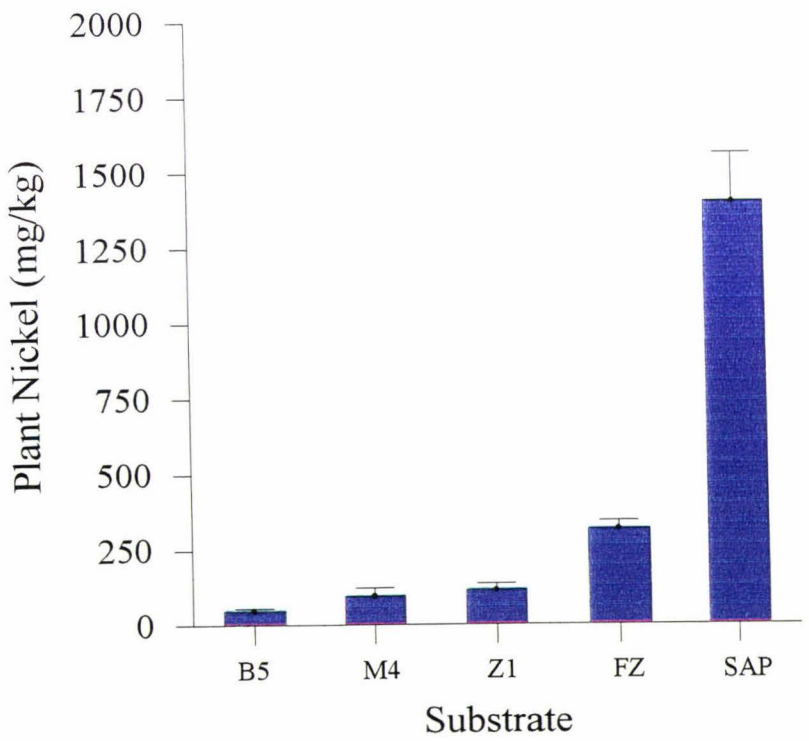

Figure 14. Nickel hyperaccumulation (n=8) by *Alyssum bertolonii* grown on Western Australian laterites. Error Bars are Standard Error of the Mean.

It should be noted that an amendment of an acidic compound (elemental sulphur or perhaps locally available acid mine tailings) to lower the pH may dramatically improve the hyperaccumulation potential of *Alyssum bertolonii* when grown on these materials. Given the total nickel concentration of these laterites I believe this work should be carried out in the near future to assess the feasibility of a small mine-site-based phytoextraction operation.

4.5.2 Cobalt Accumulation in *Alyssum bertolonii* grown on Lateritic Substrates

The figure below (Figure 15) shows the cobalt accumulation response for *Alyssum bertolonii* when grown upon the five lateritic materials from Western Australia.

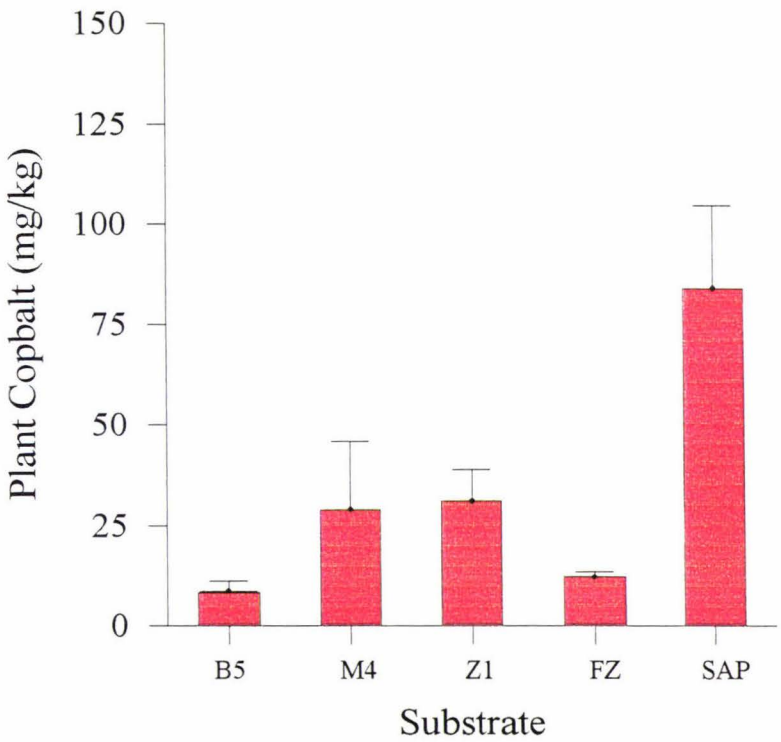

Figure 15. Cobalt accumulation (n=8) by Alyssum bertolonii grown on Western Australian laterite. Error Bars are the Standard Error of the Mean.

The response data indicate that none of the lateritic materials is capable of inducing cobalt hyperaccumulation in *Alyssum bertolonii*. The plant, however, did respond well to the SAP laterite. This level of cobalt accumulation is similar to that found in many species from the copper-cobalt flora of Zaïre. Brooks *et al.* (1980) discussed cobalt accumulation in this metallogenic province and reported

hyperaccumulation of cobalt on substrates containing <1% cobalt. The nickeliferous substrates under investigation contained 0.1% cobalt in FZ and 0.15% cobalt in SAP. Therefore the observed cobalt accumulation of 12 mg/kg and 84 mg/kg respectively may be indicative of *Alyssum bertolonii* response to this substrate concentration.

The influence of unknown accumulation controls cannot be ignored. The solvent extractions used to indicate cobalt bioavailability did not indicate the observed accumulation response in *Alyssum bertolonii*. A pH dependence involved in cobalt accumulation cannot be correlated between the laterites. The effect of appreciable substrate magnesium and calcium on cobalt accumulation in *Alyssum bertolonii* has not been studied previously. The ratio of magnesium to calcium may indicate a possible control on cobalt accumulation. As the ratio of magnesium to calcium approaches 100 the level of cobalt accumulation is seen to rise, as indicated by the Boddington Gold Mine samples and the Anaconda Nickel SAP sample. Above a magnesium-calcium ratio of 100 cobalt accumulation decreases considerably.

Much work needs to be done on the influences involved in cobalt hyperaccumulation by *Alyssum bertolonii*. Determination of the *Alyssum-bertolonii*-available cobalt fraction of a substrate also requires further experimentation as does any possible methods of increasing the bioavailable cobalt fraction.

The definition of a nickel, and more recently cobalt, hyperaccumulating species as described by Brooks *et al.* (1977) is that the plant must accumulate the metal to a concentration of >1000 mg/kg in dry herbage. Perhaps a more prudent method of describing these unusual plants would be to classify the plants metal as a function of the bioavailable metal content of the substrate. This would be a measure of the hyperaccumulation potential of the substrate related directly to the quantitative extractability of the plant when grown upon it. Classification of the above data on *Alyssum bertolonii* grown on SAP (containing 2165 mg/kg total cobalt) laterite would then represent cobalt hyperaccumulation.

4.5.3 The General Accumulation Response of *Alyssum bertolonii* when grown on Metalliferous Laterites from Western Australia

The cobalt accumulation responses of *Alyssum bertolonii* to the auriferous lateritic material (B5, M4 and Z1) indicates that although the total concentrations of

cobalt were much higher than those for nickel, the plant appears to preferentially accumulate nickel by a factor of approximately four. The accumulation response of *Alyssum bertolonii* to the nickeliferous lateritic material (FZ and SAP) indicates that an even stronger preference for nickel accumulation is in effect. The level of preferential nickel accumulation appears to be approximately 12 times the amount of cobalt accumulated from the same material.

Modification of the pH of these laterites by addition of a locally available amendment, such as acid mine tailings, would be a cost-effective method of improving the nickel hyperaccumulation potential of these materials. This type of experimental work should be carried out as the total metal concentrations of nickel and cobalt in the laterites is sufficient for phytoextraction.

Given the negative effects on nickel hyperaccumulation in *Berkheya coddii* by the presence of calcium and magnesium, it is now necessary to determine the effect of these elements on nickel and cobalt hyperaccumulation in *Alyssum bertolonii*.

4.6 Nickel and Cobalt Phytomining – A best-case scenario

It should be noted that the ammonium-acetate-extractable nickel in the laterite samples was similar by comparison with previously published data. Robinson (1997a) reported nickel uptake by *Alyssum bertolonii* of approximately 1800 mg/kg for an ammonium-acetate-extractable substrate nickel concentration of 1.7 mg/kg. The average level of plant nickel achieved during this trial was 1399 mg/kg for the SAP laterite having an ammonium acetate extractable nickel concentration of 1.6 mg/kg. Oddly enough, if we assume that ammonium acetate extractability is our bioavailable metal indicator, both FZ and M4 laterites have comparable levels of nickel extractability yet produced significantly lower levels of nickel accumulation. It must be assumed that other chemical influences are affecting the accumulation potential of this plant in these substrates.

