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Soil Amendments Affecting Nickel and Cobalt Uptake by *Berkheya coddii*: Potential Use for Phytomining and Phytoremediation

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Plants with inordinately high concentrations of heavy metals ('hyperaccumulators') can be used for phytoremediation (removal of contaminants from soils) or phytomining (growing a crop of plants to harvest the metals). Pot trials were used to investigate the effects of $MgCO_3$, $CaCO_3$, sulphur, chelating agents (NTA, DTPA, EDTA) and acid mine tailings on nickel and cobalt uptake by the South African nickel hyperaccumulator *Berkheya coddii*. Plants were grown in a nickel-rich ultramafic ('serpentine') soil diluted with pumice. Both $MgCO_3$ and $CaCO_3$ caused significant decreases in the uptake of both metals, as well as decreasing their solubility in the soil. After the addition of $MgCO_3$, there was a significant increase in soil pH, so the reduction in plant-metal uptake could not be solely attributed to the action of magnesium alone. Since $CaCO_3$ had no significant effect on soil pH, this indicated that calcium inhibits the uptake of both cobalt and nickel. All three chelating agents caused a significant reduction in plant uptake of nickel, despite increasing the solubility (plant availability) of these elements in the soil. Cobalt uptake was unaffected. Sulphur, and the addition of acid mine tailings, caused a highly significant increase in nickel and cobalt uptake, relative to the controls. Sulphur could be used as a low-cost soil amendment to enhance the metal uptake of crops grown on ultramafic soils. Thus, land management procedures would enhance phytoremediation and phytomining operations for nickel and cobalt.

Key words: Nickel, cobalt, phytoremediation, phytomining.

INTRODUCTION

Recently, the use of plants to extract heavy metals from soils (phytoextraction) has received much attention due to the possibility of decontaminating some of the Earth's ever-increasing burden of polluted soils (phytoremediation—Chaney, 1983; Baker and Brooks, 1989; McGrath *et al.*, 1993; Salt *et al.*, 1995; Robinson *et al.*, 1997*a*, *b*), and more recently as a method to commercially mine metals from low-grade ore bodies (phytomining—Nicks and Chambers, 1995, 1998; Anderson *et al.*, 1998; Brooks, 1998; Chaney *et al.*, 1998).

In a phytoextraction operation, a crop of plants is grown in soil containing elevated concentrations of one or more heavy metals. The plants accumulate the metals, either naturally, or they are induced to do so by soil amendments. When mature, the crop is harvested, removed and burnt. This leaves a small volume of ash containing a high concentration of the target metal(s). This ash, termed 'bio-ore', can then be smelted to recover the metal, or, if the metal is of low value, stored in a small area where it does not pose a risk to the environment. The plants used in a phytoextraction operation should ideally have a large biomass production and accumulate high concentrations of metal in the above-ground portions (hyperaccumulator plants—Brooks *et al.*, 1977). Species exhibiting both these qualities are somewhat rare.

Berkheya coddii is an asteraceous perennial plant found naturally on serpentine soils in southern Africa (Morrey *et al.*, 1992). Robinson *et al.* (1997*a*) reported that *B. coddii* had a high biomass production (22 t ha^{-1} per annum) combined with a high uptake of nickel (up to 1 % dry weight) when grown on nickel-rich soils. This makes it an ideal candidate for nickel phytoextraction. The high nickel uptake was, however, dependent on there being sufficient 'bioavailable' nickel in the soil. Sequential croppings of *B. coddii* growing on ultramafic soil will lead to a reduction in uptake of nickel, due to a reduction of soluble nickel in the soil. Robinson *et al.* (1998) used sequential extractions to estimate the number of crops attainable on an ultramafic 'serpentine' soil before nickel uptake dropped to 70 % of its original value.

Adding a compound to the soil that solubilizes non-available heavy metals should increase metal uptake by plants. Evidence for this comes from Blaylock *et al.* (1997) and Huang *et al.* (1997) who showed that the aerial parts of Indian mustard (*Brassica juncea*) plants accumulated lead to a level of 1·5 % d.wt 1 week after addition of EDTA to the soil. Although the plants then rapidly died, this is not important for a phytoextraction operation as dead tissue can be harvested and burnt as easily as live material. The disadvantage with this strategy is the cost involved in adding such compounds, plus the risks that they may pose to the environment. Robinson *et al.* (1997*a*) found that attempts to enhance nickel uptake in *B. coddii*, by adding EDTA and citric acid to the substrates, actually caused a decrease in nickel uptake, despite causing an increase in the concentration of soluble nickel. This loss was attributed to

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competition with the plant's own nickel-binding agents, thereby causing the nickel to diffuse downwards to the plant's root system. This earlier study, however, used EDTA at only one concentration and we wished to investigate its effect over a wide range of different concentrations.

