ACTA BIOLOGICA CRACOVIENSIA Series Botanica 46: 75–85, 2004

UPTAKE OF CADMIUM, LEAD, NICKEL AND ZINC FROM SOIL AND WATER SOLUTIONS BY THE NICKEL HYPERACCUMULATOR *BERKHEYA CODDII*

JOLANTA MESJASZ-PRZYBYŁOWICZ[1*], MIROSŁAW NAKONIECZNY[2], PAWEŁ MIGULA[2**],
MARIA AUGUSTYNIAK[2], MONIKA TARNAWSKA[2], WOLF UWE REIMOLD[4***],
CHRISTIAN KOEBERL[5****], WOJCIECH PRZYBYŁOWICZ[1] AND ELŻBIETA GŁOWACKA[3]

[1]*Materials Research Group, iThemba Laboratory for Accelerator Based Sciences,*
P.O. Box 722, Somerset West 7129, South Africa
[2]*Department of Animal Physiology and Ecotoxicology;* [3]*Department of Zoology,*
University of Silesia, ul. Bankowa 9, 40-007 Katowice, Poland
[4]*School of Geosciences, University of Witwatersrand,*
Johannesburg 2050, South Africa
[5]*Department of Geological Sciences, University of Vienna,*
Althanstrasse 14, A-1090 Vienna, Austria

Received October 15, 2003; revision accepted July 7, 2004

Berkheya coddii Roessler (Asteraceae), an endemic herbaceous and perennial nickel-hyperaccumulating plant growing on Ni-enriched ultramafic soils in South Africa, is perceived as a promising species for phytoremediation and phytomining due to its large biomass production and high Ni content. Total concentrations of a number of elements in mature leaves, soil and related bedrock were obtained. The average Ni concentration in leaves was 18,000 $\mu g \cdot g^{-1}$ dry mass, whereas in soil and bedrock the total amount of Ni was 1,300 $\mu g \cdot g^{-1}$ and 1,500 $\mu g \cdot g^{-1}$, respectively. Exceptionally high average Ni concentrations (55,000 ± 15,000 $\mu g \cdot g^{-1}$, n = 6) were found in *B. coddii* leaves from Songimvelo Game Reserve, including the highest-ever reported concentration of Ni in leaves (76,100 $\mu g \cdot g^{-1}$ – maximum value in a single sample). Young plants grown in pots with ultramafic soil accumulated small quantities of Cd, Pb or Zn, but the concentrations of these elements increased after the addition of metal solutions to the soil. Excised shoots immersed in concentrated solutions of Cd, Ni, Pb or Zn accumulated large amounts of these metals in the leaves.

Key words: *Berkheya coddii*, hyperaccumulation, nickel, zinc, lead, cadmium, phytoextraction, phytoremediation, caulofiltration.

INTRODUCTION

There is much concern about environmental pollutants, including heavy metals, which occur in high concentrations at many sites worldwide. Most remediation technologies are based on mechanical and physicochemical methods involving soil removal or replacement, as well as chemical cleaning methods such as vitrification or leaching. These methods are expensive, are usually harmful to the natural soil environment, and generate large amounts of waste (Cunningham, 1995; Glass, 1998, 2000). A far cheaper and safer way to remove heavy metals involves bioremediation, which is a microbe-based technology, and phytoremediation in which vascular plants remove pollutants from the environment or

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*e-mail: mesjasz@tlabs.ac.za **migula@us.edu.pl ***reimoldw@geosciences.wits.ac.za ****christian.koeberl@univie.ac.at

PL ISSN 0001-5296

render them harmless (Salt et al., 1998). Phytoremediation has public appeal because it can leave a fertile ecosystem (Salt et al., 1995; Kumar et al., 1995; Raskin and Ensley, 2000; Garbisu and Alkorta, 2001). The idea of using plants to extract metals from contaminated soils was first suggested by Chaney (1983). In many countries, phytoremediation is perceived as a useful method for the reclamation of agricultural and post-industrial lands. In Poland, Silesia was contaminated with heavy metals (especially Cd, Pb and Zn) in past decades owing to the massive presence of heavy metal industry and uncontrolled emissions of pollutants (Gzyl, 1995). Some Polish institutions are involved in remediation studies which include the use of microorganisms (Galiulin et al., 1998) as well as the use of whole plants for phytoextraction from sewage sites (Pogrzeba et al., 2001).