Cobalt accumulation was low, reflecting the lower accumulation coefficient of this metal with respect to nickel. Ammonium-acetate- and RO-water-extractable cobalt indicated that Z1 and SAP laterites had a bioavailable cobalt fraction above 1 mg/kg. The artificial substrate trial discussed in Chapter Two indicates that this level

of ammonium acetate extractability should produce cobalt accumulation to approximately 300 mg/kg for a mixed nickel-cobalt substrate. The observed plant cobalt concentration 84 mg/kg would suggest that other substrate-related influences are affecting accumulation. A polymetallic phytoextraction scenario based on the above data for *Alyssum bertolonii* would be as follows;

Assuming an annual unfertilised biomass production of 9 tons per hectare the amount of nickel and cobalt yielded would be 13 kg and 0.8 kg respectively. The current price of nickel is US$ 10.50/ kg and US$ 33/kg for cobalt. This equates to a nickel revenue of US$ 137 and a cobalt revenue of US$ 26 or a total metal revenue of US$ 163 per hectare.

4.7 Nickel Accumulation by *Berkheya coddii* grown on Lateritic Substrates

Responses to the auriferous materials (B5, M4 and Z1) supplied by Boddington Gold Mine and the nickeliferous FZ material supplied by Anaconda Nickel Ltd. show a poor accumulation potential (Figure 16) as determined by ammonium acetate extraction. The M4 and FZ responses are anomalous given their ammonium-acetate-extractable nickel concentrations are similar to that of the SAP material. This may indicate the presence of accumulation-inhibiting elements other than magnesium and calcium. Both M4 and FZ laterites are highly ferruginous materials originating from the upper laterite profile. The abundance of iron may be influencing the hyperaccumulation potential of *Berkheya coddii*. Experimentation on this hypothesis should be carried out.

The response by *Berkheya coddii* to the nickeliferous laterite SAP, supplied by Anaconda Nickel, produced a weak hyperaccumulation response as one would expect from this species under such low bioavailable nickel concentrations. The SAP laterite is the only material capable of inducing *Berkheya coddii* to hyperaccumulate nickel to the required plant concentration of >1000 mg/kg. In light of work by Robinson *et al* (1999a) on the effects of magnesium and calcium on nickel hyperaccumulation in *Berkheya coddii* it would appear that the magnesium concentration of the SAP substrate (9.7%) should have significantly reduced nickel accumulation to approximately 150 mg/kg. Likewise, an alkaline pH of 8.5 should have significantly reduced accumulation of nickel, and cobalt. The ammonium-

acetate-extractable nickel concentration from the SAP substrate was 1.56 mg/kg. This level of ammonium acetate extractability in the artificial substrate experiment produced accumulation of 10 mg/kg in the nickel-only substrate and 40 mg/kg in the mixed nickel-cobalt substrate.

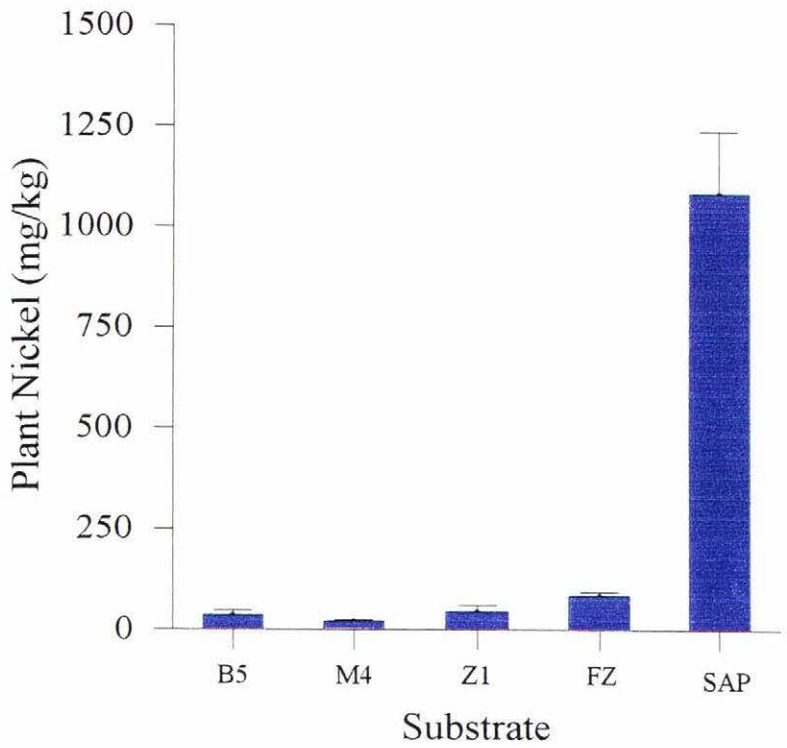

Figure 16. Nickel hyperaccumulation by *Berkheya coddii* grown on Western Australian laterite (n=8).

The above factors should have resulted in a very poor nickel uptake response by *Berkheya coddii*. Possible explanations for this hyperaccumulation response are briefly outlined below.

1. Nickel-binding ligands produced by the roots of *Berkheya coddii* could be stripping nickel from its host mineralogy more efficiently than was detected by ammonium acetate extraction.
2. The apparent lack of a detrimental effect to nickel accumulation caused by a high magnesium concentration in the SAP material probably reflects the relatively poor solubility of magnesium in the laterites compared to the carbonates used by Robinson *et al.* (1999a). The rhizospheric effect (outlined above), caused by ligand production in the roots of *Berkheya coddii*, could also be masking the negative effect of magnesium.

3. However, the most likely explanation for this accumulation response is that the relatively high pH (8.5) of the SAP substrate is reducing cation mobility (bioavailability) of other elements resulting in relatively high nickel accumulation due to the high abundance of nickel in this material. Hedley *et al.* (1982) reported from a study of major cations and anions taken up by rape plants (*Brassica napus* var. Emerald) that in periods of lower pH conditions in the rhizosphere, the plants absorbed more cations than anions resulting in a further decrease in pH.

4.7.1 Cobalt Accumulation of *Berkheya coddii* grown on Lateritic Substrates

The B5 laterite supplied by Boddington Gold Mine and the SAP laterite supplied by Anaconda Nickel Ltd., indicate a weak accumulation potential when compared to the remaining three substrates (M4, Z1 and FZ). Bioavailable cobalt determination using the different solvent extractions failed to correlate with plant accumulation responses. The cobalt accumulation response (Figure 17) for *Berkheya coddii* when grown on the B5 laterite supplied by Boddington Gold Mine and the SAP laterite solvent extractions failed to identify the observed accumulation response by *Berkheya coddii*. The artificial nickel-cobalt mixed substrate experiment indicated that an ammonium acetate extractable cobalt concentration of 2 mg/kg in the substrate would produce a cobalt accumulation up to 150 mg/kg in the plant. Therefore the observed level of cobalt accumulation for the SAP substrate can be assumed to be indicative of this species.

Cobalt accumulation by *Berkheya coddii* grown on the B5 substrate may relate to a root-produced ligand process stripping cobalt from the substrate and/or promoted by a low pH of 4.5. The preferential accumulation of cobalt in the presence of nickel, identified in the artificial substrate experiment, cannot be ignored in light of these data. The B5 substrate contained twice as much cobalt as nickel which would result in an over-emphasis of this response. The SAP substrate contained considerably more nickel than cobalt, which would suppress the interference relationship, resulting in a higher nickel accumulation than cobalt.

The accumulation responses therefore may, in part, be related to cobalt-bearing mineralogies within this substrate and their reactivity within the rhizosphere. The influence of other unknown controls upon cobalt accumulation must also be

considered as important and further work to this end should be carried out, considering the global price of cobalt is more than three times that of nickel.

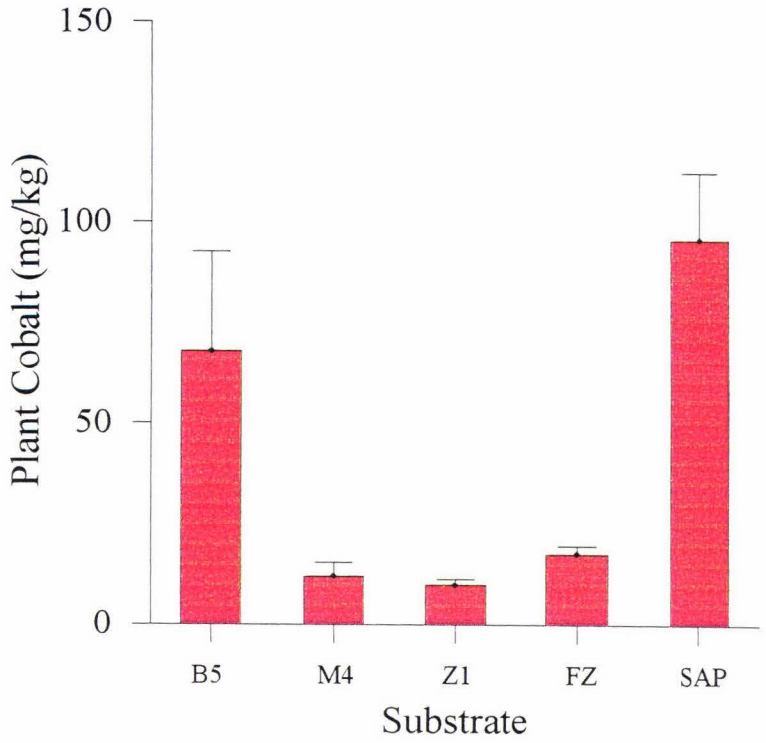

Figure 17. Cobalt hyperaccumulation by *Berkheya coddii* grown on Western Australian laterite (n=8).