Soil acidification has been shown to increase the solubility of metals in ultramafic soils (Robinson *et al.*, 1996), and so this may also increase metal uptake by the plant. Ultramafic soils usually have a large Mg/Ca quotient that can also be important in metal uptake (Robinson *et al.*, 1996). Chaney *et al.* (1998) have stated that in the case of *Alyssum*, addition of magnesium to the substrate caused enhanced uptake of nickel by the plant. We wished to establish whether this was the case for *B. coddii*, another hyperaccumulator of nickel.

The aims of the present study were therefore threefold: (1) an investigation of the effects on cobalt and nickel uptake by plants following soil amendments with EDTA, DTPA and DTA; (2) determination of the effect on cobalt and nickel uptake by plants resulting from soil acidification; and (3) investigation of magnesium and calcium amendments (as carbonates) on uptake of cobalt and nickel by plants.

MATERIALS AND METHODS

Environmental control and growth media

Experiments were conducted in shade houses at the Horticultural and Food Research Institute, Palmerston North, New Zealand. The experiments were conducted from November 1998 to February 1999. Temperatures ranged from 15–25 °C and the average vapour pressure deficit was 0·7 kPa. Plants were watered daily. Fertilization was carried out by addition of 'Osmocote' slow-release fertilizers at manufacturer's recommended rates. Pots were arranged in a randomized block design.

All experiments were conducted using mixtures of pumice/serpentine and pumice/serpentine/acid mine tailings prepared by mixing the dry materials in a concrete mixer. Table 1 shows the elemental composition of the substrates used. Nearly all of the nickel and cobalt was contained in the serpentine soil, which also had a high Mg/Ca quotient. Both pumice and the acid mine tailings had negligible amounts of nickel and cobalt. The acid mine tailings (AMT) were collected from the Waihi gold mine, Waihi, New Zealand. These tailings contain negligible concentrations of heavy metals, and have a pH of 2·2 due to pyrite oxidation. Pots were prepared 1 month before planting to allow equilibration of the substrate.

Effect of $CaCO_3$, $MgCO_3$ and sulphur additions on nickel and cobalt uptake by plants

The effect of $CaCO_3$, $MgCO_3$ and sulphur on nickel and cobalt uptake was studied by growing specimens of *Berkheya coddii* in 500 ml plastic pots for a period of 15 weeks in a 1 : 1 (by volume) pumice : serpentine soil mixture. To this was added incremental amounts of the aforementioned chemicals. For each amendment there were five different treatments with replicates of four plants within each group. The magnesium and calcium salts were added at rates of 0, 1·25, 2·5, 5 and 10 g kg^{-1} of soil. Elemental finely ground sulphur was added at a rate of 0, 0·625, 1·25, 2·5 and 5 g kg^{-1} of soil. The above-ground biomass of the plants was excised after the 15-week period and analysed for nickel and cobalt.

Effect on metal uptake by plants when acid mine tailings (AMT) were added to the substrate

The effect of soil acidification was investigated by adding increasing amounts of AMT to serpentine soil. Four replicates of treatments containing 33 % (by volume) serpentine soil were prepared with the following additions of AMT: 0 (control), 4, 8, 17, 25 and 33 %. The deficit was made up with pumice. The above-ground biomass of the plants was excised after the 15-week period and analysed for nickel and cobalt.

Effect of NTA, DTPA and EDTA on plant metal uptake

Sixty plants were grown in pots containing 500 g of a 1 : 1 (by volume) mixture of pumice : serpentine for a period of 15 weeks. For each chelation treatment, four replicates were prepared with the following additions: 0 (control), 5, 10, 20 and 40 g kg^{-1} of soil. All chelating agents were added as 5 % solutions prepared using sodium salts (trisodium NTA, trisodium DTPA, and disodium EDTA). Reagents were