Plants used in phytoextraction are hyperaccumulator species, as well as fast-growing crop plants with high biomass production (Brooks and Robinson, 1998). For naturally occurring plants with an unusual ability to concentrate exceptionally high amounts of heavy metals, the term 'hyperaccumulator' was first introduced by Jaffré and Brooks (Jaffré, 1976; Brooks et al., 1977) and later defined by Reeves (1992). About 400 hyperaccumulating plant species have been identified in world flora, most of them (318) being Ni hyperaccumulators (Brooks, 1998; Reeves and Baker, 2000). Other elements that may be hyperaccumulated include Al, As, Cd, Co, Cu, Mn, Pb, Se, Tl and Zn. Most hyperaccumulators accumulate one specific element, but some such as *Thlaspi caerulescens* are capable of concentrating a few metals. Unfortunately, a characteristic feature of many hyperaccumulators is small biomass production, a serious limitation for phytoremediation (Brooks and Robinson, 1998). However, a few species with a higher biomass production have already been evaluated for commercial phytoextraction – phytomining. The first trials undertaken included hyperaccumulators such as *Streptanthus polygaloides* with Ni (Nick and Chambers, 1995; 1998), *Alyssum* species with Co and Ni (Robinson et al., 1997a; Brooks et al., 2001; Li et al., 2003a,b), *Thlaspi caerulescens* with Zn and Cd (Hammer and Keller, 2003; Zhao et al., 2003), *Iberis intermediata* with Tl (Anderson et al., 1999; Leblanc et al., 1999) and *Berkheya coddii* with Ni (Robinson et al., 1997b; Robinson et al., 1999; Brooks et al., 2001).

Berkheya coddii Roessler (Asteraceae), an endemic plant species of the ultramafic South African flora (Morrey et al., 1989), is one of the most promising plants to be used for phytoextraction purposes on contaminated soils. It is a perennial, fast-growing herbaceous plant able to accumulate nickel from the soil, at certain sites reaching average values as high as 38,000 $\mu g \cdot g^{-1}$ of dry weight of leaves (Augustyniak et al., 2002). Biomass of 22 t dry weight per ha can be obtained after moderate fertilization without a decrease in nickel concentration (Robinson et al., 1997). *B. coddii*, presently cultivated commercially in South Africa (Rustenburg Base Metals Refiners), has also been tested for phytoextraction in New Zealand and the United States. The feasibility of recovering nickel and producing biofuels from nickel-containing biomass of *B. coddii* has also been investigated in Japan (le Clercq et al., 2001).

In the present study, trace element concentrations were determined in ultramafic bedrock, related soil and leaves of *B. coddii* plants growing under natural conditions at the same locality, to show the possible transfer of the elements into the plant. The next step was to compare and quantify the uptake of Cd, Ni, Zn or Pb by young plants of *B. coddii* growing in pots on ultramafic soil enriched with these metals, and by excised shoots from solutions containing the same heavy metals. The findings are discussed in terms of applications for extraction of heavy metals from industrial tailings and waste water effluents at disposal sites.

MATERIALS AND METHODS

Bedrock, related soil and plant samples were collected in summer (January) and autumn (March) at the Agnes Mine and the Songimvelo Game Reserve (Barberton area, Mpumalanga Province, NE South Africa) as described in a previous paper (Augustyniak et al., 2002). Element concentrations in the bedrock, soil and plant samples were determined by X-ray fluorescence spectrometry (XRF), instrumental neutron activation analysis (INAA) and atomic absorption spectrophotometry (AAS). After plant remains larger than 2 mm were sieved from the soil, rock and soil samples were powdered with an automatic agate mill. XRF analyses were made at the University of the Witwatersrand, Johannesburg, South Africa, for determination of major and some trace elements (Ba, Cu, Nb, Ni, Sr, Y, V and Zr) following procedures described by Reimold et al. (1994). For INAA, subsamples of ~150 mg were sealed in polyethylene (PE) vials. The sample vials were packed together with well-characterized reference materials into a larger PE-irradiation vial.