4.7.2 The General Accumulation Response of *Berkheya coddii* when grown on Metalliferous Laterites From Western Australia

Nickel accumulation by *Berkheya coddii* grown on the Boddington Gold Mine samples was very poor (Figure 16). Ammonium-acetate extraction indicated that the M4 material had sufficient bioavailable nickel (1.8 mg/kg) to support hyperaccumulation. Robinson *et al* (1997b) reported that accumulation of approximately 1800 mg/kg could be achieved from a similar substrate concentration. The level of accumulation observed on M4 laterite cannot be explained in terms of the test criteria (Table 9) used to define the controls on nickel accumulation in this experiment.

The pH of the laterite is within normal plant growth and metal bioavailability limits. A relatively low total nickel concentration accompanied by sufficient RO water, ammonium acetate, and ammonium thiocyanate extractability indicate that M4 should have produced a discernible accumulation response. The concentrations of magnesium and calcium are insufficient to influence accumulation. I can only

assume that other chemical properties of this material are inhibiting nickel accumulation in *Berkheya coddii* possibly caused by the presence of high concentrations of iron (or other elements) in the substrate effecting the plants ability to accumulate the metal or possible to produce the metal binding ligands required for metal uptake.

The Anaconda Nickel material produced accumulation in the SAP laterite only. Nickel bioavailability as determined by ammonium-acetate extraction indicated that both substrates had the potential to produce hyperaccumulation. The fact that the FZ laterite produced a higher concentration of ammonium-acetate-extractable nickel and yet could not support nickel hyperaccumulation is curious. The pH of the substrate should have been suitable to produce a discernible accumulation response. This again may indicate the effect of high iron oxides concentrations upon the hyperaccumulation potential of these plants. The SAP response is unusual, even though the total nickel concentration was 2.7%, the bioavailable nickel fraction was only 1.7 mg/kg. Therefore, the SAP laterite has the same hyperaccumulating potential as FZ and M4 and most of the nickel is bound in crystalline lattices.

The apparent lack of a detrimental effect to nickel accumulation caused by a high magnesium concentration in the SAP material probably reflects the relatively poor solubility of magnesium in the laterites compared to the carbonates used in the recent report by Robinson *et al.* (1999a).

Discernible cobalt accumulation occurred on the B5 and SAP substrates only. The level of accumulation in *Berkheya coddii* was low by any standard at 68 mg/kg and 96 mg/kg respectively, probably reflecting the cobalt accumulation coefficient relative to ammonium-acetate-extractable cobalt in these substrates. The use of ammonium acetate in determining the bioavailable cobalt fraction assumes that cobalt has similar chemical properties to nickel. This assumption allows for a loose correlation of results between studies. The response of *Berkheya coddii* grown on either laterite should have been highlighted by the ammonium-acetate extraction. Evidence of B5 or SAP having an appreciable substrate accumulation potential was not detected during the solvent extractions. Conversely, the Z1 laterite had

appreciable ammonium-acetate-extractable cobalt but failed to support an appreciable accumulation response.

The above data lead to two conclusions,

1. Ammonium-acetate-extractable cobalt is not a suitable method of determining the bioavailable cobalt fraction from the laterite samples investigated.
2. The plant-available cobalt fraction and accumulation potential of these laterites is controlled, in part, by hitherto unknown properties, possibly a high iron oxide concentration.

4.7.3 Nickel and Cobalt Phytomining – A best-case scenario

An average plant nickel concentration of 1081 mg/kg was achieved on the SAP laterite, which had an ammonium acetate extractable nickel concentration of 1.6 mg/kg.

Cobalt accumulation of 96 mg/kg was achieved by *Berkheya coddii* on the SAP substrate. The artificial substrate trial discussed in Chapter Two indicates that a similar level of solvent-extractable cobalt would produce accumulation to a similar level in a mixed nickel-cobalt substrate. The cobalt accumulation response of *Berkheya coddii* grown on this substrate is therefore considered to be representative of this plant's accumulation potential. A polymetallic phytoextraction scenario based on the above data for *Alyssum bertolonii* would be as follows;

Assuming an annual unfertilised biomass production of 22 tons per hectare the amount of nickel and cobalt yielded would be 23.8 kg and 2.1 kg respectively. The current price of nickel is US$ 10.50/kg and US$ 33/kg for cobalt. This equates to a nickel revenue of US$ 250 and a cobalt revenue of US$ 69 or a total metal revenue of US$ 319 per hectare.

4.8 Conclusions

Ore quality determined that the total concentrations of nickel and cobalt were well within the range of substrate concentrations necessary for accumulation to

occur. Reagent extractability identified M4, FZ and SAP materials as having suitable bioavailable nickel fractions of >1 mg/kg. This experimental work also indicated that a deionised water extraction and a 1% solution of ammonium thiocyanate removed similar amounts of nickel from the laterites as a 1 M ammonium-acetate extraction. This leads me to conclude that a water extraction is just as effective at determining the bioavailable nickel fraction from a laterite as ammonium acetate.

Ammonium-acetate-extractable cobalt indicated that Z1 and SAP had potential to produce discernible accumulation in both species. However, the level of bioavailable cobalt is significantly lower than reported under hyperaccumulators in the wild. Artificial substrate experiments indicated that an ammonium-acetate-extractable cobalt concentration of 2 mg/kg from a mixed nickel-cobalt substrate would induce *Berkheya coddii* to accumulate 150 mg/kg cobalt. *Alyssum bertolonii* required an ammonium acetate extractable concentration of 2 mg/kg to phytoextract 25 mg/kg.

There was no correlation between ammonium-acetate, RO-water and ammonium thiocyanate extractions for determination of the bioavailable cobalt in the laterites investigated. Often a deionised water extraction, indicating a soluble cobalt fraction, did not compare favourably with a weak aqueous solution of ammonium acetate or ammonium thiocyanate. Ammonium-thiocyanate cobalt extractability was affected by changing substrate pH. An effective solvent extractant was not found to indicate a substrate's accumulation potential. This conclusion reflects a lack of understanding of cobalt's chemical behaviour in the presence of these compounds and the controls on cobalt extractability and accumulation in lateritic substrates.

Gold extractability using ammonium thiocyanate and ammonium thiosulphate indicated that both compounds would remove gold from the laterites. However, ammonium thiocyanate failed to complex gold in the alkaline laterites supplied by Anaconda Nickel Ltd., reflecting a pH dependence for gold chelation using this chelator. A pH dependency of gold chelation was also observed for ammonium thiosulphate. The B5 and FZ samples had similar total gold concentrations, but significantly different gold extractabilities. Ammonium thiosulphate also showed a minimum detectable gold extractability related to a minimum total gold concentration of approximately 0.2 mg/kg in the substrate. Above this substrate

concentration, the total gold concentration had little effect on ammonium thiosulphate extractability.

Both *Alyssum bertolonii* and *Berkheya coddii* hyperaccumulated nickel from the SAP substrate and accumulated cobalt to approximately 100 mg/kg dry weight. The accumulation responses for the other substrates were poor by comparison. The Boddington Gold Mine laterites all contained considerably more cobalt compared to nickel which resulted in suppression of nickel accumulation in these substrates. The Anaconda Nickel Ltd. samples had nickel concentrations 10 times those of cobalt, indicating that cobalt interference should not lower nickel accumulation. This assumption applies only if the nickel-cobalt interference relationship is based on the ratio of these two elements in the substrate and not their absolute concentrations. Ammonium acetate identified M4, FZ and SAP as having suitable nickel bioavailability. Cobalt extractability indicated that only Z1 had a plant-available cobalt fraction that would result in discernible cobalt accumulation. The level of cobalt extractability was well below the level required to hyperaccumulate this metal using either plant.

Plant accumulation responses did not reflect the bioavailable metal concentrations determined by reagent extraction. Nickel extractability should have resulted in hyperaccumulation on M4, FZ and SAP laterites. The poor response from M4 and FZ indicates the presence of, as yet unknown, accumulation-inhibiting elements. Both laterites have high concentrations of iron which may be influencing the hyperaccumulation potential in both *Alyssum bertolonii* and *Berkheya coddii*. However, this hypothesis requires further experimentation to determine its validity. Reagent-extractable cobalt identified Z1 laterite as having an accumulation potential, although not sufficiently high enough to produce hyperaccumulation. This extractability was not reflected in plant-accumulation levels for either species. Appreciable cobalt accumulation occurred on B5 and SAP laterites. The level of reagent-extractable cobalt and consequent anomalous accumulation responses from both *Alyssum bertolonii* and *Berkheya coddii* was possibly caused by elemental interference to cobalt accumulation in the laterites. Interference caused by high magnesium or calcium concentrations (Robinson *et al.*, 1999a) does not appear to explain the observed plant-uptake responses. Controls on cobalt accumulation by

Alyssum bertolonii and *Berkheya coddii* when grown on these laterites is therefore unknown and should be investigated with vigour because it is over twice the value of nickel.