TABLE 1. *Elemental concentrations (%) in the substrates used in this study*

Element	Serp.	Pumice	AMT	Element	Serp.	Pumice	AMT
Al	1·53	6·00	3·47	Na	0·009	2·200	< 0·1
Ca	0·262	0·660	0·240	Ni	0·609	< 0·001	< 0·001
Co	0·054	< 0·001	< 0·001	P	0·034	0·149	< 0·01
Cr	0·785	0·001	n.d.	S	0·052	—	< 0·001
Cu	0·010	0·002	0·001	Si	15·7	26·2	51·7
Fe	23·42	2·00	1·15	Ti	0·013	0·150	0·143
K	0·017	1·30	0·39	V	0·002	0·002	n.d.
Mg	15·92	0·40	0·19	Zn	0·014	—	0·0416
Mn	0·287	0·082	0·039	Density	1·3	0·8	1·3

Analyses performed by X-ray fluorescence spectrometry. Serp, Soil from Serpentine Rd between Rai Valley and Nelson, New Zealand; AMT, pyritic pit wall material from the Waihi gold mine, Waihi, New Zealand.

added at the end of the 15-week period and the plants were then harvested 1 month later.

Determination of nickel and cobalt concentrations of plants

Plant material was placed in a drying cabinet at 70 °C until a constant weight was reached. Approximately 0·1 g samples of plant material were weighed accurately into 15 ml borosilicate test tubes. The samples were ashed overnight at 500 °C. Five ml volumes of warm (80 °C) 2 M HCl were added to each, and the samples were mixed and shaken to dissolve the ash. Nickel and cobalt in the solutions were determined by flame atomic absorption spectroscopy (FAAS).

Determination of the solubility of nickel and cobalt in the soil

Soils were dried at 70 °C. Subsamples (2 g) were weighed accurately into 50 ml centrifuge tubes and 20 ml of 1 M ammonium acetate (pH 7·0) was then added to each. This extractant was used so that the results could be compared with those of other studies (e.g. Robinson *et al.*, 1998) that showed that ammonium acetate extracts could be used to predict the nickel content of a plant grown in the extracted soil. The tubes were agitated for 24 h and the mixtures filtered. Nickel and cobalt were determined in these extracts by FAAS.

RESULTS AND DISCUSSION

Effect of $MgCO_3$ and $CaCO_3$ soil amendments on nickel and cobalt uptake by Berkheya coddii

The addition of magnesium and calcium carbonates caused a significant ($P < 0{\cdot}05$) decrease in the plant uptake of both nickel and cobalt (Figs 1 and 2). Soil pH was significantly increased by the addition of $MgCO_3$ (Table 2) though not by $CaCO_3$. Thus the decrease in plant metal uptake must be attributed either to increased pH or to some other action of magnesium, whereas for calcium, pH change was not a factor. The ammonium-acetate-extractable fraction of nickel and cobalt in the soil was decreased in all treatments relative to the control, although the decrease was not correlated with the amount of calcium or magnesium added. The results of this part of the investigation, though inconclusive to some extent, clearly showed that addition of magnesium to the substrate did not increase either cobalt or nickel uptake by the plants; this directly contradicts the findings of Chaney *et al.* (1998) for *Alyssum*.

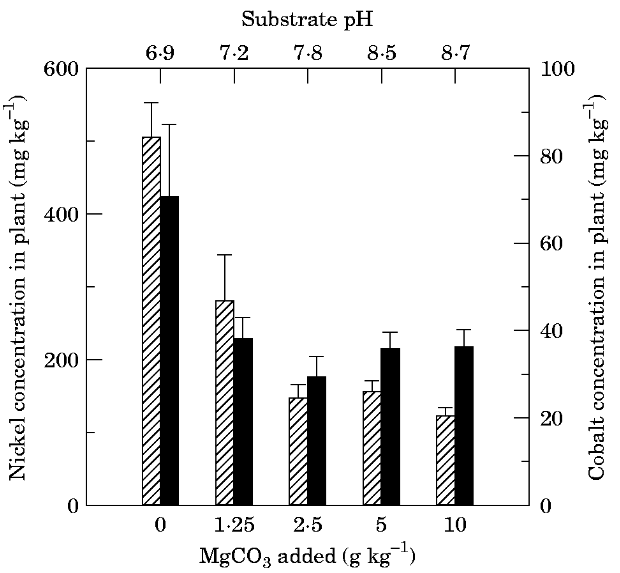

FIG. 1. Metal concentrations (Co, ■; Ni, ▨) in *Berkheya coddii* after addition of magnesium carbonate to the substrate. Bars ($n = 4$) are s.e.m.

