Uptake and distribution of nickel and other metals in the hyperaccumulator *Berkheya coddii*

B. H. Robinson[1], E. Lombi[2], F. J. Zhao[2] and S. P. McGrath[2]

[1]HortResearch, Private Bag 11 030, Palmerston North, New Zealand; [2]Agriculture and Environment Division, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

Author for correspondence:
B. H. Robinson
Tel: +64 63568080
Fax: +64 63546731
Email: brobinson@hortresearch.co.nz

Received: *21 October 2002*
Accepted: *21 January 2003*

doi: 10.1046/j.1469-8137.2003.00743.x

Summary

• *Berkheya coddii* is a fast-growing, high biomass nickel (Ni) hyperaccumulator plant that has recently attracted attention for its possible use in the phytoextraction of Ni. The mechanisms of Ni accumulation, however, are not well understood in this plant.

• Plants were grown hydroponically in varying Ni concentrations to assess the uptake and distribution of Ni, and other metals, at the whole plant level. X-ray microanalyses (EDXA) of frozen hydrated tissues were conducted to determine the distribution of Ni at the cellular level in the leaves.

• Most Ni was found in the shoots, especially in the leaves. Leaf Ni concentration increased with age, whereas older stem sections had lower Ni concentrations than new growth. EDXA analyses revealed that the cuticle of the upper epidermis had a significantly higher Ni concentration than the rest of the leaf. The Ni concentrations in the other leaf tissues were not significantly different.

• This pattern of distribution contrasts sharply with some other hyperaccumulator species that commonly show a preferential accumulation of Ni and other metals in the vacuoles of the epidermal cells.

Key words: *Berkheya coddii*, nickel (Ni), hyperaccumulation, compartmentation, phytoextraction.

© *New Phytologist* (2003) **158**: 279–285

Introduction

Nickel (Ni) hyperaccumulator plants represent over three-quarters of known metal hyperaccumulators, with a total number of 318 taxa reported to date (Baker *et al.*, 2000; Reeves & Baker, 2000). Recently, *Berkheya coddii* has attracted attention because of its high Ni concentration and rapid biomass production. Robinson *et al.* (1997) reported an annual biomass production of 22 t ha^{-1} and up to 1% (w : w) Ni in the dry above-ground biomass. *Berkheya coddii* is an asteraceous summer-green perennial plant that is found on ultramafic (serpentine) soils in southern Africa (Morrey *et al.*, 1992).

The phenomenon of Ni hyperaccumulation by plants is not well understood. Krämer *et al.* (1997) reported that at low substrate Ni concentrations the rates of Ni uptake and root-to-shoot translocation in both the hyperaccumulator *Thlaspi goesingense* and the nonNi-hyperaccumulator *Thlaspi arvense* were similar. It was suggested that Ni tolerance alone could explain Ni hyperaccumulation when plants were grown in presence of large concentrations of available Ni. More is known in terms of Ni tolerance in hyperaccumulator plants where two mechanisms, complexation and compartmentalization, appear to be responsible for Ni detoxification. The complexation of Ni by histidine and organic acids such as citrate, malate and malonate has been reported in a number of hyperaccumulator plants (Lee *et al.*, 1977, 1978; Krämer *et al.*, 1996; Brooks *et al.*, 1998). Similar to what has been observed in cadmium (Cd) and zinc (Zn) hyperaccumulation, Ni has been reported to accumulate in the epidermal cells in *Senecio coronatus* and several species of *Alyssum* (Mesjasz-Przybylowicz *et al.*, 1994; Psaras *et al.*, 2000). In particular, Küpper *et al.* (2001) showed that Ni was preferentially compartmentalized in the vacuoles of epidermis cells in *T. goesingense*, *Alyssum bertolonii* and *Alyssum lesbiacum*. Using cell fractionation, Krämer *et al.* (2000) also showed that intracellular Ni

is predominantly localized in the vacuoles of *T. goesingense*. This pattern of cellular distribution is consistent with the finding by Persans *et al.* (2001) who reported a high-level expression of a vacuolar metal ion transporter protein (TgMTP1) in *T. goesingense*. This transporter may account for the enhanced accumulation of Ni in leaf vacuoles of *T. goesingense*. Most of the information regarding the mechanism of Ni hyperaccumulation has been obtained in two genera of the Brassicaceae: *Thlaspi* and *Alyssum*. Very little is known about Ni hyperaccumulation in plants belonging to other families. Therefore, it is not known whether the same mechanisms are shared by all Ni hyperaccumulators.

