*International Journal of Phytoremediation,* 5(3):235–244 (2003)

ISSN: 1522-6514
DOI: 10.1080/16226510390255689

# Nickel and Cobalt Phytoextraction by the Hyperaccumulator *Berkheya coddii*: Implications for Polymetallic Phytomining and Phytoremediation

**S. M. Keeling,[1,2*] R. B. Stewart,[1] C. W. N. Anderson,[1] and B. H. Robinson[3]**
*[1] Soil and Earth Sciences, Institute of Natural Resources, Massey University, Palmerston North, New Zealand; [2] Currently, School of Earth Sciences, James Cook University, Cairns, Australia; [3] HortResearch Ltd., Palmerston North, New Zealand*

* Corresponding author: Soil and Earth Sciences, Institute of Natural Resources, PB 11-222, Massey University, Palmerston North, New Zealand.

## ABSTRACT

We investigated the potential of the South African high-biomass Ni hyperaccumulator *Berkheya coddii* to phytoextract Co and/or Ni from artificial metalliferous media. Plant accumulation of both metals from single-element substrates indicate that the plant/media metal concentration quotient (bioaccumulation coefficient) increases as total metal concentrations increase. Cobalt was readily taken up by *B. coddii* with and without the presence of Ni. Nickel uptake was, however, inhibited by the presence of an equal concentration of Co. Bioaccumulation coefficients of Ni and Co for the single element substrates (total metal concentration of 1000 $\mu g\ g^{-1}$) were 100 and 50, respectively. Cobalt phytotoxicity was observed above a total Co concentration in plant growth media of 20 $\mu g\ g^{-1}$. Elevated Co concentrations significantly decreased the biomass production of *B. coddii* without affecting the bioaccumulation coefficients. The mixed Ni–Co substrate produced bioaccumulation coefficients of 22 for both Ni and Co. Cobalt phytotoxicity in mixed Ni–Co substrate occurred above a total Co concentration of 15 $\mu g\ g^{-1}$. When grown in the presence of both Ni and Co, the bioaccumulation coefficients of each metal were reduced, as compared to single-element substrate. This may indicate competition for binding sites in the root zone. The interference relationship between Ni and Co uptake demonstrated by *B. coddii* suggests a significant limitation to phytoextraction where both metals are present.

**KEY WORDS:** *Berkheya coddii*, cobalt, nickel, hyperaccumulation, phytomining, phytotoxicity.

## I. INTRODUCTION

Phytoextraction is a process that has applications for the recovery of metal from substrates such as soil, particularly those associated with sub-economic mineralizations and contamination by industry. Numerous sites across the globe that are enriched with heavy metals could potentially be phytoremediated (decontamination by phytoextraction) and/or phytomined (commercial production of metals via cropping). This technology also has potential application in the minerals industry for the phytomining and phytoremediation of low-grade ores and metalliferous mine spoils.

Hyperaccumulator plants are plant species that are able to accumulate abnormal concentrations of various metal in their tissues. Brooks *et al.* (1977) defined hyperaccumulation of Ni and Co as being concentrations greater than 1000 $\mu$g g$^{-1}$ (ppm) of dried plant material. The hyperaccumulation level varies for different metals, e.g., 10,000 $\mu$g g$^{-1}$ Zn and Mn (Brooks *et al.*, 1977).

The South African Ni hyperaccumulator *Berkheya coddii* is an asteraceous perennial plant that typically grows to 2 m in height and is confined to ultramafic outcrops in the Eastern Transvaal near Barberton (Brooks, 1998). *Berkheya coddii* is one of over 300 known Ni hyperaccumulators with reported foliar Ni concentrations in excess of 17,000 $\mu$g g$^{-1}$ (1.7%) dry mass (Howes, 1991; Howes *et al.*, 1998; Morrey *et al.*, 1989).

Phytoextraction using hyperaccumulating plants was first proposed by Chaney (1983) and later by McGrath *et al.* (1993), who demonstrated that these plants could be used to recover soil contaminants. The technique required cultivating the crop of hyperaccumulators selected for the ability to take up the target contaminants on the polluted soil. The above-ground biomass would be harvested and combusted to produce an ash containing the contaminant metals. This metal-rich ash, typically only 7% of the original weight of the dried biomass, would then be stored or treated in a smelter to recover the metals for sale to offset the cost of soil remediation. The technique was termed phytoremediation and is the subject of a considerable research effort worldwide (Brooks, 1998; Raskin *et al.*, 1994; Salt *et al.*, 1995).