The level of metal production calculated from both phytomining scenarios using SAP laterite from Anaconda Nickel Ltd. would be improved by two methods at the Murrin Murrin mine site:

1. Application of an acidic compound, such as locally available acid-mine tailings, would lower the substrate pH and increase the bioavailability of both metals thus increasing plant accumulation levels.

2. Application of a fertiliser, such as mine camp effluent, would promote plant growth and increase biomass production in *Berkheya coddii*, thus improving the metal yield.

It is believed that a series of small field trials, at one hectare scale, designed to ascertain the above effects should be carried out.

Another approach to improving the metal production of the polymetallic phytoextraction programme would be to cultivate both *Alyssum bertolonii* and *Berkheya coddii* on the same substrate. As indicated above, both species show hyperaccumulation potential on the SAP laterite. Little is known regarding the changes in biomass production resulting from this type of horticultural practice. However, if *Alyssum bertolonii* could produce 50% of the biomass, generated when grown individually, under a canopy of *Berkheya coddii*, the metal production revenue per hectare would increase to US$ 400. The Murrin Murrin 9 open pit currently being mined has a surface expression of 5 km by 9 km, or 4,500 hectares. If this open pit, once excavated to the SAP interface, were planted under the above conditions the metal yield would be worth US$ 1.8 million.

Extraction of the metal/s from the plant tissues raises the question, 'what type of metallic product should, or could, be produced?"

The most publicised method of extraction is combustion to produce a high-grade oxide 'bio-ore', which is then treated in a similar fashion to existing ores to produce a finished metal (perhaps coupled to a small thermal power station to increase revenue). In South Africa, a major nickel mining company is currently phytoremediating nickel contaminated soils using *Berkheya coddii* and extracting the recovered nickel by blending the plant material with existing nickel ores prior to metal recovery in the milling circuit.

Another alternative would be to ascertain a suitably valuable metallic compound, such as nickel nitrate, that could be easily produced from the simple heavy metal storage compounds found in these plants to maximise the return.

It should also be noted that the revenue derived from phytoextracting these metals may not necessarily represent an additional component to existing metal production levels, but simple provide the capital to finance a rehabilitation programme.

4.8.1 Further research arising from this project.

The efficiency of ammonium thiosulphate at removing cobalt was not effected by substrate pH. The ammonium thiosulphate extractability of cobalt in the laterites indicated low levels of cobalt mobility in the range 0.4-0.7 mg/kg for substrate cobalt concentrations ranging from 1269 to 2165 mg/kg and could be used to model cobalt bioavailability in these laterites. This would require further experimental work.

The artificial substrate experiment identified an interference relationship where cobalt is preferentially absorbed in the presence of nickel at equal concentrations to a substrate concentration of approximately 350 mg/kg. The value of cobalt metal is considerably higher than that of nickel and research on the controls of, and improvement to, cobalt phytoextraction from naturally occurring mineralisations of nickel and cobalt should be continued in earnest.

The relationship, if any, between substrate iron, and other cation and anion, concentrations and nickel and cobalt accumulation potentials should be determined to indicate whether or not this has influenced the accumulation levels observed in this experiment.

The accumulation potential of the SAP material should have been considerably lower than observed in appreciation of our current understanding of pH and elemental effects on plant accumulation. Given the harsh substrate conditions of the SAP laterite both plants hyperaccumulated nickel. This raises the question, what level of nickel phytoextraction could be achieved from this substrate if the pH was lowered to a more plant-amenable level? Further research concerning pH modification should be carried out on the material.

Chapter Five - Ammonium Thiocyanate Induced Gold Accumulation using Lateritic Substrates

5.1 Introduction

During the development and exploration of a bauxite resource near Boddington, 130 km south-east of Perth, Western Australia a significant gold mineralisation was discovered within the laterite profile. By 1988 the mineable reserve had been delineated as a staggering 45 Mt averaging 1.8 ppm (mg/kg) gold or 2.7 Moz of gold contained. This was the first major gold resource discovered in a lateritic terrain and/or associated with bauxite mineralisation and fuelled an extensive re-evaluation of existing exploration data and target definition procedures. Trace element signatures in lateritic terrains are now used almost exclusively in lateritic gold exploration.

The development of the Boddington Gold Mine served to establish laterite gold mining technologies worldwide, from which numerous other successful laterite gold operations followed. The eventual resource uncovered at Boddington was much larger than the initial 2.7 Moz delineated. Recent depletion of laterite ores at the site prompted a 'deeps' exploration drilling programme which has uncovered a world class hard-rock resource of 15.5 Moz, now in the final stages of mine planning evaluation.

Boddington Gold Mine has a considerable stockpile of sub economic and auriferous waste material, including mine tailings which now must be rehabilitated in accordance with Western Australian mining law. Rehabilitation of drastically disturbed land, such as those found in mining environments, is an expensive exercise since there is no financial benefit to the mining company concerned. Hyperaccumulating plants offer a short term solution to financing a rehabilitation programme by yielding a metal commodity. Often these plants can ameliorate current ground conditions allowing other species to flourish. The discovery of thiocyanate-induced gold accumulation in plants by Anderson *et al.* (1998) sparked

interest in the possibility of 'farming a crop of gold'. Numerous glasshouse trials have been undertaken at Massey University to investigate chelator-induced gold accumulation. Some used artificial substrates amended with gold solutions or fine elemental gold powder and others used auriferous acid mine tailings. This investigation focuses on the use of existing auriferous lateritic material from Boddington Gold Mine (grading from 0.2-1.6 mg/kg gold) as substrates for an induced gold uptake plant trial.

The final substrate metal concentrations for the laterites after preparation of the media for planting are shown in Table 1. Once the laterites were air dried and crushed to below 5 mm, two parts of the laterite was blended with one part of finely sieved pumice to 'lighten' the substrate. Several laterites had a high clay content and one had a very low clay content prompting the decision to blend pumice into the media to promote root development and general plant growth for this three month trial.

Table 17. Revised gold concentations for media used in an induced gold accumulation pot trial, including pH. Concentrations in µg/kg. Standard Errors is approx. ±51%.

Ore Type	pH	Total Gold	Growth Media Gold
B5	4.9	510	340
M4	6.4	1616	1077
Z1	5.3	210	140
FZ	7.7	680	453
SAP	8.5	200	133

The blended media now have average gold concentrations of ca. 1000 µg/kg or less. These concentrations are more akin to current sub economic mineralisation that falls outside modern mining standards. Previous experimentation on induced gold accumulation using chelation has dealt with substrate gold concentrations in the range of 1000-5000 µg/kg, making these substrates economic by today's modern mining methods and thus would not be given over to phytomining technology. These concentrations are suited to investigating the effects of different induction techniques because they afford a readily available source of substrate gold. The substrates investigated here represent low gold concentrations in naturally occurring waste materials and are therefore better suited to studying the feasibility of an actual induced gold-accumulation field trial.

The experiment is divided into two parts. The first experiment (Pretreatment) was carried out to further understand gold mobility in the laterites and to determine chelator potential for these laterite ores so that a suitable application protocol could be developed. The second experiment carried forward the trends uncovered in the pretreatment experiment and extended these while trialing a statistically robust sample population.

5.2 Induced Gold Accumulation – Pretreatment Experiment

The solvent extractable gold data (Table 12) illustrates the low bioavailable gold fraction, determined using ammonium thiocyanate and ammonium thiosulphate, at natural pH levels of the lateritic material. Given the requirements of pH-dependent gold solubility and chelator efficiency, it now became apparent that insufficient gold mobilisation would occur using a neutral solution of chelator or without first modifying the substrates pH using an acidic amendment.

The decision to continue experimentation using ammonium thiocyanate only was taken because the resultant size of the trial using both chelators was beyond the scope of this project. Ammonium thiocyanate was chosen in preference to ammonium thiosulphate because this chelator favours the acid conditions necessary for gold solubility. It was therefore decided to test the effectiveness of applying an amendment of acidified ammonium thiocyanate in an attempt to temporarily reduce the substrate's pH sufficiently to mobilise gold while allowing the plant to survive long enough under such harsh conditions to accumulate the chelated metal.

This experiment determines the most effective method for delivering the chelating compound ammonium thiocyanate to a laterite ore for induced accumulation of gold.

The species under investigation (*Acacia longifolia*, *Brassica juncea*, *Linum usitatissimum* and *Iberis intermedia*) were chosen for the following reasons.

The use of *Acacia longifolia* was undertaken to investigate the potential of a native Western Australian species to induced accumulation of gold using ammonium thiocyanate. Because I was supplied metalliferous material from Western Australia I

wanted to investigate a native species from this area so that any further work arising from this preliminary investigation could be performed in the field without consideration of the impact of exotic species on the environment. This plant has also been reported as cyanogenic by Seigler *et al.* (1989).

The decision to use *Brassica juncea* was based solely on previous work carried out by Anderson *et al.* (1998) on induced accumulation of gold by this species using ammonium thiocyanate as a chelating agent.

The use of *Linum usitatissimum* in this investigation was to determine the potential of this highly cyanogenic species to induced accumulation of gold using ammonium thiocyanate. This species produces large quantities of thiocyanate from it's roots in response to fungal attack (Reynolds, 1931). Thiocyanate levels in this plant are also known to increase considerably during flowering. It was hypothesised that this physiological response and subsequent production of thiocyanate in the rhizosphere may promote the uptake of thiocyanate solubilised gold.