In this paper, we investigate some aspects of Ni hyperaccumulation in *B. coddii* because of its high biomass, high Ni accumulation and its consequent potential to be used for the phytoextraction (either phytoremediation or phytomining) of Ni. Compartmentalization of metals seems to be a key mechanism of tolerance in hyperaccumulator plants (for a recent review see McGrath *et al.*, 2002). Therefore, the aim of this study was to determine the distribution of Ni and other elements, at whole plant and cellular scale, in *B. coddii*.

Materials and Methods

Plant growth and analyses

Seedlings of *B. coddii* were germinated in a mixture of Perlite and vermiculite moistened with deionized water. After 3 wk, seedlings were transferred to 1-l vessels containing 1000 μM $Ca(NO_3)_2$, 500 μM $MgSO_4$, 50 μM K_2PO_4, 100 μM KCl, 10 μM H_3BO_4, 1.8 μM $MnSO_4$, 0.2 μM $NaMoO_4$, 0.31 μM $CuSO_4$, 50 μM Fe(III)-EDDHA (ethylenediamine-di(*o*-hydroxyphenylacetic acid)) and 1 μM $ZnSO_4$ (Shen *et al.*, 1997). Solution pH was maintained at 6.0 with 2000 μM 2-(*N*-morpholino) ethanesulfonic acid (MES), pH adjusted with KOH. After 2 wk, nickel (Ni, as $NiSO_4$) was added to the nutrient solution at concentrations of 0.5, 50, 100, 250 and 500 μM. Each Ni treatment was replicated in three vessels, each containing three plants. The nutrient solutions were aerated continuously and renewed every 3 d. The experiment was carried out in a controlled environment room with the following conditions; 16 h daylength with a light intensity of 350 μmol photons m^{-2} s^{-1} supplied by fluorescent tubes, 20°C day/16°C night temperatures, and 60–70% relative humidity. Seventeen weeks after the treatments were started, the plants were removed from the nutrient solutions and washed thoroughly in deionized water. Roots, leaves and stems of plants of each pot were collected separately. The plant material was frozen in liquid nitrogen, freeze-dried for 72 h, and the dry weight recorded. Dried plant material was finely ground and subsamples (0.5 g) were digested with a mixture of HNO_3 and $HClO_4$ (Zhao *et al.*, 1994). The total concentration of Ni and other elements were determined by inductively coupled plasma emission spectroscopy (ICP-AES, Fison Accuris, Ecublens, Switzerland). Water-soluble Ni in leaves was extracted by shaking 0.5 g of ground plant material with 20 ml of 1000 μmol MES buffer at pH 6.0 for 45 min (Cakmak & Marschner, 1987). The samples were then filtered and the Ni concentration determined as described earlier.

Distribution of the Ni and other elements in leaves and stems of different age

To investigate the effect of age of tissue on element concentrations, three plants from the 100 μM treatment were selected for an investigation into the distribution of metal concentrations with plant height. The above-ground portions of these plants were divided into five parts of equal length, with the oldest at the base and youngest at the apex. The total stem lengths of the plants were 80, 57 and 62 cm and each plant had only one shoot. Leaves and stems were analysed separately as described earlier.

Cellular distribution of Ni and other elements in leaves

Leaf specimens (three per treatment) were taken for analysis from plants grown in the presence of 100 and 500 μM Ni. A small section of the leaf was excised, mounted in an aluminium (Al) sample holder, then rapidly frozen in melting nitrogen slush. The sample was transferred to a preparation chamber set at −180°C and fractured. The surface of the sample was evaporatively coated with carbon and transferred to a cooled stage (−170°C) inside a scanning electron microscope (XL 40; Philips, Eindhoven, The Netherlands). Energy dispersive X-ray microanalysis was performed in the scanning electron microscope using an acceleration voltage of 30 keV. Spectra from 0 to 20 keV were collected at increments of 10 eV per channel and analysed using the program SUPERQUANT (EDAX, San Francisco, CA, USA). A calibration between peak : background ratios for specific elements and their concentration in standard solutions was used to estimate concentration (van Steveninck & van Steveninck, 1991). Two-dimensional distribution patterns of Ni, Ca and K were collected by scanning an area of the specimen for up to 1 h and integrating the counts for each element within their respective spectrum windows into dot-maps. These maps are semiquantitative and show the metal distribution in each tissue type.