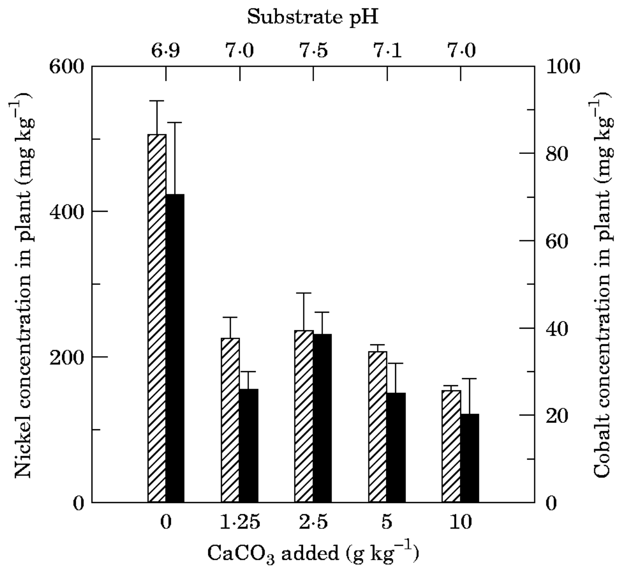

FIG. 2. Metal concentrations (Co, ■; Ni, ▨) in *Berkheya coddii* after addition of calcium carbonate to the substrate. Bars ($n = 4$) are s.e.m.

Effect of sulphur and acid mine tailing soil amendments on metal uptake by plants

There was a highly significant ($P < 0{\cdot}01$) positive correlation between the concentration of sulphur added, and the nickel and cobalt concentrations in the plants (Fig. 3). At the highest rate of sulphur addition to the soil (5 g kg^{-1}), the mean nickel and cobalt concentrations in *Berkheya coddii* were 1331 and 290 mg kg^{-1}, representing three- and five-fold increases relative to the controls (400 and 56 mg kg^{-1}), respectively. There were no significant differences in biomass production between treatments at pH 4·6 and above.

Lowering of the pH by addition of AMT caused a sharp increase in the metal concentrations in the plants in the pH range 6·9 to 6·0 then thereafter a much smaller increase, or even limiting value, between pH 6·0 to 4·2 (Fig. 4). The maximum improvement of nickel and cobalt concentrations was achieved at pH 4·6 and there would be no advantage, or desirability, in using a lower pH for practical field conditions. The addition of sulphur and AMT also caused a

TABLE 2. *The pH and ammonium-acetate-extractable nickel and cobalt concentrations (mg kg^{-1} = ppm) for soils treated with sulphur, calcium and magnesium carbonates, and acid mine tailings (AMT)*

	Treatment (g kg^{-1})					
	0	0·625	1·25	2·5	5	10
Soil						
Ni	4·1	4·6	4·2	7·4	12·6	—
Co	2·0	1·2	1·6	1·0	2·1	—
pH	6·9	6·3	6·1	5·7	5·5	—
$CaCO_3$						
Ni	4·1	—	4·0	2·3	2·6	3·1
Co	2·0	—	0·9	0·4	1·5	1·4
pH	6·9	—	7·0	7·5	7·1	7·0
$MgCO_3$						
Ni	4·1	—	2·5	2·3	2·9	3·7
Co	2·0	—	1·2	1·3	1·3	1·9
pH	6·9	—	7·2	7·8	8·5	8·7
Acid treatment (serp/AMT/pumice)*	4/0/8	4/0·5/7·5	4/1/7	4/2/6	4/3/7	4/4/4
Ni	4·9	4·3	5·3	13·5	13·5	15·6
Co	1·2	1·5	0·8	1·9	2·1	1·3
pH	6·9	6·5	6·0	4·6	4·2	4·2

* By weight.

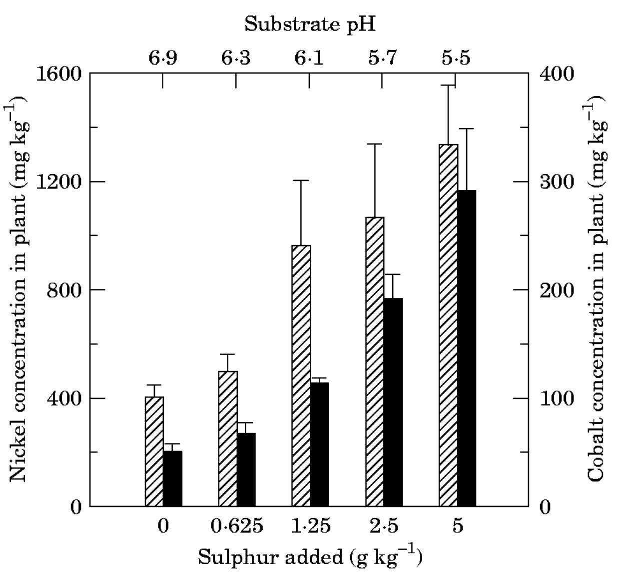

FIG. 3. Metal concentrations (Co, ■; Ni, ▨) in *Berkheya coddii* after change of pH by addition of sulphur to the substrate. Bars (n = 4) are s.e.m.