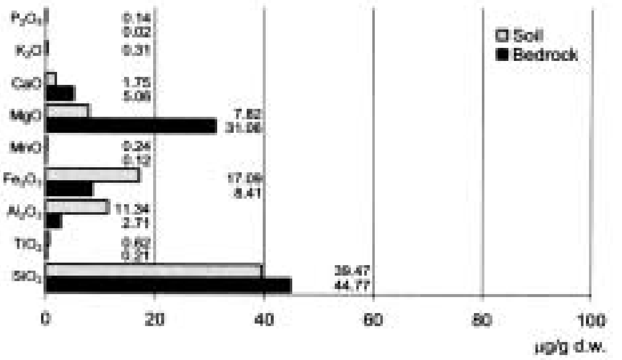

Fig. 1. Average concentrations (µg . g^{-1}) of main elements in bedrock and soil from the Agnes Mine as determined by XRF (n = 3).

Granite standard AC-E and granite USGS G-2, Allende meteorite standard reference powder, and mineralized gabbro PGE standard WMG-1 (Canmet) were used as reference materials for quantification. The packed samples were irradiated at the TRIGA Mark II reactor at the Atominstitut of the Austrian Universities in Vienna, Austria, for 8 h at a neutron flux of $\sim 2 \cdot 10^{12} \cdot n \cdot cm^{-2} \cdot s^{-1}$. For further processing, samples were transferred to the Department of Geological Sciences at the University of Vienna after a cooling period of 5 days. Details of the method (including information on standards, instrumentation, data reduction, precision and accuracy) are given by Koeberl (1993).

For the experiment on accumulation of metals, whole young plants as well as excised shoots of *B. coddii* were used. The experiments were conducted under field conditions, with the plants protected from rain. Plants were collected from their natural stand at the Agnes Mine in March.

Young plants of *B. coddii* (10–15 cm high) were dug up with soil to a depth of ~40 cm the day before the start of the experiment, placed in plastic pots, and divided into four experimental groups. The plants were carefully watered every day with the same amount of the appropriate metal solution. The control group of plants was watered with the same amount of tap water each day. On day 7 of the experiment, the plants were cut off at the soil level and dried.

At the beginning of the parallel experiment, shoots 40–50 cm long were cut from mature plants. Twenty excised vegetative shoots were put into tap water solutions containing one of four metal chlorides (cadmium, lead, nickel or zinc), thus establishing four experimental groups. Metal concentrations were up to 10 mM, depending on metal solubility. In addition, 20 shoots (control group) were placed in tap water. The solutions were changed daily. From day 2 to day 7 of the experiment, two whole shoots from each solution were randomly selected, removed and initially air-dried. Leaves detached from shoots were divided into stock samples (one per group) for metal concentration determinations. The analyses were done at the University of Silesia in Katowice (Poland). There, the samples were dried again for 3 days at 60˚C, weighed, and six ~300 mg subsamples were completely dissolved in ultrapure 65% nitric acid. Mineralization conditions were as follows: 24 h at room temperature, the next 3 hours at 50˚C, and thereafter at 130˚C until colorless, clear solutions were obtained. Mineralized samples were diluted with deionized water to a final volume of 4 ml. The abundance of selected metals (Cd, Cu, Fe, Ni, Pb and Zn) was determined by flame or flameless (graphite furnace) Atomic Absorption Spectrophotometry in a Solar Unicam 939 spectrophotometer (van Straalen and van Vensem, 1986; Augustyniak and Migula, 2000). The data obtained were analyzed using a Statistica© software package. Means and standard deviations were calculated for data for each group and every day of the experiment. The Tukey's t-test (Spjotvalla-Stoline test) was applied to estimate significant differences of metal levels in plant material using the ANOVA/MANOVA module for $p < 0.05$.

RESULTS

SOIL AND RELATED BEDROCK

The soil and related bedrock shows a distinct ultramafic (komatiitic) composition, with $SiO_2 < 45$ wt% and MgO > 30 wt% (Fig. 1). The soil is richer in Al and Fe than the bedrock, but depleted in Mg and Ca. It is not clear whether these effects represent mixing of soils derived from several bedrock types across the slope from which sampling took place, or whether it could be the result of hydrothermal processes. The trace element data for the bedrock and soil also show some interesting differences (Tab. 1). While the Ni values are not very different, the soil is enriched with Co, Cr, and in particular Au. The very high Au value of the soil is a clear indication that hydrothermal processes have affected the soil (as also evidenced by the comparatively high content of Ba, Br, Na and rare earth elements).

