Short communication

Elemental patterns in Ni hyperaccumulating and non-hyperaccumulating ultramafic soil populations of *Senecio coronatus*

R.S. Boyd [a,*], M.A. Davis [b], K. Balkwill [c]

[a] *Department of Biological Sciences, Auburn University, AL 36849-5407, USA*
[b] *Department of Biological Sciences, University of Southern Mississippi, Hattiesburg MS 39406-5018, USA*
[c] *C.E. Moss Herbarium, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, WITS 2050, Johannesburg, South Africa*

Abstract

Nickel hyperaccumulation can defend plants against herbivores and pathogens. However, variability in plant tissue elemental concentrations in space and time will influence the effectiveness of this defense. We investigated a South African Ni hyperaccumulator, *Senecio coronatus* Thunb. (Harv.), for variation in nine elements (Ni plus Ca, Cu, Fe, K, Mg, Mn, P and Zn) between populations and between above-ground and below-ground plant organs (leaves, roots). Plant material was collected from four populations growing on ultramafic soils in the vicinity of Badplaas, Mpumalanga Province, South Africa. Concentrations of Ca, Cu, Fe, K, Mg, Mn, Ni, P and Zn were determined in dry-ashed samples. Two-way analysis of variance of data for each element revealed considerable variation in *S. coronatus* plant chemistry. Leaf concentrations of all elements except Cu were generally greater than root concentrations. Population-level variation was found for Ca, Fe, Mn, P, Ni and Zn, and of these all but P showed significant two-way interactions as well. Significant positive correlations were found between some pairs of elements: in hyperaccumulator roots (Ni–Ca, K–Mg), non-hyperaccumulator roots (Fe–Mn, Fe–Zn, Fe–Cu, Cu–Zn), hyperaccumulator leaves (P–Mg, P–Fe, P–Mn, Fe–Mg) and non-hyperaccumulator leaves (P–Mn, P–Ca, Ca–Mn). Two populations hyperaccumulated Ni in leaves (means of 12,000 and 8800 µg Ni/g) whereas the others did not (means of 120 and 130 µg Ni/g). Such extreme population-level variation in Ni accumulation ability is unusual among Ni hyperaccumulator species: its physiological basis and possible consequences for plant elemental defense deserve further investigation.

1. Introduction

Hyperaccumulator plants take up unusually large amounts of elements from soils and sequester them in their tissues. According to the review by Reeves and Baker (2000), approximately 415 hyperaccumulators have been identified, with about 75% of these hyperaccumulating Ni. Reeves (1992) defined a Ni hyperaccumulator as a species for which at least one wild-collected specimen has been shown to contain at least 1000 µg Ni/g in aboveground tissues (on a dry mass basis). Most Ni hyperaccumulator taxa grow on ultramafic (serpentine) soils (Reeves et al., 1999; Reeves, 2003), which are relatively

unusual due to their low Ca:Mg ratios and often high levels of some heavy metals, including Ni (Nagy and Proctor, 1997).

High levels of elements (especially metals) in plants may defend them against some natural enemies, such as certain herbivores and pathogens (Boyd, 2004, 2007). Martens and Boyd (1994) suggested these as "elemental defenses," distinguishing them from the organic chemicals typically discussed as chemical defenses in plants (e.g., Agrawal and Fishbein, 2006). Hyperaccumulated metals such as Ni have been shown to have defensive effects against some herbivores and pathogens (Boyd, 2004), but the least concentration needed to produce defensive effects is unclear. Coleman et al. (2005) suggested that Ni concentrations in plant tissues far lower than the minimum hyperaccumulator level can have defensive benefits for plants. This question is under active investigation.

* Corresponding author.
  E-mail address: boydrob@auburn.edu (R.S. Boyd).

Plant defenses can vary at both temporal and spatial scales and this variation can influence herbivore responses (Shelton, 2004). Studies have documented this variation for some organic plant defense chemicals (e.g., Brenes-Arguedas and Coley, 2005; Brenes-Arguedas et al., 2006) but few studies have examined elemental defenses in this light (see Galeas et al. (2007) for a recent exception). A few investigations have documented variation in Ni hyperaccumulation due to leaf age (Boyd et al., 1999; Boyd et al., 2004) and some studies have documented such variation as it impacts phytoremediation (Anderson et al., 1997) or phytomining (Nicks and Chambers, 1995), but the topic is generally unexplored.