Nicks and Chambers (1994, 1995, 1998) reported a novel application for hyperaccumulating plants, termed phytomining, by cultivating a crop of a Ni accumulator on a substrate containig Ni concentrations that were considered sub-economic by today's modern mining methods. They showed that the California Ni hyperaccumulator *Streptanthus polygalides* could recover over 100 kg ha$^{-1}$ of Ni from a serpentine soil. Robinson *et al.* (1997) achieved a similar Ni recovery rate from serpentine soil using *B. coddii*.

As a result of the large-scale development of lateritic Ni–Co deposits in Australia, significant volumes of waste materials require rehabilitation. Similarly, the shallow depths to which laterite miners excavate these deposits exposes large areas of (often) bare saprolitic rock. These sub-economic materials often still contain minerals at levels that are suitable for phytoextraction. For example, saprolitic pit-floor waste rock from the Murrin Murrin dry Ni–Co laterite mine in Western Australia, operated by Anaconda Nickel Limited, contained 3.3% Ni, 900 $\mu$g Co g$^{-1}$, and 6.5% Mg (Keeling, 2000).

Since the value of Co on the world market is often 3–4 times that of Ni, it is surprising that little quantitative research has emerged reporting Co accumulation or Ni–Co co-accumulation in *B. coddii*. It was therefore decided to investigate the Ni, Co, and Ni–Co uptake potential of *B. coddii* under controlled greenhouse conditions using pot trial experiments of mono- and bi-metal substrates as a prelude to investigating its response to polymetallic lateritic materials.

## II. MATERIALS AND METHODS

All experiments were conducted in climate-controlled glasshouses in a plant growth unit. Glasshouse temperatures were regulated between 15°C (night) and 25°C (day) and growth lamps maintained a daylight period of 14 hours per day. The single metal substrates were prepared by blending 10 g of finely ground solid Ni or Co sulphate to 10 kg of a potting mix composed of 50% peat and 50% finely sieved pumice with additions of lime, dolomite, and "Osmocote" (a three-month slow-release fertilizer) at rates recommended by the manufacturer. These 1000 $\mu$g g$^{-1}$ Ni- and Co-only substrates were then further blended at a ratio of 1:3 with the same potting mix (devoid of Ni and Co) to produce a final substrate concentration range of 1000, 333, 111, 37, 12, and 4 $\mu$g g$^{-1}$ Ni or Co, respectively. The mixed Ni–Co substrate was prepared by blending equal amounts of both the single metal substrates together to produce potting medium containing concentrations of both elements at 500, 333, 111, 37, 12, and 4 $\mu$g g$^{-1}$ Ni and Co. Six-week-old seedlings of *B. coddii* propagated from commercially available germination mix were transplanted into 280 mL pots containing the prepared substrates. Eight replicates of each treatment were prepared. After two weeks, fresh seedlings were added to pots with poor survival. After 12 weeks, all above-ground biomass was harvested, oven dried at 70°C overnight, and dry weights (DW) were recorded to determine rates of biomass production. Sub-samples of biomass from each soil treatment were then ground, weighed, and ashed at 540°C in a muffle furnace until fully combusted. The resulting ash was accurately weighed and digested in near-boiling 2-M HCl, agitated, and analyzed by flame atomic absorption spectroscopy (FAAS). All data were tested for normality and analyzed using ANOVA due to the observed normal distribution.

## III. RESULTS AND DISCUSSION

### A. Nickel and Cobalt Accumulation from Single-Element Substrates

Table 1 shows the plant–metal concentrations in *B. coddii* over the range of total substrate metal concentrations used in this experiment. Plant uptake of Ni from both the single-element and mixed Ni–Co substrates was considerably lower than previously published data (Brooks, 1998; Robinson *et al.*, 1997). Paucity of accumulation is often reported in the literature when comparisons between natural

**TABLE 1.** Mean Ni and/or Co concentrations ± standard error of the mean ($\mu$g g$^{-1}$) and mean biomass production (g plant$^{-1}$) for *B. coddii* (n = 8).