The amount of total Ni was determined to be ~1,500 µg . g^{-1} in bedrock and 1,300 µg . g^{-1} in soil. The concentrations of elements of interest from the metal uptake experiment were as follows: Zn – 65 µg . g^{-1} in soil and 53 µg . g^{-1} in bedrock; Fe – 125,300 µg g^{-1} in soil and 58,870 µg . g^{-1} in bedrock; Cu – 49.3 µg . g^{-1} in soil and 59.5 µg . g^{-1} in bedrock. Pb and Cd concentrations in ultramafic rocks were typically low, within ranges of 0.1–1 µg . g^{-1} and 0.03–0.05 µg . g^{-1}, respectively (Kabata-Pendias and Pendias, 1985) and were not detected in soil or bedrock by XRF and INAA. The concentrations of all trace elements determined in the bedrock, related soil and plant leaves are shown in Table 1; the data indicate different levels of bioaccumulation by *B. coddii.*

PLANT ANALYSIS

Ni concentration in leaves of *Berkheya coddii*

The average concentration of Ni in *Berkheya coddii* leaves collected at the Agnes Mine in January and analyzed by INAA, was 17,900 µg . g^{-1}. The phytoextraction coefficient (PC), that is, the ratio between µg metal/g dry weight of tissue and µg metal/g dry weight of substrate (Kumar et al., 1995), was 13.63. Leaf material was collected from the same location in March and analyzed by AAS. The average Ni concentration in leaves from young small plants was 28,200 µg . g^{-1} (PC = 21.48) and 19,700 µg . g^{-1} in leaves from mature vegetative shoots (PC = 15.00).

During a preliminary survey at an additional ultramafic location in the Songimvelo Game Reserve (in March), collected leaves showed exceptionally high average concentrations of Ni, equal to 54,600 ± 1,500 µg . g^{-1} (n = 6) in plants, with the highest sample value reaching 76,100 µg . g^{-1}. This is the highest Ni concentration ever recorded in leaves of *B. coddii*. The Ni concentrations in related soil collected near the plant roots and analyzed by XRF and INAA showed typical values for ultramafic soil: 1,280 µg . g^{-1}.

Young plants in pot culture

Table 2 presents the results of experiments with small young plants growing in pots in ultramafic soil from locations at the Agnes Mine and supplemented with different solutions of Cd, Ni, Pb or Zn. After 7 days of treatment, the Ni content in leaves was lower ($p < 0.05$) in the group watered with Zn than in control plants. Lead amounts were significantly higher in leaves of plants from soil supplemented with Pb than from control plants and plants watered with Cd, Ni and Zn. Concentrations of Cd, Cu, Fe and Zn did not differ significantly between treatments.

TABLE 1. Trace elements in soil, related bedrock and leaves of the Ni hyperaccumulating plant *Berkheya coddii* from the Agnes Mine, determined using XRF and INAA. n.d. – not detected

Elements	Bedrock [µg . g^{-1}]	Related soil [µg . g^{-1}]	*Berkheya coddii* leaves [µg . g^{-1}]
Ni	**1 473**	**1 313**	**17 900**
Na	1 643	6 638	90
Sc	16	26	0.05
Cr	2 600	4 208	2.98
Fe	**58 870**	**125 300**	**289**
Co	80	142	26
Cu	**59.5**	**49**	**n.d.**
Zn	**53**	**65**	**28**
As	2.2	33	0.6
Se	0.7	2.03	0.08
Br	0.3	3.85	18.6
Rb	6.5	8.7	12
Zr	14.7	68	2.5
Sb	0.097	0.48	0.11
Cs	0.6	0.9	0.17
Ba	23	110	6.67
La	0.65	7.16	0.15
Ce	2.8	18.5	0.2
Nd	2.1	7.95	0.16
Sm	0.47	1.78	0.04
Eu	0.21	0.65	0.03
Gd	0.42	2.13	0.07
Tb	0.07	0.37	0.012
Yb	0.37	1.36	0.039
Lu	0.06	0.198	0.005
Hf	0.38	1.58	0.026
Ta	0.12	0.2	0.013
Au [ng . g^{-1}]	0.5	64	4.45
Th	0.9	1.6	0.02
U	0.15	0.28	0.06

Excised shoots in water solution

Excised plant shoots remained in good condition during the whole experiment and no losses in leaf turgor (wilt) were observed. After 7 days the excised shoots took up extremely high amounts of metals from solution in comparison with the control immersed in tap water (Tab. 3). Nickel concentrations in leaves of the group treated with 10 mM Ni solution were significantly higher than in the control group and the group treated with Pb solution. Leaves from shoots treated with Zn or Cd solution showed significantly higher concentrations of these metals compared to all other groups. Fe concentra-