Statistical treatment of data

Correlation analyses and ANOVA were performed using the GENSTAT 5 package (Genstat 5 Committee, 1993). Data that were not normally distributed were logarithmically transformed before analysis. Least significant difference (LSD) was used for comparison between the treatment means.

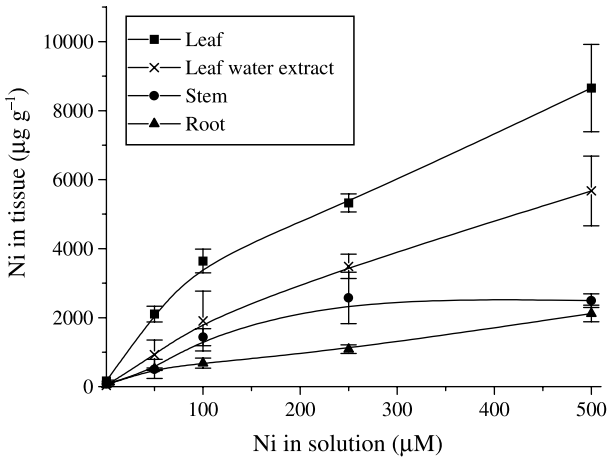

Fig. 1 Nickel (Ni) concentration in various tissues of *Berkheya coddii* as a function of Ni concentration in nutrient solution; $n = 3$. Squares, leaf; crosses, leaf water extract; circles, stem; triangles, root.

Results

Effect of Ni treatments on biomass, metal uptake and Ni solubility in *B. coddii*

At the end of the experiment, the average dry weight of the plants was 20.0 ± 1.5 g and there were no signs of toxicity in any of the treatments. The roots, stems and leaves accounted for 49%, 32% and 19% of the total dry biomass, respectively. There were no significant differences in biomass between treatments ($P > 0.1$), indicating that *B. coddii* is tolerant to at least 500 μM Ni in hydroponic solution under the conditions used in the experiment.

The concentration of Ni in the different parts of *B. coddii* plants increased with increasing Ni concentration in the hydroponic solution (Fig. 1). The results show that leaves had the highest Ni concentration, and that most of the metal accumulated in the leaves was stored in a water-soluble form. In the 500 μM Ni treatment, 65% of the total Ni accumulated in the leaves was water-soluble. The concentration of Ni in stems was consistently lower than in leaves but higher than in roots.

Although the leaves represented less than one-fifth of the total plant weight, almost half (48%) of the total Ni accumulated by plants growing in 500 μM Ni treatment was stored in these organs. The remaining amount of accumulated Ni was almost evenly distributed between the roots (29%) and the stems (23%).

The average concentrations of calcium (Ca), potassium (K), iron (Fe) and copper (Cu) in roots, stems and leaves, and their relationship to solution Ni concentration are reported in Table 1. Unlike the observation for Ni, there was a general decrease in the concentrations of these elements with an increasing Ni concentration in the hydroponic solution. With the exception of Ca, this decrease was more marked in the leaves and stems than in the roots. The concentration of K, Fe and Cu in leaves of plants grown in the 500 μM Ni solution decreased by 47%, 85% and 46%, respectively, compared with the control.

Distribution of Ni and other elements in leaves and stems of different age

The distribution of Ni and other macronutrients and micronutrients in leaves and stems was investigated in plants grown in the 100 μM Ni treatment. The average Ni concentration decreased from 4375 μg g^{-1} in the oldest leaves to 3210 μg g^{-1} in the youngest leaves (Fig. 2). A similar trend was observed for some of the other elements measured (Ca, K, magnesium (Mg), P, Fe and zinc (Zn)). For these elements, however, the leaf primordia (fraction 1) had anomalously high concentrations (data not shown).

The change in Ni concentration in the stem was more marked with height, and opposite to that in the leaves. Nickel concentrations in stems increased from 692 μg g^{-1} at the base to 1814 μg g^{-1} at the apex. All the other elements analysed followed a similar trend (data not shown).