The decision to use *Iberis intermedia* in this investigation was made subsequent to results from the artificial induced gold trial (see Chapter Three). This known hyperaccumulator of thallium (Leblanc *et al.*, 1997) responded exceedingly well to ammonium thiosulphate induced gold accumulation and its response to ammonium-thiocyanate-induced gold solubilisation was also significant.

Table 18 identifies the plant-substrate combinations investigated in this induced gold accumulation pretreatment experiment. Ammonium thiocyanate will induce gold accumulation in almost any plant species. Total gold concentrations of the laterites (Table 8 and Table 13) indicated that all were suitable for an induced gold hyperaccumulation trial experiment. The number of plant-substrate combinations tested here was dictated by the availability of plants required to numerically support the actual trial. The pretreatment experiment used substrate amendments of 10 mL 2M HCl (T1), a 1% solution of ammonium thiocyanate (T2) and a combination of both a 1% solution of ammonium thiocyanate and 10 mL 2M HCl (T3).

Acacia longifolia responded positively to all treatments. The general response trends determined from herbage analyses to come out of the study are that,

1 With the exception of the SAP material only, all responses show an increase in gold accumulation with the addition of hydrochloric acid.

2 The effect of the T2 treatment was relatively small in comparison with T1 indicating that ammonium thiocyanate has little effect on gold mobility at native pH levels.

3 The T3 treatment indicated that acidified ammonium thiocyanate greatly improved the accumulation potential of gold relative to the T2 treatment.

4 Substrate acidity controls chelator potential and subsequent bioavailability of gold.

Table 18. Induced gold accumulation experiment pretreatment (n=3) using 2M HCl (T1), 1% NH_4 SCN (T2) and a combination of both (T3), including total substrate gold and pH. Concentrations are in µg/kg (ppb). Standard Errors is approx. ±68%.

Ore Type	Tot. Au	pH	Treat.	*Acacia longifolia*		*Brassica juncea*	*Linum usitatissimum*
				Foliage	Roots	Foliage only	Foliage only
B5	340	4.9	T1	71	26	NT	66
			T2	7.1	739	NT	>0.005
			T3	62	538	NT	138
M4	1080	6.4	T1	33	32	NT	0.48
			T2	29	1,347	NT	6,854
			T3	49	9,381	NT	19,606
Z1	140	5.3	T1	461	255	>0.005	186
			T2	120	980	1,441	69
			T3	201	5,157	165	116
FZ	450	7.7	T1	24	126	NT	NT
			T2	14	13	NT	NT
			T3	46	25	NT	NT
SAP	130	8.5	T1	>0.005	3	NT	NT
			T2	>0.005	>0.005	NT	NT
			T3	36	34	NT	NT

NT = Not tested.

5.2 Results and Discussions

The initial pH of the substrate can be seen exerting control over the concentration of gold accumulated in herbage. For example, the total gold concentration of Z1 and SAP materials are similar at approximately 150 µg/kg. The pH values of these materials were considerably different at 5.3 and 8.5 respectively. The gold accumulated in the foliage of *Acacia longiflora* under a T3 treatment is considerably different for the two substrates with Z1 supporting accumulation to 201

μg/kg and SAP a mere 36 μg/kg. This reflects not only that ammonium thiocyanate can chelate more efficiently at lower pH's but also that the addition of acid lowered the pH of Z1 sufficiently to mobilise a greater fraction of the gold present than in the SAP material.

The results from analysis of root samples are included here to illustrate the levels of gold concentrations accumulated relative to herbage concentrations. The Boddington Gold materials all show a one to two order of magnitude increase in root gold concentrations compared with the herbage values for the T2 and T3 treatments. The effect of a T1 treatment on root gold is not as clear as that shown for the T2 and T3 treatment. It would appear that the T1 treatment produced a negative response to root gold accumulation in the B5 and Z1 materials, while having little impact on gold values for M4 and SAP materials and a positive response in FZ. This demonstrates that an acid amendment will mobilise gold without necessarily inducing accumulation, whereas an ammonium thiocyanate amendment will render gold bioavailable if the substrate acidity is low enough to support chelation. What is apparent is that the root gold concentrations far exceed any herbage values indicating that gold accumulation in the roots is significant. Further research in chelator-induced gold accumulation continues elsewhere at Massey University using numerous foliar and root crop species.

Brassica juncea was only trialled on the Z1 laterite because this species is already known to hyperaccumulate gold under ammonium thiocyanate induction. The Z1 material was chosen because of it's moderately low pH and low total gold concentration. The results indicate that *Brassica juncea* does not tolerate the addition of acid favourably, as is evident from the T1 and T3 responses. This may have resulted in damage to the roots of the plant or transport mechanisms in the rhizosphere. The T2 treatment produced appreciable gold accumulation. This ammonium-thiocyanate-only treatment produced a 1441 μg/kg gold accumulation from a 140 μg/kg gold laterite substrate, an accumulation coefficient of 10.

The use of *Linum usitatissimum* was restricted to the Boddington Gold materials for several reasons. Firstly, *Linum* would be relatively easy to grow at the Boddington mine site and secondly this species was selected to investigate the effect

of a cyanogenic species to ammonium-thiocyanate-induced gold accumulation. It was felt that this hypothesis should be restricted to material derived from a gold mining operation.

Linum usitatissimum produced the best accumulation levels for all plant-substrate combinations in this experiment. The B5 and Z1 samples show little response to chelator-induced accumulation. This probably reflects the low gold concentration of the substrate and the chelator's ability to complex and transport this small gold fraction to the root zone. The M4 samples show considerable gold accumulation for both T2 and T3 treatments, both well above the 1000 μg/kg threshold defined by Anderson *et al.* (1998). The T3 treatment produced gold accumulation above 19000 μg/kg. This is the best result for this experiment and comes off a substrate gold concentration of 1077 μg/kg, an accumulation coefficient of 19.

In general, the data indicate that by far the most effective method of mobilising gold within these laterites, for the purpose of phytoextraction, is to apply the chelator as an acidified amendment. Gold solubility is primarily dependent upon the pH of the substrate with higher rates of gold solubility occurring in more acid conditions. In order to solubilise gold to sufficient concentrations required for significant plant uptake, an acidified pulse of chelator should be applied. The acidic strength of the treatments were found to be sufficient to lower the pH in order to strip out and mobilise the gold from it's host mineralogy in an aqueous plant-available form. This experiment determined that the acidity and chelator strength of the T3 treatment was sufficient to solubilise gold long enough for plant accumulation to occur while being weak enough as not to damage the root system of the plant before significant gold accumulation could occur.

5.3 Ammonium-Thiocyanate-Induced Gold Accumulation using Lateritic Substrates

The pretreatment experiment highlighted the need to acidify the chelator amendment in order to solubilise the gold and thus allowing the chelator to work as a gold transport mechanism in the rhizosphere. Analyses indicated that gold was

mobilised by an amendment of acid but not necessarily accumulated in the plant whereas an acidified amendment of the chelator produced significant gold accumulation. These findings resulted in a new application protocol for the addition of ammonium thiocyanate substrate amendments.

Mobilisation of gold using an acid amendment has significant implications in terms acidic root exudates. Gardner *et al.* (1982) have shown that acid is secreted from root systems, especially in *Lupinus* species. Marschner (1995) reports that mucilage (a gelatinous material secreted by root hairs) contains low-molecular-weight compounds, such as organic and amino acids, which are capable of mobilising heavy metals in soils. The rate of mucilage production is in part controlled by the physical properties of the soils and soil pH. This would suggest that addition of acid would result in increased production of mucilage in the rhizosphere and consequentially increase the level of heavy metal absorption across the root membrane.

The pre-treatment T2 (1% ammonium thiocyanate solution only) and T3 (1% ammonium thiocyanate solution and 10 mL 2 M HCl) treatments have been retained with the addition of another more acid amendment of 1% ammonium thiocyanate solution containing 20 mL 2 M HCl. These amendments for this experiment have been designated T1, T2 and T3 respectively.

5.3.1 Ammonium-Thiocyanate-Induced Gold Accumulation on Boddington B5 Laterite

The graph below show the response of the selected species to ammonium thiocyanate induced gold accumulation when grown on B5 material supplied by Boddington Gold Mine. The B5 laterite with a gold concentration of 510 µg/kg is the most acidic material trialled at a pH level of 4.9.

The pretreatment experiment indicated that an acidified amendment of ammonium thiocyanate would induced gold accumulation to 62 µg/kg in *Acacia longifolia* and 138 µg/kg in *Linum usitatissimum* when grown on this substrate.