TABLE 2. Concentrations of Zn, Ni, Pb, Cd, Fe and Cu in leaves of *Berkheya coddii* young plants on 7th day of experiment. ± means and SD; the same symbols denote significant differences between experimental groups for the same determined metal (in columns), ANOVA, Tukey's t-test, $p < 0.05$

Groups (supplemented element)	Determined element					
	Zn $[\mu g \cdot g^{-1}]$	Ni $[\mu g \cdot g^{-1}]$	Pb $[\mu g \cdot g^{-1}]$	Cd $[\mu g \cdot g^{-1}]$	Fe $[\mu g \cdot g^{-1}]$	Cu $[\mu g \cdot g^{-1}]$
Control	39 ± 17	28200 ± 5100●	1.09 ± 0.42●	1.00 ± 0.16	360 ± 190	4.4 ± 2.1
Ni	40 ± 17	24900 ± 3000	1.41 ± 0.74◆	1.48 ± 0.96	650 ± 240	5.3 ± 0.9
Zn	120 ± 140	19000 ± 8300●◆❄	0.24 ± 0.10❄	0.83 ± 0.78	900 ± 670	3.2 ± 0.6
Pb	45 ± 9	31300 ± 2000◆	3.30 ± 2.00●◆❄■	0.58 ± 0.33	440 ± 70	5.7 ± 3.2
Cd	45 ± 13	31000 ± 3200❄	0.53 ± 0.25■	1.95 ± 1.37	700 ± 380	5.3 ± 4.8

TABLE 3. Concentrations of Zn, Ni, Pb, Cd, Fe and Cu in leaves of excised shoots *Berkheya coddii* on 7th day of experiment. ± means and SD; the same symbols denote significant differences between experimental groups for the same determined metal (in columns), ANOVA, Tukey's t-test, $p < 0.05$

Groups (supplemented element)	Determined element					
	Zn $[\mu g \cdot g^{-1}]$	Ni $[\mu g \cdot g^{-1}]$	Pb $[\mu g \cdot g^{-1}]$	Cd $[\mu g \cdot g^{-1}]$	Fe $[\mu g \cdot g^{-1}]$	Cu $[\mu g \cdot g^{-1}]$
Control	31 ± 11●	19700 ± 1530●	1.34 ± 1.36	0.66 ± 0.18●	114 ± 270●◆❄	8.6 ± 5.2
Ni	33 ± 15◆	31000 ± 3600●◆	0.50 ± 0.12	0.77 ± 0.14◆	159 ± 78	6.0 ± 0.3
Zn	4100 ± 1400●◆❄■	25700 ± 2000	0.06 ± 0.04	1.17 ± 0.21❄	70 ± 9●	8.9 ± 3.6
Pb	26 ± 16❄	18900 ± 7600◆	720 ± 1030	0.43 ± 0.39■	87 ± 59◆	5.5 ± 2.3
Cd	62 ± 8■	25700 ± 2700	0.16 ± 0.05	8900 ± 3500●◆❄■	48 ± 8❄	7.4 ± 3.1

tions were significantly lower in groups treated with Zn, Cd or Pb solutions. The concentration of Pb in leaves from shoots treated with this metal was higher, but the difference is not significant because of the high SD. Concentrations of Cu did not differ significantly between groups. The bioconcentration factors (BCF), that is, the ratio of the metal concentration in an organism to the concentration of the same metal in the ambient medium (equivalent to PC in toxicology) for the added metals are as follows: Ni – 52.46, Zn – 6.36, Pb – 0.35, Cd – 7.98.

The dynamics of metal uptake from day-to-day monitoring of excised shoots over the 7 days of the experiment are illustrated in Figures 2–5. Control leaves (day 0) had a high concentration of Ni (14,600 $\mu g \cdot g^{-1}$) typical for Ni hyperaccumulators, with the amounts of other elements within the typical range: Zn – 11, Pb – 0.8, Cd – 0.6, Fe – 276, Cu – 7 $\mu g \cdot g^{-1}$ (Kabata-Pendias and Pendias, 1985).