Cellular distribution of Ni and other elements in leaves

Results from X-ray microanalyses (EDXA) of frozen-hydrated leaves showed that the upper cuticle had a significantly higher ($P < 0.001$) Ni concentration than all the other leaf tissues analysed (Fig. 3a). This distribution pattern was similar in

Table 1 Concentration of macronutrients and micronutrients, in roots, stems and leaves of *Berkheya coddii* as a function of solution nickel concentration

Solution nickel (μM)	Calcium (mg g^{-1})			Potassium (mg g^{-1})			Iron (μg g^{-1})			Copper (μg g^{-1})		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
0.5	0.38	1.47	1.42	1.11	1.67	2.82	1019	77	378	178	31	18
50	0.39	1.43	1.21	1.43	1.22	2.60	1195	75	185	186	22	11
100	0.39	1.08	1.09	1.29	1.92	2.67	1386	98	194	179	34	14
250	0.29	0.85	1.00	1.36	1.41	2.03	1068	46	69	84	12	8
500	0.20	0.85	0.99	1.11	1.53	1.22	1336	44	55	96	15	8
P	< 0.05	< 0.05	ns	ns	ns	< 0.01	ns	< 0.01	< 0.05	ns	< 0.001	< 0.001
LSD	0.15	0.51	3.70	0.74	0.51	6.74	564	23	218	145	8.3	4.3

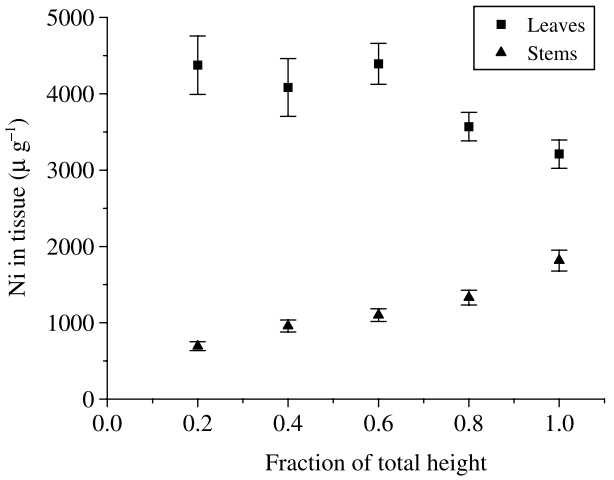

Fig. 2 Concentration of nickel (Ni) in leaves (squares) and stems (triangles) of *Berkheya coddii*, with height, grown hydroponically in the presence of 100 μM Ni. Error bars represent the standard error of the mean.

leaves of plants grown in both the 100 and 500 μM Ni treatments (Fig. 3a). The concentration of Ni in the cuticle almost doubled from the 100 μM Ni treatment to the 500 μM Ni treatment, this is consistent with a twofold increase in Ni concentration in leaves, as determined by Inductively Coupled Plasma Emission Spectroscopy (ICP-ES) (Fig. 1). By contrast, the concentrations of Ni in the upper and lower epidermal cells, palisade, spongy mesophyll and lower cuticle were not significantly different from each other ($P > 0.05$).

Calcium was evenly distributed in the upper and lower epidermis and in the palisade and spongy mesophyll cells (Fig. 3b). However, Ca concentrations were found to be significantly higher in both the upper and lower cuticles. The cellular distribution of K was more even than Ni and Ca, with no preferential accumulation (Fig. 3c). The concentration of both Ca and K was lower in the leaves of plants grown in 500 μM Ni compared with those from the 100 μM Ni treatment. This result is in agreement with the changes in total metal concentrations reported in Table 1.

Two-dimensional distributions of Ni, Ca and K across leaf sections were also obtained using EDXA. These analyses were restricted to Ni, Ca and K because only these elements were present at concentrations high enough to enable an EDXA dotmap to be produced. Figure 4 shows a micrograph of a leaf section (including upper cuticle, upper epidermis cells and palisade cells) along with the distributions of Ni, Ca and K. This analysis revealed a high concentration of Ni in the upper cuticle. Potassium appears to be evenly distributed across the leaf tissues whereas Ca is more abundant in the apoplast than in the internal cellular compartment.

Discussion

Berkheya coddii showed all the characteristics typical of hyperaccumulator plants: it did not display phytotoxicity symptoms when exposed to elevated Ni concentrations (up to 500 μM); Ni concentrations in the plants were well above the 1000 μg g^{-1} reported as the lower limit for Ni-hyperaccumulation (Brooks *et al.*, 1977); the concentration of Ni in the shoots was higher than in the roots. The results obtained confirm the elevated tolerance of this plant to large