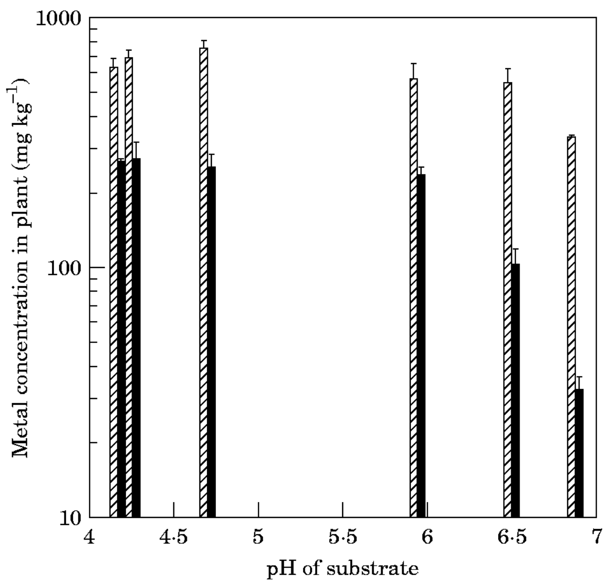

FIG. 4. Metal concentrations (Co, ■; Ni, ▨) in *Berkheya coddii* after change of pH by addition of acid mine tailings (AMT) to the substrate. Bars (n = 4) are s.e.m.

decrease in soil pH, plus an accompanying increase in the ammonium-acetate-extractable fraction of cobalt and nickel in the soil (Table 2).

The addition of sulphur and AMT to serpentine soil changed both pH and the concentration of available sulphur to the plant. Thus the increase in plant metal uptake cannot be attributed to either factor alone. Acidification is known to increase the solubility of nickel and cobalt in ultramafic soils (Robinson *et al.*, 1996). It has also been shown that plant-metal uptake is proportional to the soluble fraction of metal in the soil for *B. coddii* (Robinson *et al.*, 1997*a*). Addition of sulphur could be a relatively low-cost means to enhance the extraction of nickel and cobalt from crops of hyperaccumulators.

It might be argued that addition of AMT with its higher aluminium concentration (3·47 %) compared with the serpentine substrate (1·53 %) could increase the plant-available aluminium to phytotoxic levels. However, as stated above, we could find no statistically significant difference in plant biomass at pH 4·6 and above, though there was a significant decrease at the lowest pH value. It is also true that the bulk composition of the substrates was not

TABLE 3. *Ammonium-acetate-extractable nickel and cobalt (mg kg⁻¹ = ppm) in soils after chelate addition*

Treatment (g kg^{-1})	0	0·5	1	2	4
NTA					
Ni	6·1	18·0	10·1	9·4	15·8
Co	2·2	5·7	3·7	4·1	5·5
DPTA					
Ni	6·1	12·7	17·1	17·7	22·1
Co	2·2	7·2	9·1	11·8	15·7
EDTA					
Ni	6·1	12·3	12·7	15·4	40·8
Co	2·2	8·6	7·0	9·2	21·0

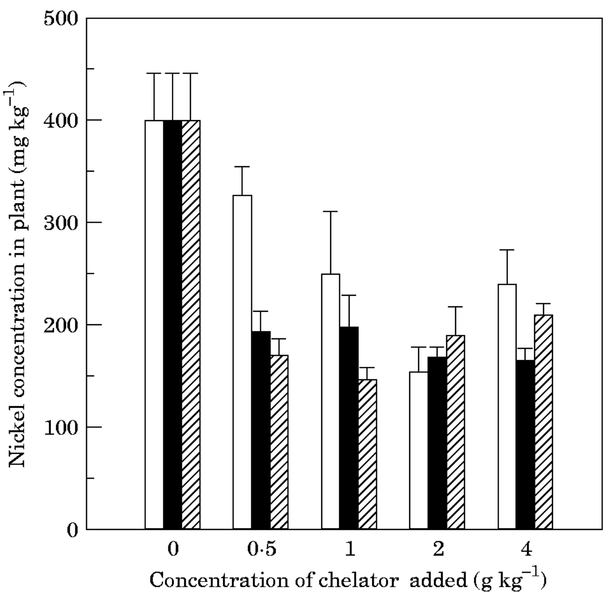

FIG. 5. Nickel concentrations (EDTA, ■; DPTA, ▨; NTA, □) in *Berkheya coddii* after addition of chelating agents to the substrate. Bars ($n = 4$) are s.e.m.