Intense accumulation took place immediately in all groups of treated plants. The most spectacular results were obtained for Pb, Cd and Zn after 2 days of the experiment, with a high increase in concentrations of these elements compared to the low levels present in the control group. After day 2, the concentrations of the metals still significantly differed at the new high levels, showing dynamic changes in the plants during the whole experiment (Figs. 2–5).

In leaves from shoots immersed in Ni solution, concentrations of this metal increased significantly from day 3 of the experiment, reaching a maximum (42,200 $\mu g \cdot g^{-1}$) on day 5. The next two days showed a decrease in concentrations (32,300 $\mu g \cdot g^{-1}$ and 30,900 $\mu g \cdot g^{-1}$) but these concentrations were still double those of the control leaves before the experiment (14,600 $\mu g \cdot g^{-1}$). The Zn concentration was significantly higher from day 3, with a maximum reached on day 5. The Fe concentration decreased significantly from day 3 (Fig. 2).

Leaves from shoots immersed in zinc chloride solution showed significantly increased Zn concentrations from day 2, with the highest amount reached on day 6 (6,100 $\mu g \cdot g^{-1}$, BCF = 9.38). Cd concentrations increased from day 5. In contrast, the amounts of Fe and Pb in these leaves decreased significantly from day 5 and day 2, respectively (Fig. 3).

Leaves from shoots immersed in Pb solution showed a significant increase in the concentration of this element only on day 2 (2,900 $\mu g \cdot g^{-1}$) and 3 (4,000 $\mu g \cdot g^{-1}$) versus the control, due to high SD values for the analyses from each day's samples (BCF = 1.39 and 1.94, respectively) (Fig. 4). The concentration of

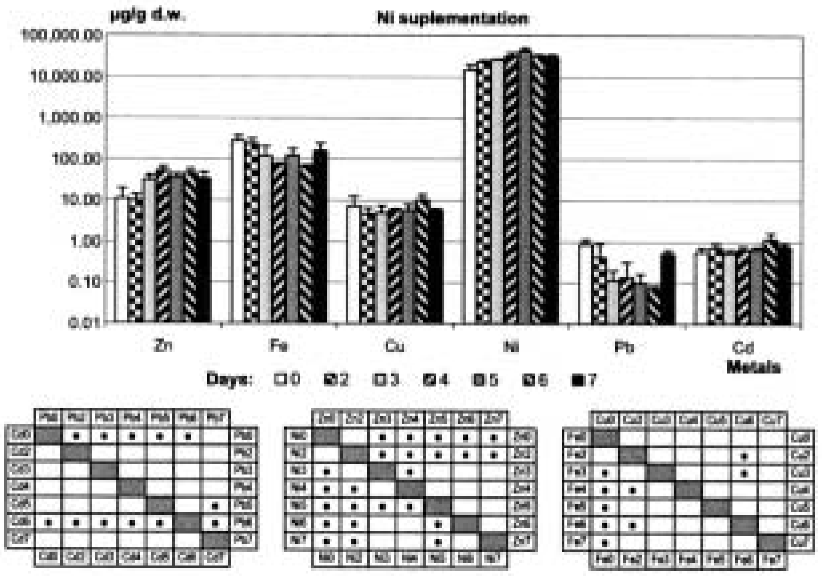

Fig. 2. Changes in concentrations (µg . g^{-1}) of selected elements in leaves from excised shoots of *Berkheya coddii* immersed in 10 mM solution of Ni during 7 days; means and SD. Black dots in the diagrams below denote statistically significant differences in element concentrations between days of the experiment.