| Substrate | 4 | 12 | 37 | 111 | 333 | 500 | 1000 |
|---|---|---|---|---|---|---|---|
| Ni only | | | | | | | |
| Plant Ni | 40 ± 37 | 177 ± 120 | 735 ± 301 | 2903 ± 693 | 5180 ± 944 | nt | 5809 ± 893 |
| Survival | 3 | 5 | 7 | 7 | 7 | | 4 |
| Biomass | 0.28 | 0.94 | 0.67 | 0.41 | 0.43 | | 0.29 |
| Co only | | | | | | | |
| Plant Co | 30 ± 13 | 157 ± 116 | 522 ± 285 | 1178 ± 548 | 1328 ± 966 | nt | 3295 ± 822 |
| Survival | 6 | 4 | 8 | 7 | 7 | | 4 |
| Biomass | 0.41 | 0.50 | 0.34 | 0.37 | 0.24 | | 0.32 |
| Ni–Co mix | | | | | | | |
| Plant Ni | 36 ± 12 | 100 ± 48 | 117 ± 43 | 124 ± 80 | 411 ± 43 | 534 ± 149 | nt |
| Plant Co | 41 ± 18 | 135 ± 68 | 693 ± 244 | 712 ± 508 | 2116 ± 307 | 2097 ± 296 | nt |
| Survival | 6 | 7 | 7 | 4 | 4 | 3 | |
| Biomass | 0.51 | 0.29 | 0.42 | 0.12 | 1.09 | 0.70 | |

nt = not tested.

stands of hyperaccumulator plants and pot trials are made. This may result from unfavorable environmental conditions as compared to the plants' native habitats or from effects relating to the controlled short-term growth experiments performed in greenhouse plant trials. An investigation of the direct effects of studying metal uptake in pot trial experiments, as compared to metal uptake under field conditions, is required.

The bioaccumulation coefficient for metal uptake from the single element substrates is higher for Co than for Ni, below a total substrate metal concentration of 333 $\mu$g g$^{-1}$. Above a total metal substrate concentration of 333 $\mu$g g$^{-1}$, however, the uptake of Co from the Co-only substrate decreases relative to Ni uptake from the Ni-only substrate (Table 1). This decrease in the Co uptake may be attributed to visible Co phytotoxicity above a substrate concentration of 333 $\mu$g g$^{-1}$, which was exhibited as stuniting, yellowed and brittle leaves, and poor root development. No visible effect of Ni phytotoxicity was evident over the experimental range (4–1000 $\mu$g g$^{-1}$). Nickel and Co uptake (Figure 1) increased in the plants with increasing total metal concentration in the substrates. These data indicate that a total substrate metal concentration of approximately 168 $\mu$g g$^{-1}$ is required for hyperaccumulation ($<$1000 $\mu$g g$^{-1}$) to occur (Figure 1), representing an Ni bioaccumulation coefficient of 6 for *B. coddii*. Cobalt hyperaccumulation occurred above a total substrate concentration of 125 $\mu$g g$^{-1}$, representing a Co bioaccumulation coefficient of 8 for *B. coddii*.

### B. Nickel and Cobalt Accumulation from the Mixed Nickel–Cobalt Substrate

In comparison to Ni-only substrate, Ni uptake is much less in the Ni–Co substrate (Figure 2). A total substrate Ni concentration of 400 $\mu$g g$^{-1}$ is required form Ni