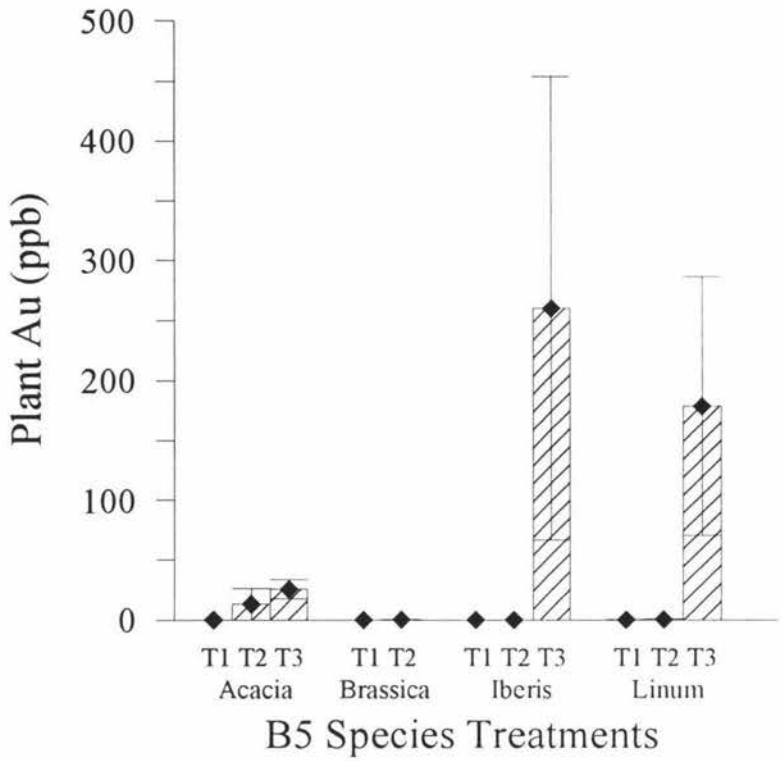

Figure 18. Acidified ammonium-thiocyanate-induced gold accumulation for species grown on Boddington B5 laterite.

The *Acacia longifolia* trial resulted in highly variable gold uptake for common treatments compared to the pretreatment results. The data does indicate good correlation between increasing acidification and gold accumulation. The T1 treatment produced a gold accumulation below detection limits, compared to 71 µg/kg in the pretreatment. The T2 treatment generated gold accumulation of 13 µg/kg compared to 7 µg/kg in the pre-treatment. The T3 treatment produced a 25 µg/kg accumulation suggesting that further increases in acidification may improve these results. The species appeared to be acid tolerant over the experimental range with numerous plants surviving the treatments to the two week harvest point. These data also indicates the relative tolerance of *Acacia longifolia* to ammonium thiocyanate at a 1% concentration.

Further work should be carried out using higher concentrations of ammonium thiocyanate and higher rates of acidification. This would determine whether *Acacia longifolia* has applications in induced gold accumulation on acidic substrates (e.g.

acid mine tailings) where chelation would be used to mobilise the gold fraction. On the basis of the current data however, *Acacia longifolia* would not be suitable for an induced gold accumulation programme on the B5 laterite, even considering its large biomass.

Brassica juncea responded poorly to all treatments. The relatively low total substrate gold concentration (510 µg/kg) compared to previous data (1000-5000 µg/kg) may reflect the relationship between a chelators potential to make gold bioavailable and the substrate gold content and characteristic. The gold-bearing mineralogy may also be exerting an influence on the level of accumulation using ammonium thiocyanate.

The plant's ability to accumulate gold could have been reduced by the maturity of the specimens at the time of planting. The plants used in this trial were mid-way through development to a reproductive stage. The *Brassica* genus is known to be cyanogenic with detectable thiocyanate complexes in all plant organs (Bradshaw *et al.*, 1990). The level of thiocyanate in various plant tissues is also known to be seasonal with higher levels occurring in spring and summer. Hak-Yoon *et al.* (1980) reported that the thiocyanate content in leaves and stems of several cultivars of *Brassica oleracea* L. were highest in 15 day seedlings, after which the thiocyanate content decreased rapidly. In light of this it could be surmise that the *Brassica juncea* specimens used in this trial may have been unable to accumulate thiocyanate-chelated gold because physiologically speaking they were beyond any form of thiocyanate tolerance associated with early life, or that the ammonium thiocyanate gold-uptake pathway in *Brassica juncea* has ceased to operate at this stage of plant development. Perhaps the thiocyanate pathway to gold accumulation in *Brassica juncea* only occurs in immature plants and/or is seasonally dependent. This experiment was performed in early autumn. An alternative explanation, deserved of further experimentation with this substrate, is that other unknown elements and conditions of the B5 laterite have conspired to inhibit gold uptake by *Brassica juncea*.

The thallium hyperaccumulator *Iberis intermedia* was induced to accumulate 260 µg/kg gold for the T3 amendment. The T1 and T2 treatments returned gold concentrations at or below detection limits. The T3 response for *Iberis intermedia* is the best result from the B5 substrate trial. The induced gold accumulation results of

Iberis intermedia grown on artificial gold substrates and the data presented here indicate that further work should be carried out on this plant, although it was the first to succumb to chelator toxicity and/or acid damage resulting in post amendment death.

Linum usitatissimum responded in a similar fashion to *Iberis intermedia* in that only the T3 treatment produced a detectable gold accumulation. The T1 and T2 treatments returned gold concentrations at or below detection limits. The T3 treatment resulted in a gold accumulation of 178 μg/kg. The plants used in this trial were no older than two week seedlings. A comparison of data here and that reported for the pre-treatment highlights the erratic nature of induced gold accumulation from these low gold concentration laterite materials. The T1 pretreatment amendment produced a similar result to the trial experiment treatment using three times the volume of acid. The author believes there to be a strong relationship between the substrate gold concentration, chelator concentration and subsequent plant-gold accumulation. The lower the substrate gold concentration the greater the concentration of chelator required to mobilise this small gold fraction to produce a detectable or appreciable plant gold concentration.

It was hoped that the above cyanogenic plant would allow for a greater accumulation of thiocyanate-chelated gold. If we compare these data with the results from *Brassica juncea*, in light of their cyanogenic physiology's, one could speculate that cyanogenesis in early plant life plays a role in thiocyanate-induced gold accumulation. *Linum usitatissimum* is known to exude thiocyanate into the rhizosphere in response to fungal attack, although this behaviour has not been ascertained it could be assumed that a biochemical pathway in the root system has supported the higher concentrations of gold found in this species. Also, the higher levels of thiocyanate in the plants in early stages of development may necessitate the existence of a thiocyanate pathway in the root system thereby providing gold thio-complexes access to the plants. As the plants mature the thiocyanate defence system becomes less important and is essentially turned on and off as required during vegetative and reproductive phases resulting in lower levels of thiocyanate complexed gold accessibility across the root membrane.

5.3.2 Ammonium-Thiocyanate-Induced Gold Accumulation on Boddington M4 Laterite

The Boddington M4 laterite contained the highest concentration of acid-digestible total gold (1600 μg/kg) of all the Western Australian materials. This translates to a substrate gold concentration of 1077μg/kg. Gold accumulation for *Acacia longifolia* grown on B5 material showed a positive correlation with increasing chelator acidity. The T1 treatment produced a gold concentration near detection limits. The T2 and T3 treatments produced gold accumulations of 494 and 884 μg/kg respectively. These results are considerably higher than the results from similar treatments in the pretreatment experiment. The markedly different results between the two sets of experiments could be explained in terms of the relative cyanogenic status of the plants with respect to their age.

These data would suggest that continued work using *Acacia longifolia*, the M4 substrate and further increases in acidified ammonium thiocyanate loading would produce higher accumulation levels more suitable for an induced-gold phytoextraction feasibility study.

The results for *Brassica juncea* again indicate little gold uptake considering a substrate gold concentration in excess of 1000 μg/kg and adequate chelator extractable gold. In light of the work of Anderson *et* al. (1998) using the same species-chelator combination and the species reported cyanogenic behaviour (Hak-Yoon *et* al., 1980) I believe that the plants used in this experiment were too mature to be used in an induced gold experiment.

Iberis intermedia accumulated gold under T2 and T3 treatments of 971 μg/kg and 314 μg/kg respectively. The T1 treatment resulted in gold accumulation below detection limits. The data B5 data indicates that an amendment of ammonium thiocyanate with 20 mL 2 M HCl or more will induce a three fold increase in plant gold relative to substrate gold concentrations. The lower-than-expected analysis for the T3 treatment may indicate a maximum level of acid tolerance although results from the B5 substrate, with a lower pH level, would have us believe that gold heterogeneity is influencing the observed results.

The response of *Linum usitatissimum* grown on M4 laterite produced the best results from this experiment, although they are much lower than pretreatment analyses. The T3 treatment produced gold accumulation to 2236 μg/kg from a data range of 716-3442 μg/kg. A much less acidic amendment in the pretreatment resulted in gold accumulation to 19,606 μg/kg! The application of acid is playing a major role in stripping out and mobilising gold from less reactive sites than would be seen by ammonium-thiocyanate chelation within the substrate.