identical because of use of pumice to adjust total weights to 100%. However, although pumice has low concentrations of elements such as calcium, magnesium and iron that could conceivably affect the results, such effects will be minor compared with the result of lowering the pH.

Effect of chelate addition on metal uptake by Berkheya coddii

Compounds to augment phytoextraction have to be inexpensive and relatively non-toxic, as large amounts may need to be added. EDTA has been shown by Blaylock *et al.* (1997) and Huang and Cunningham (1996) to induce hyperaccumulation of lead in crops such as *Zea mays* and *Brassica juncea* that, under natural conditions, do not hyperaccumulate this element. EDTA is a well known chelating agent that bonds with many metals including nickel. It currently costs around $US40 kg^{-1} and is therefore relatively inexpensive. DTPA and NTA are other strong chelating agents which are more costly than EDTA but were included for comparison with other studies such as those of Chaney *et al.* (1998) who have proposed that EDTA and NTA can increase bioavailability of nickel and other elements. Both chelating agents are degraded in the natural environment within a few months (Means *et al.*, 1980).

The addition of NTA, DTPA and EDTA caused a highly significant decrease ($P < 0{\cdot}001$) in plant-nickel concentrations in all treatments relative to the control (Fig. 5). Cobalt uptake was unaffected and the data are not presented here. This was in spite of an increase in the extractable nickel and cobalt concentrations in the soils (Table 3). There was no reduction in the biomass yield since the reagents were added at the end of the 15-week period during which the plants had been allowed to grow under constant conditions and without soil amendments. These results are consistent with those reported by Robinson *et al.* (1997*a*) where EDTA and citric acid caused a decrease in nickel uptake.

CONCLUSION

These experiments indicate that neither soil amendments with calcium and magnesium carbonates, nor the addition of chelating agents are effective in increasing metal uptake by *Berkheya coddii* on serpentine soils. Instead these soil amendments caused a significant decrease in metal uptake. The addition of chelators to the soil associated with *B. coddii* has the opposite effect to their addition to soils supporting non-hyperaccumulator plants, where there is increasing rather than decreasing metal uptake.

Addition of elemental sulphur has the potential to enhance metal crops when the plants are grown on soils with a soluble metal fraction less than that required for maximum plant uptake. Currently, sulphur addition would be a relatively low cost ($US0·25 kg^{-1} compared with $US40 for EDTA) operation. Income from the sale of cobalt, as well as from enhanced nickel yields could more than offset the small cost of the sulphur. In our experiments, the cobalt concentrations in plants growing in soils treated with sulphur at a rate of 5 g kg^{-1} increased five-fold, whereas for nickel the increase was three-fold. For a 20 t ha^{-1} crop of *Berkheya coddii*, the gross value of the metals, at current world prices, would be $44 for nickel and $48 for cobalt ($92 in total) before addition of sulphur. After addition of sulphur, the respective values increase to $132 and $240 (total $372): i.e. the total value increases four-fold. The total yields of cobalt and nickel reported from our experimental soil with low plant-available concentrations of these elements, would be much higher in the case of high-availability soils, such as those from South Africa where the plant grows naturally and contains, on average, ten-times the nickel concentrations reported here. Despite these expected higher overall yields, the relative increases in metal concentrations in plants after addition of sulphur to serpentine soil with a high nickel phytoavailability might well be less than those here. Although it is impossible to extrapolate quantitatively from one scenario to the other, it does appear that enhancement of high-value cobalt is greater than lower-value nickel when sulphur is added to the substrate.

Except for addition of AMT, our experiments failed to show any statistical difference in biomass yields among the different treatments used. It therefore appears that sulphur additions providing pH values of 4·6 or greater, are not likely to affect plant biomass and therefore total yields of cobalt and/or nickel in a phytoremediation/phytomining operation. An interesting avenue of future research would be to investigate the effects of chelating agents on other hyperaccumulator plants in comparison to non-hyperaccumulator plants grown on the same soil.

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