Senecio coronatus Thunb. (Harv.) (Asteraceae) grows in grasslands in Southern Africa, ranging from the Western Cape north to Tanzania, Zambia and Angola (Hilliard, 1977). It is an herbaceous perennial with a shortened upright subterranean stem that produces long (10–40 cm) broad (to 40 cm) simple leaves and relatively fleshy roots (Hilliard, 1977). Morrey et al. (1992) first reported that *S. coronatus* populations from ultramafic soil hyperaccumulated Ni, and further research by Mesjasz-Przybylowicz et al. (1994) revealed most of the Ni in leaves of those populations was located in the leaf epidermis. *S. coronatus* is unusual among hyperaccumulators in that some populations on ultramafic soils are reported to hyperaccumulate Ni yet others do not. For example, Boyd et al. (2002) reported mean leaf Ni concentrations of 12,100 and 680 µg Ni/g from two populations growing on ultramafic soils in Mpumalanga Province, South Africa. Mesjasz-Przybylowicz et al. (1997) documented the tissue level distribution of Ni and other elements in stems and leaves of hyperaccumulating and non-hyperaccumulating *S. coronatus* using Particle Induced X-ray Emission (PIXE). Thus, on ultramafic soils this species appears to exist as either hyperaccumulator or accumulator populations. This species also can be found growing on non-ultramafic soils in this area of South Africa (Smith et al., 2001).

The ability of *S. coronatus* to inhabit both ultramafic and non-ultramafic soils, and the very different concentrations of Ni in hyperaccumulator and non-hyperaccumulator populations on ultramafic soils, raise questions about the mechanisms that underlie this ability. Mesjasz-Przybylowicz et al. (1997) suspected that hyperaccumulator and non-hyperaccumulator ultramafic soil populations were genetically different. They based this on the observation that both phenotypes grew in areas that hosted other Ni hyperaccumulator species and these other species consistently hyperaccumulated Ni at all sites. A recent study by Mesjasz-Przybylowicz et al. (2007) explored soil Ni levels for a hyperaccumulating and a non-hyperaccumulating population, and showed 2-fold more total soil Ni for the non-hyperaccumulating population and similar levels of DTPA-extractable Ni from soils of both populations, reinforcing the hypothesis that these populations represent different *S. coronatus* genotypes. Such genetic differentiation raises questions about how such differences evolved as well as what physiological mechanisms are involved in the differing responses of these populations to soil Ni. To our knowledge, however, these questions have yet to be explored for *S. coronatus*.

We undertook this study to document variation of elemental concentrations (especially Ni concentrations) in *S. coronatus* at two levels. First, we were interested in documenting variation between populations growing on ultramafic soils. Second, we wished to examine variation in Ni concentration between above-ground organs (leaves) and below-ground organs (roots) of *S. coronatus* plants. Finally, we wished to examine correlations between elements and compare them between hyperaccumulator and non-hyperaccumulator populations, as these may provide clues regarding the physiological mechanisms that generate the different responses of these populations to Ni.

2. Materials and methods

The area east of Badplaas in Mpumalanga Province, South Africa, contains scattered outcrops of ultramafic soils (Morrey et al., 1992; Smith et al., 2001). Our study sites were located on ultramafic exposures with grassland vegetation typical for these areas. The four sites, used previously in a study of two Ni hyperaccumulator *Berkheya* species (Boyd et al., 2004), were: 1) Doyershoek, where plants were located along a firebreak on a steep hillside; 2) Groenvaly, which had plants at the foot of a ultramafic hill; 3) Groenvaly Mine, an abandoned mine site about 1 km from the Groenvaly hill; and 4) near a small airfield that was called the "Airstrip Serpentine" site.

We arbitrarily selected full-sized individuals spaced more than 1 m apart. Plants were excavated, separated into leaf and root fractions, and the roots were washed in water to remove adhering soil. Leaves were not washed to avoid leaching elements from the samples. Samples were dried at 60 °C for at least 72 h and analyzed for element concentrations. Samples were ground, dry-ashed at 485 °C, further oxidized in 1 M HNO_3, and the residues were re-dissolved in 1 M HCl. Concentrations of Ca, Cu, Fe, K, Mg, Mn, P and Zn were determined by an inductively coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000). Nickel concentrations were determined using an atomic absorption spectrophotometer (Instrumental Laboratory, IL 251).