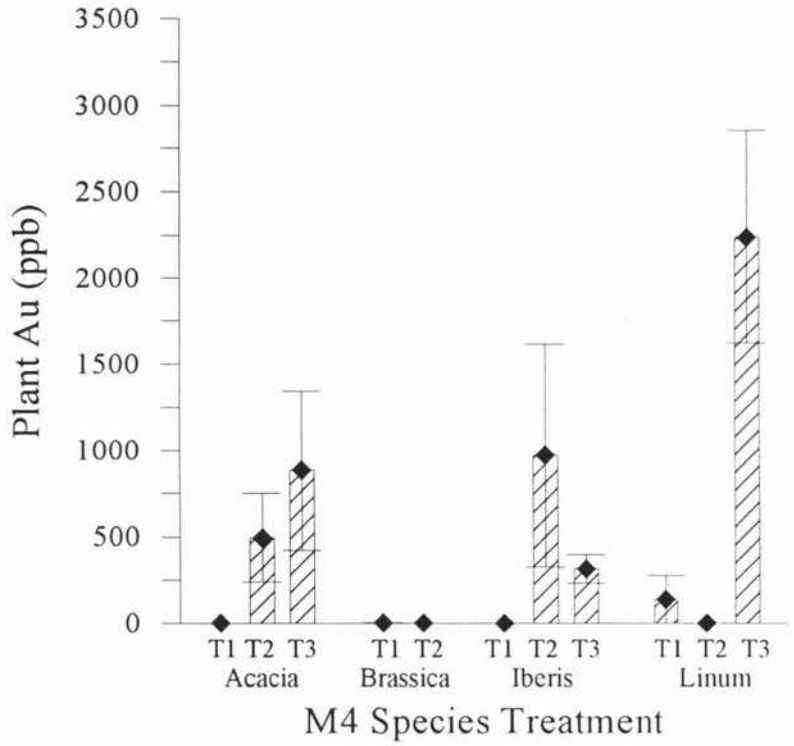

Figure 19. Acidified ammonium-thiocyanate-induced gold accumulation for selected species grown on Boddington M4 laterite. Concentrations in μg/kg.

So why have the two experiments produced such different results? The considerably lower-than-expected results for the T1 and T2 treatments may again may reflect the low level of plant cyanogenism as a function of plant maturity. The time elapsed between the pretreatment experiment and the complete trial experiment was approximately one month, which translates to an approximate plant age of four months. This degree of plant maturity indicates that the level of cyanogenic behaviour in plants treated in the complete trial was considerably lower than that of the pretreatment trial. Therefore the same influences upon gold uptake, as a function

of plant maturity, by this species may occur similar to those outlined for *Brassica juncea*.

5.3.3 Ammonium-Thiocyanate-Induced Gold Accumulation on Boddington Z1 Laterite

The Z1 laterite from Boddington Gold Mine has the lowest gold concentration (140 μg/kg) of the laterites investigated. The graph below illustrates the accumulation responses of different species to the different treatments used to induce gold accumulation with ammonium thiocyanate.

The general trend observed in this trial was that addition of improved the level of gold accumulation, a fact reflected in the pre-treatment results where an acid-only amendment induced a greater level of gold accumulation than that observed by both ammonium-thiocyanate-containing treatments. This I ascribe to the gold-bearing mineralogy of this high clay content laterite. The gold present may perhaps occur as free gold or as a chloride complex, making it more accessible to thiocyanate than gold bound in stronger sulphide complexes or silicate lattices.

Considering the low total gold concentration of approximately 140 μg/kg in the above laterite, the relative level of gold accumulation for plants grown on this material is far greater than that of all other substrates.

The effect of acidified ammonium-thiocyanate-induced gold accumulation in *Acacia longifolia* and *Brassica juncea* was weak. *Acacia longifolia* accumulated a similar level of gold here compared to the B5 trial. Both materials have low gold concentrations. This lack of appreciable gold accumulation in B5 and Z1 substrates may be attributed to the relative poor chelator potential under such low gold concentrations.

Brassica juncea performed better on Z1 substrates than either B5 with the lowest pH or M4 with the highest total gold concentration. The gold concentrations indicate a much poorer response to a higher level of ammonium thiocyanate chelation than previously reported. Anderson *et al.* (1998) reported that a 0.5%

solution of ammonium thiocyanate extracted 1.8% of the gold present in a colloidal gold ore from Waihi Gold Mine, New Zealand. Ammonium-thiocyanate-induced accumulation in *Brassica juncea* grown on this substrate resulted in a gold yield of 9000-19,000 µg/kg. A 0.64% solution extracted 9.2% of gold from an artificial gold-in-sand substrate that yielded accumulation of 2000-57,000 µg/kg from the same experiment. This highlights the control of the gold-host mineralogy in determining the level of gold accumulation and the high level of variability in results under ammonium thiocyanate chelation.

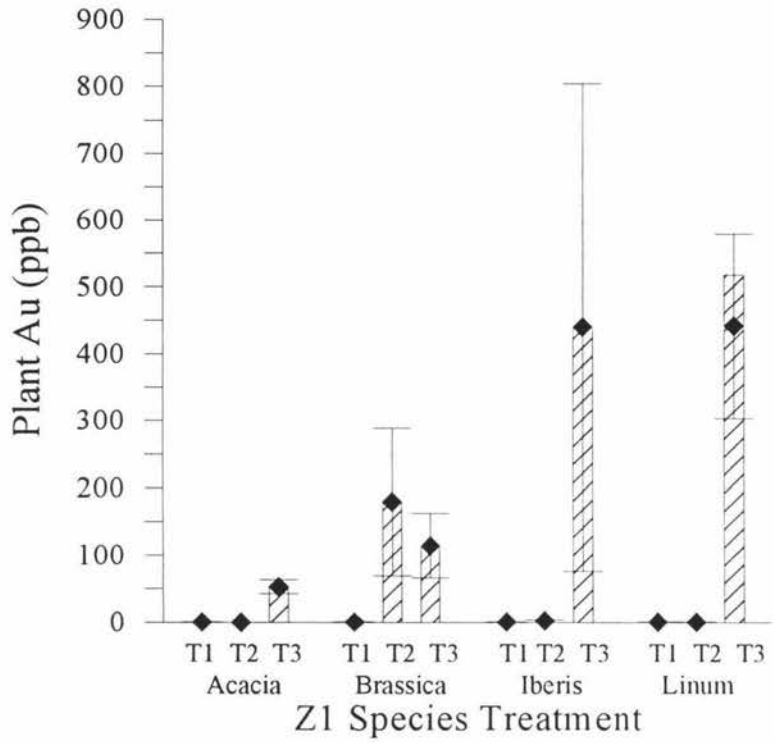

Figure 20. Acidified ammonium-thiocyanate-induced gold accumulation for selected species grown on Z1 laterite. Concentrations in µg/kg.

Iberis intermedia and *Linum usitatissimum* show similar responses to all ammonium thiocyanate amendments. The relative level of gold accumulation for plants grown on Z1 laterite compared to total gold concentrations of the laterites was high. The weak response to low acidification and relatively good response to high acidification serve again to illustrate the relationship between gold bioavailability and pH-dependant gold solubility.

The pretreatment experiment showed a firm relationship between gold accumulation and gold bioavailability as determined by substrate conditions, such as

substrate pH and the concentration of chelator applied. The efficiency of ammonium thiocyanate at inducing gold accumulation was greatly improved by acidification. These experimental trends were extended to the full trial population resulting in a similar trend in gold accumulation under the same conditions. Mean analyses of herbage from the full trial were lower than those observed in the pretreatment experiment. Analyses of root samples gave similar or lower values than those from the pretreatment experiment. These results may be explained in terms of the relative cyanogenic behaviour of the plants in relation to their maturity at the time of treatment and/or also a reflection of the narrow window of substrate conditions necessary for gold uptake influenced by small chemical changes in the environment as dictated by other mobile elements in the laterite materials investigated.

5.4 Gold accumulation in *Acacia longifolia* – uptake versus transportation

Gold accumulation levels in *Acacia longifolia* indicate that this species is not suitable for an induced phytoextraction programme, even though biomass production from this species is considerable and allows for higher overall yields at lower plant-gold concentrations than reported in previous research. *Acacia longifolia* is also classified as cyanogenic (Seigler *et al.*, 1989). The response of younger plants, than those used in this experiment, should be investigated to determine any relationships between increased levels of plant thiocyanate and thiocyanate-induced gold accumulation. However much work would need to be undertaken to determine the plant's accumulation potential. This work would include;

- Determination of the species tolerance to more concentrated amendments of ammonium thiocyanate. This may result in a higher gold accumulation rate under more acidic conditions which would counteract the phytotoxic effect of ammonium thiocyanate chelation. Chelator potential is a function of the concentration of the amendment, as well as the substrate acidity and gold concentration. Ammonium thiocyanate appears to depress gold accumulation in foliage compared with acidified treatments. It may be that ammonium thiocyanate is allowing other elements to be accumulated by the plants which in turn are phytotoxic, e.g. manganese. This

hypothesis should be investigated if further work into gold induced accumulation using laterite substrates is to continue.

- Determination of the species' acid tolerance. It appears that *Acacia longifolia* is more tolerant to the addition of acidified ammonium thiocyanate compared with the other species investigated. A large number of plants survived the two-week period between treatment and harvest. Results also indicated that *Acacia longifolia* accumulated gold without the presence of a chelator, particularly in the high clay content Z1 laterite. This may present a lower induction cost compared with ammonium thiocyanate.

- The gold storage sites and accumulation levels within plant organs and tissues must also be identified. Because *Acacia longifolia*, a woodland species, produces a large volume of timber it would have to be established whether tissues from the interior of the tree accumulated appreciable gold. This is assuming mature plants were used. Immature plants, grown from seedlings, with higher ratios of photosynthetic tissues to wood may provide a more effective alternative. If this was the case then the effect of coppicing should also be determined as this practice is known to improved nickel levels in *Berkheya coddii*.