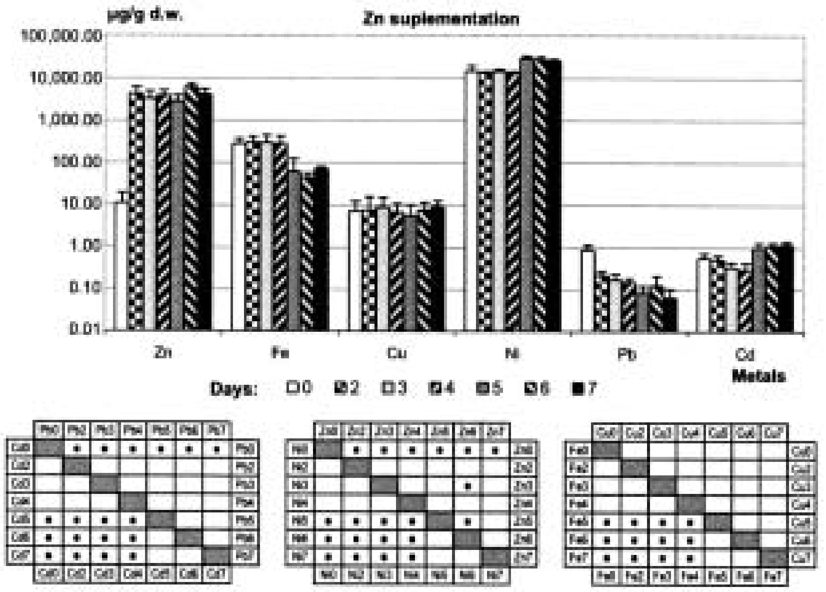

Fig. 3. Changes in concentrations (µg . g^{-1}) of selected elements in leaves from excised shoots of *Berkheya coddii* immersed in 10 mM solution of Zn during 7 days; means and SD. Black dots in the diagrams below denote statistically significant differences in element concentrations between days of the experiment.

Pb in leaves from shoots treated with this metal was higher, but the difference was not significant.

In leaves from shoots immersed in Cd solution, the concentration of this element increased from 0.6 µg . g^{-1} on day 0 to 5,200 µg . g^{-1} on day 2 (BCF = 4.65), with the highest concentration observed on day 7: 8,924 µg . g^{-1} (BCF = 7.97) (Fig. 5). This was similar to the pattern of observed changes of Fe and Pb concentrations in leaves, as well as in the Zn-treated group (without changes in Cu concentration). This was the only group in which expanded necroses on leaves were observed from day 3 of the experiment.

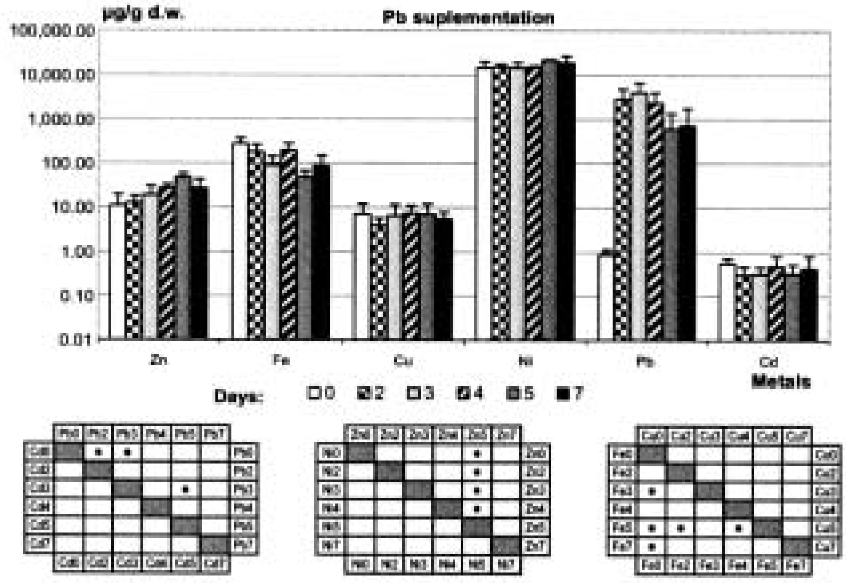

Fig. 4. Changes in concentrations (µg . g^{-1}) of selected elements in leaves from excised shoots of *Berkheya coddii* immersed in saturated solution of Pb during 7 days; means and SD. Black dots in the diagrams below denote statistically significant differences in element concentrations between days of the experiment.