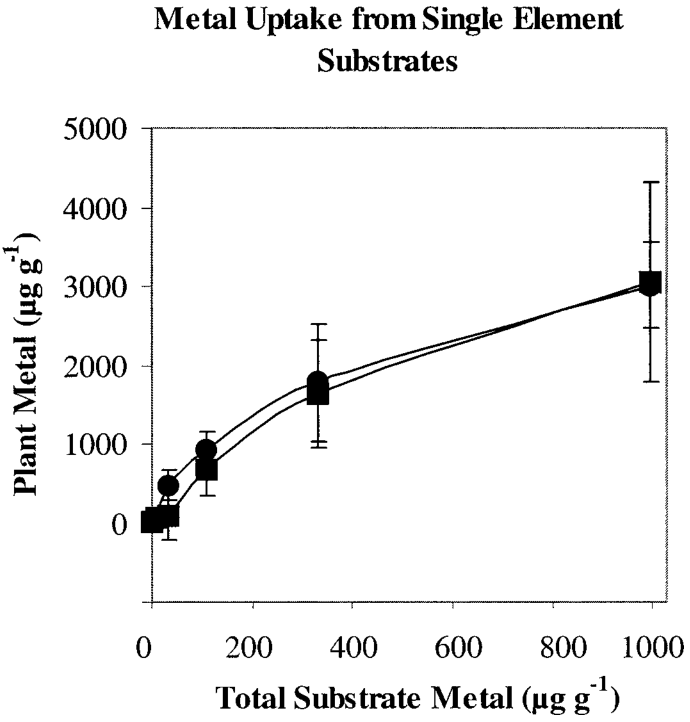

**FIGURE 1.** Mean Ni (square) and Co (circle) uptake by *Berkheya coddii* (dry wt., n = 8) as a function of total substrate metal concentrations from single-element substrates. ± standard error of the mean, concentrations in $\mu$g g$^{-1}$.

hyperaccumulation, compared with 168 $\mu$g g$^{-1}$ required in the Ni-only substrate. This is a 2.4-fold decrease in the bioaccumulation coefficients, from 6 to 2.5, compared with the single-element substrate. The decrease in the bioaccumulation coefficients is attributed to interference by Co in the substrate. Cobalt is inhibiting Ni uptake at concentrations below approximately 450 $\mu$g g$^{-1}$ by being preferentially accumulated by *B. coddii.*

The relationship between Ni and Co uptake by *B. coddii* for single-element substrates, compared to the mixed Ni–Co substrate, is best expressed using bioaccumulation coefficients quotients of both elements (Figure 3). The bioaccumulation coefficient quotient for Co indicates little deviation about a 1:1 relationship at different substrate concentrations, indicating that Co uptake from both the single- and mixed-element substrates is similar. Therefore, Co accumulation by *B. coddii* is independent of any influence from Ni uptake. However, Ni uptake is markedly different in the single- and mixed-element substrates, indicated by increasing deviation from a 1:1 relationship at different substrate concentrations.

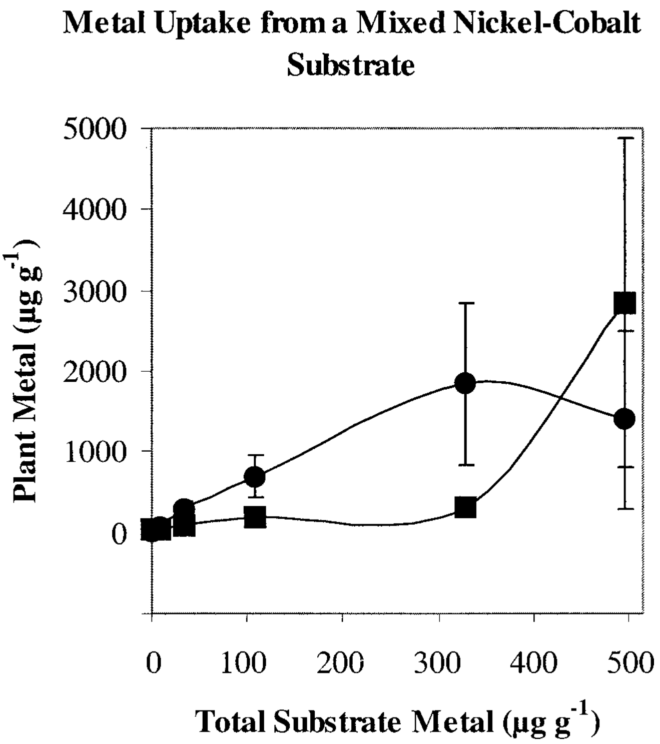

**FIGURE 2.** Mean Ni (square) and Co (circle) uptake by *Berkheya coddii* (dry wt., n = 8) as a function of total substrate metal concentrations from mixed-metal substrates. ± standard error of the mean, concentrations in $\mu$g g$^{-1}$.

## C. Biomass Production by *Berkheya coddii* Grown on Artificial Substrates

Biomass production in Ni-only substrates (Figure 4) was not significantly affected (P = 0.296) by metal concentrations over the experimental range investigated. All plants appeared healthy and vigorous at harvest, with no noticeable symptoms of Ni toxicity.