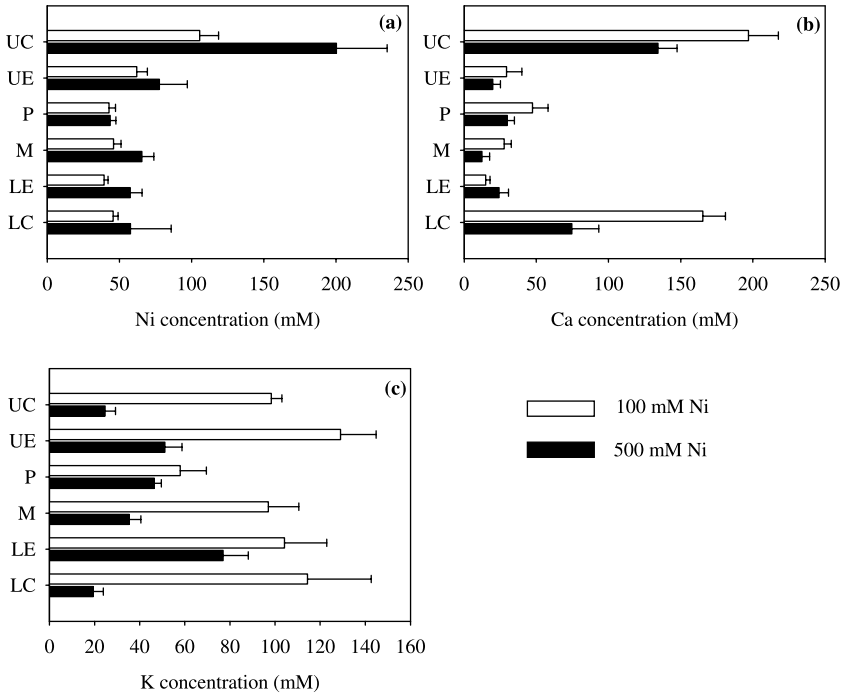

Fig. 3 Concentration of nickel (Ni) (a), calcium (Ca) (b) and potassium (K) (c) in different parts of *Berkheya coddii* leaves as determined by X-ray microanalyses of cross-sections. UC, upper cuticle; UE, upper epidermis cells; P, palisade cells; M, mesophyll cells; LE, lower epidermis cells; LC, lower cuticle; n = 8–29; Error bars represent the standard error of the mean.