Concentrations of each element were analyzed using two-way Analysis of Variance (ANOVA). Plant organ (leaf or root) and population were used as main effect factors in the ANOVA model and the interaction was also included (Abacus Concepts, 1998). Correlations were examined for all pairwise combinations of the nine elements. Data were divided into hyperaccumulator leaf, hyperaccumulator root, non-hyperaccumulator leaf and non-hyperaccumulator root datasets and each dataset was analyzed for correlations separately. Because of the large number (36) of pairwise correlations in each dataset, for each analysis we divided our α-level (0.05) by 36 (yielding a corrected $\alpha < 0.0014$) to help us focus on the strongest correlations.

3. Results

Two-way ANOVA of data for each element revealed considerable variation in *S. coronatus* plant chemistry (Table 1).

Table 1
Two-way ANOVA results of elemental concentration data for *S. coronatus* samples

Element	ANOVA factor		
	Population (df=3,51)	Plant Organ (df=1,51)	Interaction (df=3,51)
Ca	5.1 (0.0038)	170 (<0.0001)	4.9 (0.0045)
Cu	2.6 (0.064)	2.0 (0.17)	0.92 (0.44)
Fe	7.6 (0.0003)	33 (<0.0001)	5.0 (0.004)
K	0.52 (0.67)	11 (0.0017)	0.48 (0.70)
Mg	2.1 (0.12)	260 (<0.0001)	2.2 (0.10)
Mn	22 (<0.0001)	61 (<0.0001)	15 (<0.0001)
P	5.5 (0.0023)	93 (<0.0001)	1.3 (0.30)
Ni	19 (<0.0001)	23 (<0.0001)	9.6 (<0.0001)
Zn	22 (<0.0001)	61 (<0.0001)	11 (<0.0001)

Plant organ refers to either leaf or root samples. Values reported are F (α-value). The population factor includes the four populations from which samples were collected.

Significant population-level variation was found for Ca, Fe, Mn, P, Ni and Zn. The greatest variation was found for Ni (Table 2): two populations hyperaccumulated Ni in leaves (at 12,000 and 8800 µg Ni/g) whereas the others contained at least 73-fold less Ni (120 and 130 µg Ni/g). Leaf concentrations of all elements except Cu were generally greater than root concentrations. In roots the variation in Ni concentration was less: hyperaccumulator populations contained at least 48-fold more Ni (Table 2). Again, variability of Ni concentrations in roots was greater than the variability of all other elements in roots. All elements except Cu, Mg and P showed significant two-way interactions as well (Table 1), indicating that the variation associated with population differed between leaves and roots for most elements.

Strong positive correlations were found between concentrations of several elements in leaves and roots (Table 3). Leaves and roots shared no pairs of correlated elements in common. Levels of P were correlated in leaves with several other elements depending upon population: leaves from hyperaccumulator and non-hyperaccumulator plants both showed significant correlations between P and Mn levels (Table 3). Comparison of correlations for hyperaccumulator and non-hyperaccumulator roots showed no shared correlations (Table 3). Noteworthy correlations included that of Ni with Ca in hyperaccumulator roots and that of Fe with several metals (Cu, Mn, Zn) in non-hyperaccumulator roots.

4. Discussion

Most Ni hyperaccumulator species are restricted to serpentine soils and all populations hyperaccumulate (Reeves, 1992). The most striking result of this study is the presence of both hyperaccumulator and non-hyperaccumulator populations of *S. coronatus* on ultramafic soils. Populations of *S. coronatus* with contrasting accumulation abilities can be remarkably near one another: in our study the Groenvaly and Groenvaly Mine populations were separated by ca. 1 km. The difference in Ni accumulation ability between *S. coronatus* populations may be due to differences among plants, among soils, or some combination of these two factors. However, we suggest the difference is physiological rather than environmental, for two reasons. First, the floras of the sites were similar and indicative of ultramafic soils in this area, implying similarity in soil features. Second, all our study sites supported populations of *Berkheya coddii* Roessl. (Asteraceae): a Ni hyperaccumulator endemic to ultramafic soils in this area. A study by Boyd et al. (2004) of population-level variation in Ni concentrations of *B. coddii* on three of these sites (Groenvaly, Groenvaly Mine and Doyershoek) documented hyperaccumulator concentrations at all sites, again implying similarity in soil Ni concentration. Highest *B. coddii* Ni levels were found at Groenvaly (10,900 µg Ni/g) and Groenvaly Mine (10,700 µg Ni/g) whereas the population at Doyershoek had a significantly less (but still hyperaccumulator) level of Ni (8800 µg Ni/g). For *S. coronatus* (Table 2), the highest Ni concentration was found for Groenvaly and less for Doyershoek (consistent with the pattern for *B. coddii*), but the Groenvaly Mine population contained only 130 µg Ni/g. It seems likely that the two species at the Groenvaly Mine site are responding differently to the same soil Ni concentration. Thus, we suggest that the *S. coronatus* population at Groenvaly Mine lacks the physiological mechanisms for Ni hyperaccumulation.