By contrast, there was a significant decrease (P = 0.023) in biomass production when *B. coddii* was grown on Co-only substrates. The mean herbage weight at the growth maximum on 37 $\mu$g g$^{-1}$ total substrate Co was 0.65 g. Above this substrate concentration, evidence of Co phytotoxicity was seen as a reduction in plant weight and, visibly, as pronounced yellowing of leaves, leaf stems, and leaf axial regions (frequently accompanied by white spotting on foliage); stunted inter-nodal stems; and foliage that was brittle and readily broken when grasped firmly. The date indicate that *B. coddii* is more tolerant to Ni than Co at higher substrate metal concentrations. The effects of Co phytotoxicity did not, however, affect the Co bioaccumulation coefficients (Figure 3). Cobalt

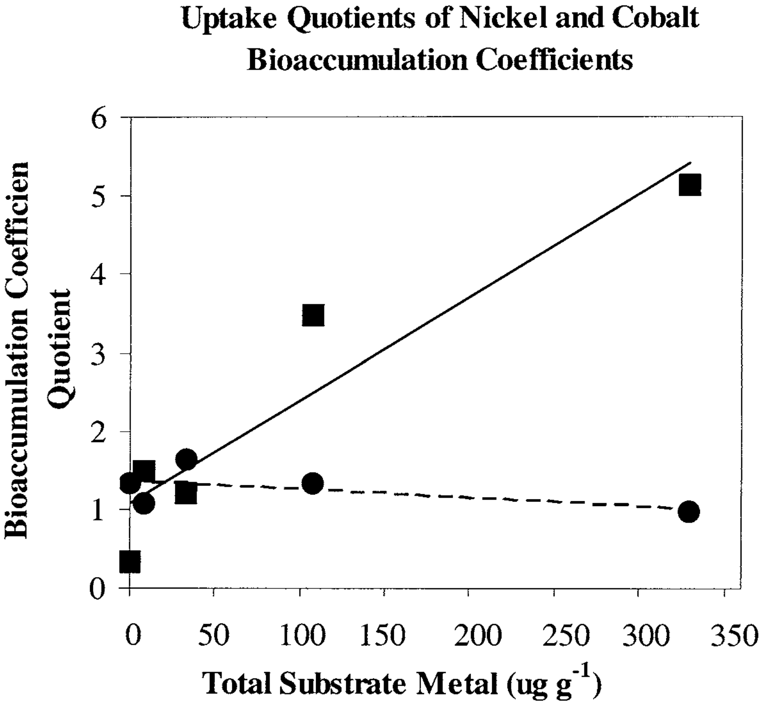

**FIGURE 3.** Bioaccumulation coefficient quotients (single-element substrates uptake/mixed-element substrate uptake) for Ni and Co uptake by *Berkheya coddii* (dry wt., n = 8) for a range of total substrate metal concentrations ($\mu$g g$^{-1}$).

accumulation in *B. coddii* may, therefore, be an externally controlled process related to Co geochemistry rather than a plant-initiated response to, or need for, Co. *Berkheya coddii* could, as a result, be classified as an indicator plants for Co (Baker, 1981).

Biomass production from the mixed Ni–Co substrate is greater at total substrate metal concentrations below 37 $\mu$g g$^{-1}$ Ni and Co. Above this concentration, biomass production decreased significantly and was attributed to Co phytotoxicity. The average herbage weight recorded at a total metal concentration of 37 $\mu$g g$^{-1}$ (the growth maximum) was 0.4 g. Observations made at harvest time recorded similar symptoms as those seen in the Co-only trial, yet not as pronounced. Phytotoxicity at total substrate metal concentrations greater than 111 $\mu$g g$^{-1}$ Ni and Co resulted in the death of 85% of the sampled population. The average weight of the surviving plants was approximately 0.1 g, indicating that Co bioavailability is the dominant factor in plant health on the mixed Ni–Co substrate at, and above, this total metal concentration.