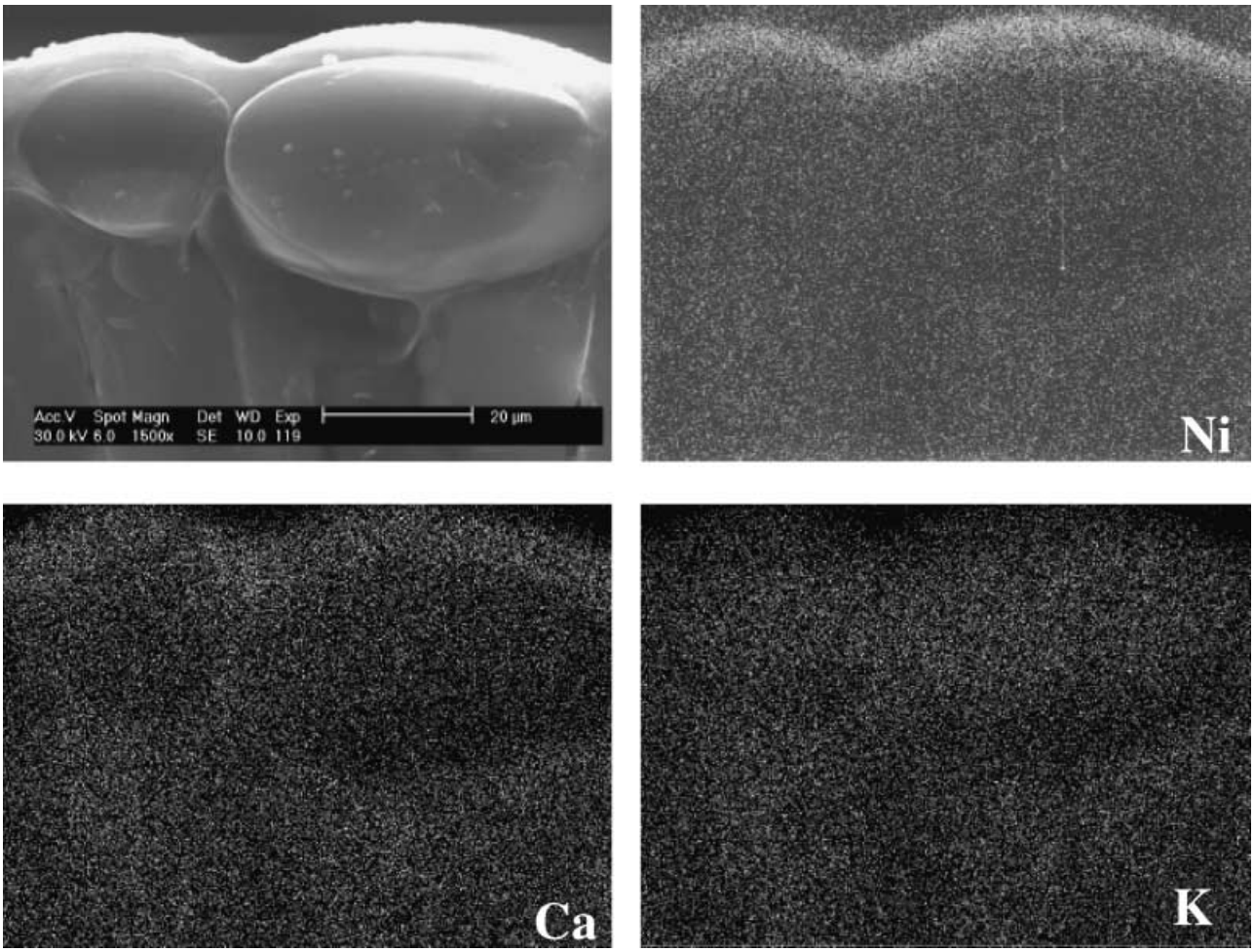

Fig. 4 Distribution of nickel (Ni), calcium (Ca) and potassium (K) across upper cuticle, upper epidermis cells and palisade of *Berkheya coddii* leaf, as revealed by X-ray microanalyses of frozen-hydrated tissues.

external and internal Ni concentrations and its efficient transport of Ni from roots to shoot.

Most Ni accumulated by *B. coddii* (71%) was found in shoots (Fig. 1). Küpper *et al.* (2000) and Zhao *et al.* (2000) suggested that the apparent metal accumulation in roots of hydroponically grown plants may be partly due to metal precipitation in the apoplast. In our experiments, EDXA analyses of *B. coddii* roots from the 500 µM Ni treatment showed high concentrations of Ni and P on the surface of the rhizodermis (data not shown), consistent with the suggestion that Ni is present as a phosphate precipitate. Hyperaccumulator plants usually store more metal in the shoots than in the roots. For example, 70–80% of the total Zn hyperaccumulated by *Arabidopsis halleri* and *Thlaspi caerulescens* was reported to be stored in the shoot (Shen *et al.*, 1997; Perronnet *et al.*, 2000; Zhao *et al.*, 2000).

Within the shoot, leaves appeared to be the primary sink for Ni in *B. coddii* (Fig. 1). Another characteristic that seems to be common to metal hyperaccumulator plants is the fact that metals are largely stored in water-soluble forms. Zhao *et al.* (2000) reported that 60–65% of the shoot Zn in *A. halleri* was water-soluble. In this study, we found that most Ni (65%) in the leaves was extractable with water at pH 6.

At the shoot level, the concentration of Ni in the leaves decreased from older to younger leaves whereas Ni concentration in the corresponding parts of the stem followed an opposite trend, with the larger concentration of Ni found near the apex (Fig. 2). The distribution pattern of nickel in *B. coddii* indicates that transpiration has a large influence on the long-distance transport of within plants.

The concentrations of K, Fe and Cu in the shoot of *B. coddii* were significantly reduced by increasing Ni concentrations in the hydroponic solution (Table 1). In the roots, however, there was no significant difference in the concentration of these elements between the treatments. This may indicate that the uptake of K, Fe and Cu was not affected, but their root-to-shoot translocation was reduced when Ni concentration in the nutrient solution increased. However, increasing Ni in the nutrient solution decreased Ca concentrations in all parts of the plants. This may indicate a competition between Ca and

Ni for plant uptake. Zhao *et al.* (2000) reported that the concentrations of K, Ca, P, Fe, Mn, Cu and Ni decreased in the shoot of *A. halleri* when the plants where exposed to increasing concentration of Zn. Similarly, Wenzel & Jockwer (1999) reported that K translocation from roots to shoots was depressed in Ni hyperaccumulators when grown in high-Ni substrates.