Further research should explore why Ni hyperaccumulation varies in this unusual species. As suggested by Mesjasz-Przybylowicz et al. (1997, 2007), the striking difference in Ni concentration among *S. coronatus* populations on ultramafic soils suggests genetically based differences between them. For *S. coronatus*, it is not known if hyperaccumulator and non-hyperaccumulator populations can interbreed nor how

Table 2
Elemental concentration means (SE) for *S. coronatus* leaf and root samples from each of the four populations studied

Element	Groenvaly		Groenvaly Mine		Doyershoek		Airstrip Serpentine	
	Leaf (N=11)	Root (N=6)	Leaf (N=6)	Root (N=6)	Leaf (N=6)	Root (N=2)	Leaf (N=16)	Root (N=6)
Ca	26,000 (1300)	2000 (180)	17,000 (2000)	1400 (100)	15,000 (2100)	2000 (110)	29,000 (1900)	1700 (150)
Cu	12 (0.98)	10 (0.27)	10 (0.24)	7.7 (0.21)	11 (0.77)	12 (1.7)	11 (0.69)	10 (0.61)
Fe	500 (26)	390 (30)	450 (49)	210 (21)	400 (28)	170 (13)	370 (20)	350 (41)
K	8700 (1200)	5800 (1300)	11,000 (1700)	5900 (830)	8400 (1100)	4300 (610)	8100 (950)	6100 (830)
Mg	15,000 (860)	1700 (200)	17,000 (2200)	1700 (240)	18,000 (1300)	1400 (250)	13,000 (800)	1700 (250)
Mn	100 (9.7)	14 (1.3)	23 (3.4)	5.7 (062)	38 (2.7)	5.6 (0.70)	27 (3.3)	8.5 (0.88)
P	750 (28)	420 (21)	720 (45)	330 (48)	610 (44)	250 (41)	560 (37)	320 (28)
Ni	12,000 (1800)	2100 (580)	130 (36)	25 (8.9)	8800 (2000)	1200 (57)	120 (29)	50 (12)
Zn	60 (2.3)	35 (5.5)	15 (0.41)	8.1 (0.50)	54 (5.5)	17 (3.5)	17 (0.78)	11 (1.1)

Concentrations are reported as µg/g dry mass.

Table 3
Statistically significant ($\alpha = 0.0014$) correlations between element concentrations in organs from hyperaccumulator and non-hyperaccumulator *S. coronatus* populations

Plant part (population type)	Significant pairwise element correlations
Leaf (hyperaccumulator)	P–Mn, P–Mg, P–Fe, Fe–Mg
Leaf (non-hyperaccumulator)	P–Mn, P–Ca, Ca–Mn
Root (hyperaccumulator)	Ni–Ca, K–Mg
Root (non-hyperaccumulator)	Fe–Mn, Fe–Zn, Fe–Cu, Cu–Zn

All significant correlations were positive.

hyperaccumulation ability might segregate in offspring. Such experiments could contribute to our understanding of the genetics of Ni hyperaccumulation, as they have for the genetics of Zn hyperaccumulation (Pollard and Baker, 1996; Macnair, 2002). They also might reveal whether *S. coronatus* is a coherent species or if it should be subdivided taxonomically. *S. coronatus* is widespread in southern Africa and Hilliard (1977) reports high variation in plant stature and leaf size. Currently, *S. coronatus* populations are known from both ultramafic and non-ultramafic soils in Mpumalanga (Smith et al., 2001) and ultramafic populations can vary significantly in Ni levels. Hyperaccumulation ability can be a useful indicator of differences between populations that in some cases have taxonomic significance (e.g., Baker et al., 1992). Our findings regarding *S. coronatus* suggest variation that, if accompanied by breeding barriers or morphological differences, might justify taxonomic separation of hyperaccumulator and non-hyperaccumulator populations. We hope our results stimulate more detailed investigations of these populations.