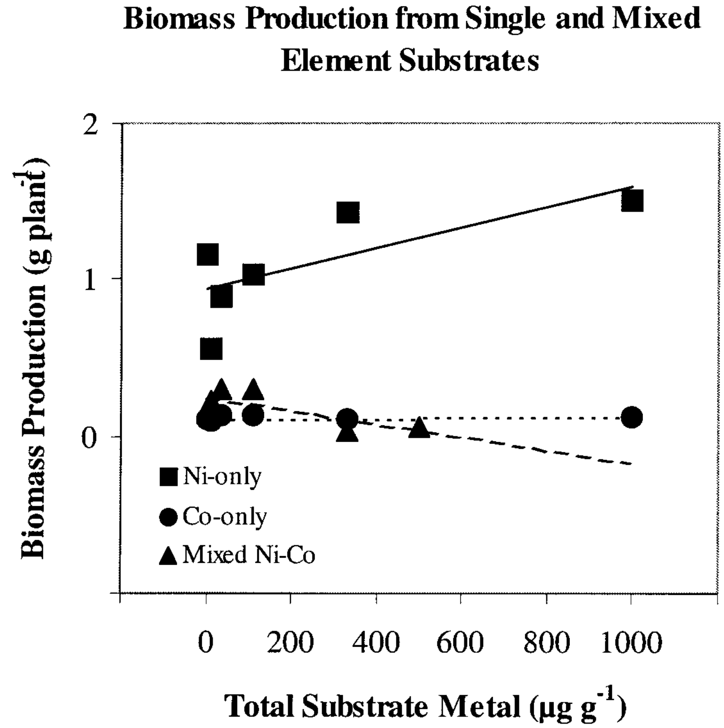

**FIGURE 4.** Biomass production (g plant$^{-1}$) of *Berkheya coddii* (dry wt., n = 8) and first order linear regressions as a function of the total substrate Ni (square), Co (circle), and mixed metal (triangle). Concentrations in $\mu$g g$^{-1}$.

## IV. CONCLUSIONS

*Berkheya coddii* hyperaccumulated Ni from the single-element artificial substrate at a total Ni level (168 $\mu$g g$^{-1}$) lower than concentrations reported from previous glasshouse research (Robinson *et al.*, 1997). Cobalt hyperaccumulation from the single-element substrate occurred above 125 $\mu$g g$^{-1}$ total substrate Co and appeared at the biomass production maximum, preceding the onset of Co phytotoxicity. The bioaccumulation coefficients for Co- and Ni-only substrates for *B. coddii* were eight and six, respectively Phytotoxicity to Co was manifest above a total substrate Co concentration of 111 $\mu$g g$^{-1}$, represented by a reduction in biomass production and yellowing of foliage leading to necrosis at high concentrations.

Plant uptake of Ni and Co from the mixed substrate showed higher Co accumulation and a reduction in overall Ni accumulation in plant materials as compared to single-element substrates. Total substrate Ni required to achieve hyperaccumulation (>1000 $\mu$g g$^{-1}$) by *B. coddii* increased from 168 $\mu$g g$^{-1}$ in the single-element substrate to 400 $\mu$g g$^{-1}$ in the mixed substrate. The bioaccumulation coefficients for Ni therefore increased 60% as compared to the Ni-only substrate.

Cobalt hyperaccumulation from both Co-bearing substrates occurred at a total Co concentration of 400 $\mu$g g$^{-1}$, above the total Co concentration that induced phytotoxicity. The Co bioaccumulation coefficients did not, therefore, change between substrates.

Biomass production rates and general plant health, for both Co-bearing substrates, indicated Co phytotoxicity at total substrate metal concentrations in excess of 111 $\mu$g g$^{-1}$. A comparison of the potential crop yields of Ni and Co illustrates the negative impact of Co phytotoxicity on biomass production and the mean Ni and Co phytoremediation potential of *B. coddii*. Biomass production values were determined assuming optimal growth conditions of 9 t ha$^{-1}$ occurred on the Ni-only substrate. Biomass production values for the Co-bearing substrates were determined using the ratio of plant weights between these substrates and the Ni-only substrate. The crop yields for single element substrates were approximately 14.5 kg Ni ha$^{-1}$ and 12.6 kg Co ha$^{-1}$. By comparison, the extrapolated metal yields for the mixed Ni–Co substrates were 0.9 kg Ni ha$^{-1}$ and 3.1 kg Co ha$^{-1}$, a reduction in Ni and Co bioaccumulation coefficients by 95% and 75%, respectively.

The phytotoxic effect of Co on general plant growth and accumulation potential in *B. coddii* is important and must be considered when evaluating Ni and Co phytoextraction potential using this species. If we assume that the Ni-only pot trial is a "best case scenario" for plant growth, then the following points can be made.