Accumulation of metals in an intracellular compartment, most likely the vacuole, of epidermis cells seems to be a common feature in hyperaccumulator plants (for a review see McGrath *et al.*, 2002). Preferential accumulation of Ni in the intracellular compartment of epidermis cells of *A. bertolonii*, *A. lesbiacum* and *T. goesingense* has been reported by Küpper *et al.* (2001). However, most of the hyperaccumulators in which metal compartmentation has been investigated are brassicaceous. In the asteraceous *B. coddii*, we found significantly higher Ni concentrations in the apoplast of the upper epidermis cells, especially within the cuticle (Figs 3 and 4). This pattern of cellular distribution of Ni resembles that of Al in mature leaves of tea (*Camelia sinensis*; Matsumoto *et al.*, 1976; Memon *et al.*, 1981) where Al was found to be localized in cell walls and cuticle. In the case of Ni, however, a lot of metal was found inside the cells, whereas Al was exclusively outside the cells. Mesjasz-Przybylowicz *et al.* (2001) studied the distribution of Ni in young apical leaves of *B. coddii* using a nuclear microprobe and freeze-dried samples. They found that the highest Ni enrichment occurred in leaf margins, mesophyll and midrib epidermis. However, these results are difficult to compare with our findings because we used frozen hydrated samples and mature leaves. In dried samples, cellular compartmentation of elements may have been affected because metals may be redistributed in the fixation and drying processes (van Steveninck & van Steveninck, 1991).

The Ni distribution in *B. coddii* differs significantly from what has previously been reported for other Ni hyperaccumulator species, where this metal appeared to be compartmented mainly in vacuoles. Vacuolar sequestration of Ni and other heavy metals has been proposed as a key tolerance mechanism in hyperaccumulator plants (Krämer *et al.*, 2000; Küpper *et al.*, 2001; Persans *et al.*, 2001). In the case of *B. coddii*, compartmentalization of Ni in the upper cuticle may help protect the cytoplasm from toxic concentrations of Ni. The disproportionately higher cuticle Ni concentrations in the 500 μM Ni treatment compared with the 100 μM Ni treatment may indicate that nickel is translocated to the cuticle when a threshold concentration in the living tissues is exceeded. However, the biological importance of cuticle-bound Ni is unclear because the total mass of Ni that is stored in the cuticle is likely to be small compared with the Ni contained in the leaf. This is due to the small total biomass of the cuticle.

Most other hyperaccumulator plants store heavy metals in the vacuoles of epidermal cells. High metal concentrations in the epidermis may indicate a predator defence role for the metal, as these are the first tissues encountered by grazing animals. However, the high Ni concentrations in the upper, but not the lower cuticle are not consistent with a predator defence role for Ni because many insect herbivores feed on the underside of leaves. Another possible role for nickel in the upper cuticle may be as a defence against ultraviolet radiation and protection of the underlying chlorophyll from solarization. This could be tested by growing plants under elevated levels of ultraviolet radiation in the presence and absence of Ni.

Conclusions

Several aspects of Ni accumulation by *B. coddii* are consistent with previous reports of metal hyperaccumulation in other plants: an efficient uptake and root-to-shoot translocation of Ni, and a high tolerance to Ni in the external medium and inside plant tissues. Similarly, depression of macronutrient and micronutrient uptake and/or translocation with increasing concentrations of the hyperaccumulated metal in soil solution has been reported previously. Unlike most hyperaccumulators, however, Ni is evenly distributed across the different leaf cell types. In *B. codii*, the Ni concentration in the upper cuticle was significantly higher than in the rest of the leaf. The importance of this distribution is unclear because the cuticle-bound Ni represents only a small fraction of the total leaf Ni.

Acknowledgements

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

References

Baker AJM, McGrath SP, Reeves RD, Smith JAC. 2000. Metal hyperaccumulator plants: A review of the ecology and physiology of a biochemical resource for phytoremediation of metal-polluted soils. In: Terry N, Bañuelos G, Vangronsveld J, eds. *Phytoremediation of contaminated soil and water*. Boca Raton, FL, USA: Lewis Publishers, 85–107.

Brooks RR, Chambers MF, Nicks LJ, Robinson BH. 1998. Phytomining. *Trends in Plant Science* **3**: 359–362.

Brooks RR, Lee J, Reeves RD, Jaffré T. 1977. Detection of nickeliferous rocks by analysis of herbarium species of indicator plants. *Journal of Geochemical Exploration* 7: 49–57.

Cakmak I, Marschner H. 1987. Mechanism of phosphorus induced zinc deficiency in cotton. III. Changes in physiological availability of zinc in plants. *Physiologia Plantarum* **70**: 13–20.

Genstat 5 Committee. 1993. *GENSTAT 5*. Oxford, UK: Clarendon Press.

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC. 1996. Free histidine as a metal chelator in plants that accumulate nickel. *Nature* **379**: 635–638.