We found significant variation in concentrations of most elements between populations (Table 1), indicating high variability for elements other than Ni (although not as extreme). We also found significant differences in element concentrations between roots and leaves in most cases. For Ni, the much greater concentration of Ni in *S. coronatus* leaves compared to roots is typical of hyperaccumulator plants (e.g., Robinson et al., 2003). Correlations between elements documented some significant positive relationships, but the only correlation involving Ni was its positive correlation with Ca in hyperaccumulator plant roots. This is interesting, as the usually low Ca:Mg ratio of serpentine soils is reported as a chemical feature that challenges plant growth (Nagy and Proctor, 1997). One explanation for this correlation is shared uptake mechanisms for both Ni and Ca. We note that, in contrast to our correlation results, Mesjasz-Przybylowicz et al. (1997) documented different correlations in *S. coronatus* using Proton Induced X-Ray Emission (PIXE) analysis at the tissue level. They reported significant positive correlations between Ni and three elements (Mn, Fe, and Zn) in stems of hyperaccumulating plants but found no significant correlations in non-hyperaccumulating plants.

Hyperaccumulated Ni may defend plants against some natural enemies (Boyd, 2004). For *S. coronatus*, Boyd et al. (2002) showed that hyperaccumulator leaves were less damaged by snails than non-hyperaccumulator leaves in choice experiments. If Ni does confer protection, then leaves of hyperaccumulator *S. coronatus* are much better defended than are roots, as leaves contained at least 5-fold more Ni (Table 2). Similarly, leaves and roots of hyperaccumulator populations are also better defended by Ni than those of non-hyperaccumulator populations. Non-hyperaccumulator populations may rely on organic chemicals for defense or may face lower levels of herbivore attack: it would be interesting to measure herbivore abundance on these sites to determine if it correlates positively with Ni concentrations in plants. Noret et al. (2007) postulated that metalliferous sites (such as serpentine soils) might have less herbivore activity than non-serpentine sites: it is possible that there is significant variation between serpentine sites as well, but such questions are in need of experimental investigation in serpentine systems (Alexander et al., 2006).

Acknowledgements

References

Abacus Concepts, 1998. StatView. SAS Institute Inc., Cary, USA.

Agrawal, A.A., Fishbein, M., 2006. Plant defense syndromes. Ecology 87, S132–S149. Supplement.

Alexander, E.B., Coleman, R.G., Keeler-Wolf, T., Harrison, S.P., 2006. Serpentine Geoecology of Western North America: Geology, Soils, and Vegetation. Oxford University Press, New York, pp. 1–512.

Anderson, T.R., Howes, A.W., Slatter, K., Dutton, M.F., 1997. Studies on the nickel hyperaccumulator, *Berkheya coddii*. In: Jaffré, T., Reeves, R.D., Becquer, T. (Eds.), The Ecology of Ultramafic and Metalliferous Areas. Centre ORSTOM de Noumea, New Caledonia, pp. 261–266.

Baker, A.J.M., Proctor, J., Van Balgooy, M.M.J., Reeves, R.D., 1992. Hyperaccumulation of nickel by the flora of the ultramafics of Palawan, Republic of the Philippines. In: Baker, A.J.M., Proctor, J., Reeves, R.D. (Eds.), The Vegetation of Ultramafic (Serpentine) Soils. Intercept, Andover, pp. 291–304.

Boyd, R.S., 2007. The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. Plant and Soil 293, 153–176.

Boyd, R.S., 2004. Ecology of metal hyperaccumulation. New Phytologist 162, 563–567.

Boyd, R.S., Davis, M.A., Wall, M.A., Balkwill, K., 2002. Nickel defends the South African hyperaccumulator *Senecio coronatus* (Asteraceae) against *Helix aspersa* (Mollusca: Pulmonidae). Chemoecology 12, 91–97.

Boyd, R.S., Jaffré, T., Odom, J.W., 1999. Variation of nickel content in the nickel-hyperaccumulating shrub *Psychotria douarrei* (Rubiaceae) from New Caledonia. Biotropica 31, 403–410.

Boyd, R.S., Davis, M.A., Balkwill, K., 2004. Nickel accumulation patterns in two South African Ni hyperaccumulator species. In: Boyd, R.S., Baker, A.J.M., Proctor, J. (Eds.), Ultramafic Rocks: Their Soils, Vegetation and Fauna. Science Reviews 2000 Ltd, St Albans, Herts, pp. 275–278.