1. *Berkheya coddii* is Ni hypertolerant over the experimental range.
2. Nickel uptake decreases significantly in the presence of Co.
3. *Berkheya coddii* readily accumulates Co. However, in the presence of Ni, hyperaccumulation of both elements appears at Co concentrations that are phytotoxic.
4. Cobalt concentrations above 111 $\mu$g g$^{-1}$ have a pronounced phytotoxic effect on biomass production in *B. coddii* without affecting the bioaccumulation coefficients of Co.

## ACKNOWLEDGEMENTS

The author gratefully acknowledges the late Emeritus Professor R. R. Brooks and Anglo American Platinum Corporation Ltd of Rothenburg, South Africa, for providing seed of *Berkheya coddii*.

## REFERENCES

Baker, A. J. M. 1981. Accumulators and excluders—strategies in the response of plants to heavy metals. *J. Plant Nutrition* **3,** 643–654.

Brooks, R. R. 1998. *Plants that Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining*. Wallingford, UK, CAB International.

Brooks, R. R., Lee, J., Reeves, R. D., and Jaffré, T. 1977. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochem. Expl.* **7,** 49–57.

Chaney, R. L. 1983. Plant uptake of inorganic waste constituents. In: *Land Treatment of Hazardous Wastes*, pp. 50–76 (Parr, J. F., Marsh, P. B., and Kla, J. M., Eds.). Park Ridge, NJ. Noyes Data Corp.

Howes, A. W. 1991. Investigations into nickel hyperaccumulation in the plant *Berkheya coddii*: Unpublished MSc thesis, Natal, South Africa, University of Natal.

Howes, A. W., Slatter, K. A., Sim, E. A., and Jones, A. N. 1998. Rehabilitating nickel contaminated soils at a base metal refinery using the nickel hyperaccumulating plant species, *Berkheya coddii*. In: *Waste Processing and Recycling in Mineral and Metallurgical Industries III* (Rao, S. R., Amaratunga, L. M., Richards, G. G., and Kondos, P. D., Eds.). Canada, The Metallurgical Society of CIM.

Keeling, S. M. 2000. The economic significance of the phytoextraction of nickel, cobalt and gold from metalliferous soils: Unpublished MSc thesis, New Zealand, Massey University, 162 p.

McGrath, S. P., Sidoli, C. M. D., Baker, A. J. M., and Reeves, R. D. 1993. The potential for the use of metal-accumulating plants for the in situ decontamination of metal-polluted soils. In: *Integrated Soil and Sediment Research: A Basis for Proper Protection*, pp. 673–676 (Eijackers, H. J. P. and Hamers, T., Eds.). Amsterdam, The Netherlands, Kluwer.

Morrey, D. R., Balkwill, K., and Balkwill, M.-J. 1989. Studies on serpentine flora: preliminary analyses of soils and vegetation associated with serpentine rock formation in the south-eastern Transvaal. *S. African J. Botany* **55,** 171–177.

Nicks, L. J., and Chambers, M. F. 1994. Nickel farming. *Discovery Magazine* **19,** 22–23.

Nicks, L. J., and Chambers, M. F. 1995. Farming of metals. *Mining Environmental Management* **11,** 15–18.

Nicks, L. J., and Chambers, M. F. 1998. A pioneering study of the potential of phytomining for nickel. In: *Plants that Hyperaccumulate Heavy Metals*, pp. 313–326 (Brooks, R. R., Ed.). Wallingford, UK, CAB International.

Raskin, I., Kumar, N. P. B. A., and Dushenkov, S. 1994. *Phytoremediation of Metals*. Nov. 15: United States of America, Phytotech, Inc., Morristown, NJ.

Robinson, B. H., Brooks, R. R., Howes, A. W., Kirkman, J. H., and Gregg, P. E. H. 1997. The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. *J. Geochem. Expl.* **60,** 115–126.

Robinson, B. H., Brooks, R. R., Kirkman, J. H., Gregg, P. E. H., and Alverez, H. V. 1997. Edaphic influences on a New Zealand ultramafic (‘serpentine’) flora: a statistical approach. *Plant Soil* **188,** 11–20.

Salt, D. E., Blaylock, M. J., Kumar, N. P. B. A., Dushenkov, V., Ensley, B. D., and Chet, I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnol.* **13,** 468–474.