Gold accumulation levels in foliage under different treatments was considerably lower than gold accumulated in the roots of *Acacia longifolia*. The level of gold accumulation in the roots was often two orders of magnitude greater than in herbage, indicating different biochemical pathways in effect between gold adsorption across the root membrane and gold transportation within the plant. This indicates that the roots absorb a larger amount of gold than is transported and stored in herbage over the same time period or that the mechanism, or pathway, is the same but more affected by the environment across the root membrane

The plant's ability to absorb gold into the roots from the soil solution is greatly improved by the addition of ammonium thiocyanate. It would appear that gold can easily make the jump across the root membrane from the soil solution, but is not readily transported and stored in herbage or the presence of the mechanism destabilises the gold complex by the very act of absorption.

The gold concentrations in roots may represent the 'lag' in transport efficiency between gold absorption and translocation for storage in foliage. It may be assumed that this level of gold concentration in the roots may represent a major gold storage site in *Acacia longifolia*. Gold accumulation in the rhizosphere has significant implications in gold exploration methods in laterite terrains, first alluded to by Baker (1973 and 1978), Mann, (1984), and Wood, (1996) on the role of organic compounds in gold transportation and mineralisation. If *Acacia longifolia* has the potential to accumulation and store these levels of gold in the roots, previously forested regions and paleosols would become important exploration targets.

5.5 Acidity, chelation and gold accumulation

The ammonium thiocyanate treatment at neutral pH produced accumulation in most species, often at lower concentrations than the acid-only treatment, indicating that ammonium thiocyanate is capable of inducing gold accumulation in any species given a readily available source of soluble substrate gold. However, the response to an acidified ammonium thiocyanate amendment was not appreciably different to an acid-only amendment and often resulted in less accumulation of gold. All pretreatment amendments using an acid-only amendment resulted in higher levels of gold accumulation than a neutral solution of ammonium thiocyanate. This shows well that in most cases gold bioavailability (solubility) is primarily controlled by acidity and that gold uptake is passive, promoted by ammonium thiocyanate chelation, and driven by transpiration.

The ammonium thiocyanate and acidified ammonium thiocyanate amendments produced considerable gold accumulations in the roots of *Acacia longifolia* and in herbage of *Linum usitatissimum*. All trials showed a trend of increasing gold accumulation with increasing acidification. The level of gold accumulation identified in this experiment falls below the range required for a feasible induced-gold phytomining operation at today's gold prices. *Linum usitatissimum* grown on the B5 laterite (1000 µg/kg gold) under a T3 treatment was the only plant-substrate-treatment combination to reach a similar average plant-gold concentration of 2000 µg/kg. Although the level of gold accumulation relative to the

substrate concentration is significant, the gold content of all species was well below that required to pay for the cost of the treatment. A brief phytomining scenario based on these results is discussed below.

5.6 Cyanogenesis and Ammonium Thiocyanate-Induced-Gold Accumulation

Acacia longifolia, *Linum usitatissimum* and *Brassica juncea* are cyanogenic. These plants produce organic thiocyanate in plant tissues. The degree of cyanogenesis is seasonal and dependent upon the age of the plant. Generally, plants are more cyanogenic in spring and summer periods. Younger plants have a higher level of thiocyanate as a defence mechanism against herbivores. *Linum usitatissimum* is a highly cyanogenic plant possessing the ability to produce rhizospheric thiocyanate in response to fungal attack.

Gold accumulation by *Brassica juncea* grown on B5 and M4 laterites was conspicuously absent, given their total gold concentrations and the previously reported accumulation potential of this plant in the pretreatment experiment. The Z1 laterite, with the lowest gold concentration of 0.14 μg/kg, produced the highest plant accumulation. The results of this experiment, in light of the work by Anderson *et al.* (1998), serve to highlight the variability of results obtained so far using ammonium thiocyanate induction. The age of plants trialled on B5 and M4 laterite were considerably greater than those used in the Z1 trial. The accumulation levels observed for the B5 and M4 substrates are below or near detection limits for all treatments. Gold accumulation for the lowest gold content substrate (Z1) indicated an appreciable effect by acidified ammonium-thiocyanate chelation. Why then has *Brassica juncea* not accumulated gold on the more concentrated substrates under acidified chelation? One could argue that gold heterogeneity is responsible. However, it seems unlikely that all samples from these trials were devoid of gold. I therefore attribute this lack of induced gold accumulation to the low level of plant cyanogenesis and maturity of seedlings at the time of treatment. Cyanogenesis may therefore play an important role in thiocyanate-induced gold accumulation within this group of plants.

Linum usitatissimum performed best under the experimental conditions. The pretreatment experiment produced ammonium-thiocyanate-assisted gold accumulation of 6800 µg/kg on a dry weight basis. The acidified amendment from this trial produced gold accumulation to 19,600 µg/kg. The full trial produced gold accumulation to 2200 µg/kg, with three times the volume of acid added, and had no discernible response under identical conditions of the pretreatment. Given that the experimental design and execution were identical for these two experiments I can only surmise that the plants themselves have produced this marked difference in accumulation responses as a result of their decreasing cyanogenic behaviour towards maturation.

Determination of the effect of thiocyanate production in the rhizosphere and gold mobility under these conditions was deemed beyond the scope of this project. No control on thiocyanate production, in response to fungal attack, in the roots was ascertained. The plants were treated at flowering when thiocyanate production in the plant was known to be at a seasonal and physiological high. Results from the pretreatment trial indicated a good response to thiocyanate-induced gold chelation. The response of *Linum usitatissimum* to acidified ammonium-thiocyanate chelation when grown on M4 and Z1 substrates produced the highest results for the selected species. Gold accumulation on the B5 substrate was also appreciable. The level of accumulation exhibited by *Linum usitatissimum* on the Z1 substrate was considerably higher than in *Brassica juncea*. This again may be explained in terms of the relative thiocyanate levels, maturity, and thiocyanate tolerance of these two species.

5.7 A Phytomining Scenario - Induced Phytoextraction of Gold from Laterite Gold Ore using *Linum usitatissimum*

The use of plants to extract substrate metals is primarily controlled by economic factors, such as the total metal yield and biomass production of the plant versus the cost of propagation, treatment and metal recovery. Obviously, the biomass yield and plant gold accumulation level dictate the total gold yield which must at least offset the cost of planting, treatment and processing of the bio-ore. *Linum*

usitatissimum accumulated 6,800 µg/kg gold in the pretreatment experiment under a non-acidified 1% solution of ammonium thiocyanate. Assuming that the cost of ammonium thiocyanate is US$ 3 per kg, a figure of US$ 1560 represents the total treatment cost per hectare when added to a root zone depth of 10 cm. The cost of incineration is approximately US$ 100 per tonne. Current phytomining models allow for a 25% energy credit associated with biomass incineration at US$ 5 per tonne. The biomass yield of *Linum usitatissimum* is 9 tonnes per hectare. The cost of processing per hectare (in US$) is therefore 1560 (chemicals) + 900 (incineration) – 45 (energy credit) = US$ 2415 per hectare. At a gold concentration of 6,800 µg/kg in dry herbage, a gold yield of 61.2 g per hectare at a current value of approximately US$ 600 is realised. Clearly this scenario is in deficit by US$ 1815. If the accumulation level of 19,600 µg/kg using acidified ammonium thiocyanate is applied to this model the gold yield is 176.4 g per hectare and the deficit is still US$ 655, excluding the cost of acidification. At these costs and level of biomass production a plant gold concentration of 27,000 µg/kg would be required before any profit were to be generated. The easiest route to altering this particularly high required plant gold concentration would be to attempt to improve the biomass yield, while maintaining the average gold concentration.

5.7.1 Further work arising from this study

The accumulation potential of ammonium thiocyanate in laterites should be clarified to establish better controls on gold bioavailability. The relationship between total gold concentration and ammonium-thiocyanate-induced gold accumulation at different concentrations should also be determined for these laterite materials. This would determine whether a lower rate of application would produce the same accumulation response at a much lesser treatment cost. If a 0.5% solution were capable of inducing a similar gold accumulation in *Linum usitatissimum* the cost of treatment would drop to US$ 780 per hectare and a plant gold concentration of 18 mg/kg would be viable. This plant gold level was achieved in the pretreatment experiment for an acidified amendment of ammonium thiocyanate.

The relationship between acidification and gold accumulation indicated the desire to source acid-tolerant species for ammonium-thiocyanate-induced gold accumulation. A search of existing botanical information may provide this. An alternative to this would be to bioengineer an acid-tolerant species with a suitably high biomass production.

The relationships between cyanogenic species, ammonium-thiocyanate tolerance and induced gold accumulation using ammonium thiocyanate should also be investigated further. If cyanogenic plants are capable of promoting ammonium-thiocyanate-induced gold accumulation, even on a seasonal or maturation basis, the level of plant gold would approach the required economic threshold.

The high levels of gold accumulated in the roots of *Acacia longifolia* indicate that work should be carried out into root gold accumulation, perhaps using a high yielding vegetable species. This may introduce a new aspect to induced gold accumulation research by moving the focus from herbage accumulation of gold to accumulation in the rhizosphere.

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