Brenes-Arguedas, T., Coley, P.D., 2005. Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species. Oikos 108, 410–420.

Brenes-Arguedas, T., Horton, M.W., Coley, P.D., Lokvam, J., Waddell, R.A., Meizoso-O'Meara, B.E., Kursar, T.A., 2006. Contrasting mechanisms of secondary metabolite accumulation during leaf development in two tropical tree species with different leaf expansion strategies. Oecologia 149, 91–100.

Coleman, C.M., Boyd, R.S., Eubanks, M.D., 2005. Extending the elemental defense hypothesis: dietary metal concentrations below hyperaccumulator levels could harm herbivores. Journal of Chemical Ecology 31, 1669–1681.

Galeas, M.L., Zhang, L.H., Freeman, J.L., Wegner, M., Pilon-Smits, E.A.H., 2007. Seasonal fluctuations of selenium and sulfur accumulation in selenium hyperaccumulators and related nonaccumulators. New Phytologist 173, 517–525.

Hilliard, O.M., 1977. Compositae in Natal. University of Natal Press, Pietermaritzburg.

Macnair, M.R., 2002. Within and between population genetic variation for zinc accumulation in *Arabidopsis halleri*. New Phytologist 155, 59–66.

Martens, S.N., Boyd, R.S., 1994. The ecological significance of nickel hyperaccumulation: a plant chemical defense. Oecologia 98, 379–384.

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

Mesjasz-Przybylowicz, J., Przybylowicz, W.J., Prozesky, V.M., Pineda, C.A., 1997. Quantitative micro-PIXE comparison of elemental distribution in Ni-hyperaccumulating and non-accumulating genotypes of *Senecio coronatus*. Nuclear Instruments and Methods in Physics Research B 130, 368–373.

Mesjasz-Przybylowicz, J., Barnabas, A., Przybylowicz, W., 2007. Comparison of cytology and distribution of nickel in roots of Ni-hyperaccumulating and non-hyperaccumulating genotypes of *Senecio coronatus*. Plant and Soil 293, 61–78.

Morrey, D.R., Balkwill, K., Balkwill, M.-J., Williamson, S., 1992. A review of some studies of the serpentine flora of Southern Africa. In: Baker, A.J.M., Proctor, J., Reeves, R.D. (Eds.), The Vegetation of Ultramafic (Serpentine) Soils. Intercept, Andover, pp. 147–157.

Nagy, L., Proctor, J., 1997. Soil Mg and Ni as causal factors of plant occurrence and distribution at the Meikle Kilrannoch ultramafic site in Scotland. New Phytologist 135, 561–566.

Nicks, L.J., Chambers, M.F., 1995. Farming for metals? Mining and Environmental Management 3, 15–18.

Noret, N., Meerts, P., Vanhaelen, M., Dos Santos, A., Escarré, J., 2007. Do metal-rich plants deter herbivores? A field test of the defence hypothesis. Oecologia 152, 92–100.

Pollard, A.J., Baker, A.J.M., 1996. The quantitative genetics of zinc hyperaccumulation in *Thlaspi caerulescens*. New Phytologist 132, 113–118.

Reeves, R.D., 2003. Tropical hyperaccumulators of metals and their potential for phytoextraction. Plant and Soil 249, 57–65.

Reeves, R.D., 1992. The hyperaccumulation of nickel by serpentine plants. In: Baker, A.J.M., Proctor, J., Reeves, R.D. (Eds.), The Vegetation of Ultramafic (Serpentine) Soils. Intercept, Andover, pp. 253–277.

Reeves, R.D., Baker, A.J.M., 2000. Metal-accumulating plants. In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. John Wiley & Sons, New York, pp. 193–228.

Reeves, R.D., Baker, A.J.M., Borhidi, A., Berazaín, R., 1999. Nickel hyperaccumulation in the serpentine flora of Cuba. Annals of Botany 83, 29–38.

Robinson, B.H., Lombi, E., Zhao, F.J., McGrath, S.P., 2003. Uptake and distribution of nickel and other metals in the hyperaccumulator *Berkheya coddii*. New Phytologist 158, 279–285.

Shelton, A.L., 2004. Variation in chemical defences of plants may improve the effectiveness of that defence. Evolutionary Ecology Research 6, 709–726.

Smith, S., Balkwill, K., Williamson, S., 2001. Compositae on serpentine in the Barberton Greenstone Belt, South Africa. South African Journal of Science 97, 518–520.

Edited by J Van Staden