Krämer U, Grime GW, Smith JAC, Hawes CR, Baker AJM. 1997. Micro-PIXE as a technique for studying nickel localization in leaves of the hyperaccumulator plant *Alyssum lesbiacum*. *Nuclear Instruments and Methods in Physics Research* **130**: 346–350.

Krämer U, Pickering IJ, Prince RC, Raskin I, Salt DE. 2000. Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. *Plant Physiology* **122**: 1343–1353.

Küpper H, Lombi E, Zhao FJ, McGrath SP. 2000. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta* **212**: 75–84.

Küpper H, Lombi E, Zhao FJ, Wieshammer G, McGrath SP. 2001. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. *Journal of Experimental Botany* **52**: 2291–2300.

Lee J, Reeves RD, Brooks RR, Jaffré T. 1977. Isolation and identification of a citrato-complex of nickel from nickel-accumulating plants. *Phytochemistry* **16**: 1503–1505.

Lee J, Reeves RD, Brooks RR, Jaffré T. 1978. The relation between nickel and citric acid in some nickel-accumulating plants. *Phytochemistry* **17**: 1033–1035.

Matsumoto H, Hirasawa E, Morimura S, Takahashi E. 1976. Localisation of aluminium in the tea leaf. *Plant and Cell Physiology* **17**: 627–631.

McGrath SP, Zhao FJ, Lombi E. 2002. Phytoremediation of metals, metalloids and radionuclides. *Advances in Agronomy* **75**: 1–56.

Memon AR, Chino M, Yatazawa M. 1981. Micro-distribution of aluminum and manganese in the tea leaf tissues as revealed by X-ray microanalyzer. *Communications in Soil Science and Plant Analysis* **12**: 441–452.

Mesjasz-Przybylowicz J, Balkwill K, Przybylowicz WJ, Annegarn HJ. 1994. Proton microprobe and X-ray fluorescence investigations of nickel distribution in serpentine flora from South Africa. *Nuclear Instruments and Methods in Physics Research* **89**: 208–212.

Mesjasz-Przybylowicz J, Przybylowicz WJ, Pineda CA. 2001. Nuclear microprobe studies of elemental distribution in apical leaves of the Ni hyperaccumulator *Berkheya coddii*. *South African Journal of Science* **97**: 591–593.

Morrey DR, Balkwill K, Balkwill MJ, Williamson S. 1992. A review of some studies of the serpentine flora of Southern Africa. In: Baker AJM, Procter J, Reeves RD, eds. *The Vegetation of ultramafic (serpentine) soils*. Intercept. Andover. 147–158.

Perronnet K, Schwartz C, Gerard E, Morel JL. 2000. Availability of cadmium and zinc accumulated in the leaves of *Thlaspi caerulescens* incorporated into soil. *Plant and Soil* **227**: 257–263.

Persans MW, Nieman H, Salt DE. 2001. Functional activity and role of cation-efflux family members in Ni hyperaccumulation in *Thlaspi goesingense*. *Proceedings of the National Academy of Sciences, USA* **98**: 9995–10000.

Psaras GK, Constantinidis T, Cotsopoulos B, Manetas Y. 2000. Relative abundance in the leaf epidermis of eight hyperaccumulators: evidence that the metal is excluded from both guard cells and trichomes. *Annals of Botany* **86**: 73–78.

Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In: Raskin I, Ensley BD, eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. New York, NY, USA: John Wiley & Sons, 193–229.

Robinson BH, Brooks RR, Howes AW, Kirkman JH, Gregg PEH. 1997. The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. *Journal of Geochemical Exploration* **60**: 115–126.

Shen ZG, Zhao FJ, McGrath SP. 1997. Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*. *Plant, Cell & Environment* **20**: 898–906.

van Steveninck RFM, van Steveninck ME. 1991. Microanalysis. In: Hall JL, Hawes C, eds. *Electron microscopy of plant cells*. London, UK: Academic Press, 415–455.

Wenzel WW, Jockwer F. 1999. Accumulation of heavy metals in plants grown on mineralised soils of the Austrian Alps. *Environmental Poll* **104**: 145–155.

Zhao FJ, Lombi E, Breedon T, McGrath SP. 2000. Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. *Plant, Cell & Environment* **23**: 507–514.

Zhao F, McGrath SP, Crosland AR. 1994. Comparison of three wet digestion methods for the determination of plant sulphur by inductively coupled plasma atomic emission spectrometry (ICP-AES). *Communications in Soil Science Plant Analysis* **25**: 407–418.