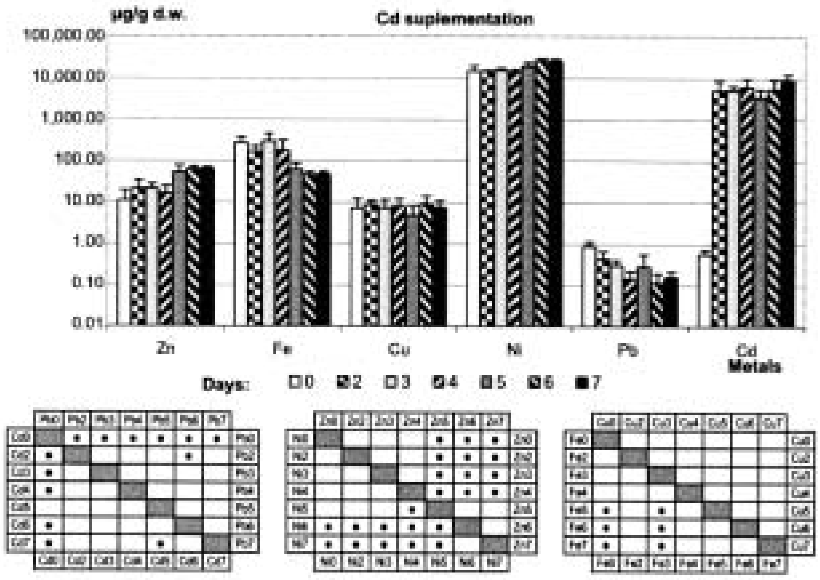

Fig. 5. Changes in concentrations (µg . g^{-1}) of selected elements in leaves from excised shoots of *Berkheya coddii* immersed in 10 mM solution of Cd during 7 days; means and SD. Black dots in the diagrams below denote statistically significant differences in element concentrations between days of the experiment.

DISCUSSION

The soil shows characteristics specific to ultramafic soils: low levels of nutrients and SiO_2, a high Mg/Ca ratio, and high levels of Fe, Ni and Cr (Fig. 1; Tab. 1). All these conditions may be stressful for plant growth and require special physiological adaptations. The ultramafic sites in the investigated region of the Barberton Greenstone Belt differ significantly even at very short distances, due to the complicated geology (Lowe and Byerly, 1999). Even within the Agnes Mine site, the concentration of Ni in *B. coddii* leaves has been found to vary from 1 wt% to 3.8 wt%, depending on the sampling site (Augustyniak et al., 2002). Comparison of element concentrations in bedrock, related soil and *B. coddii* leaves collected at the same time from exactly the same places may demonstrate different strategies of uptake in this plant species and may also reflect the availability of the elements in the soil. Following Kabata-Pendias and Pendias's (1985) division of elements into five groups corresponding to their phytoextraction coefficients (PC), the elements accumulated by *B. coddii* leaves can be ranked as follows:

1. hyperaccumulation – PC above 10: Ni
2. intensive accumulation – PC 10–1.0: Br, Rb
3. medium accumulation – PC 1.0–0.1: Zn, Sb, U, Cs, Co
4. slight accumulation – PC 0.10–0.01: Na, Au, Ta, Ba, Eu, Se, Zr, Gd, Tb, Yb, Lu, La, Sm, Nd, As, Hf, Th, Ce, Sc, Fe
5. lack of accumulation – PC below 0.01: Cr, V, Sr, Y, Nb, Tm, W (e.g., PC for Cr is 0.0007).

Berkheya coddii is a nickel-hyperaccumulating plant. The phytoextraction coefficient for this element varied from 13.6 (for leaves collected in summer) to 15.0 and 21.5 (for leaves from vegetative mature shoots and young plants, collected in autumn, respectively). The highest-ever recorded Ni concentration in leaves (average 54,600 $\mu g \cdot g^{-1}$, PC = 42.67; highest 76,100 $\mu g \cdot g^{-1}$, PC = 59.45) demonstrates the nickel hyperaccumulation capability of this plant. The highest previously reported Ni concentration in *B. coddii* leaves was 38,000 $\mu g \cdot g^{-1}$ (Augustyniak et al., 2002). One possible explanation for these phenomenal results could be a recent fire at these locations, followed by intense regrowth of young plant shoots. A similar observation was reported by Robinson et al. (1997): *B. coddii* plants excised at ground level rapidly produced new shoots with a higher Ni concentration (5,500 $\mu g \cdot g^{-1}$) than the original plants (1,800 $\mu g \cdot g^{-1}$).

During the experiments with young plants growing in pot culture, *B. coddii* took up relatively small quantities of other metals (Tab. 2). Despite the short duration of the experiment, this suggested that cadmium and lead increase nickel uptake and that zinc decreases it. These findings require confirmation.

The studies of excised shoots demonstrated that they have a high ability to accumulate heavy metals from solutions (Tab. 3). The amounts of accumulated Cd and Pb equalled the levels for whole plants kept in solutions supplemented with those metals. Even the nickel concentration increased in the shoots in Ni-supplemented solutions. This suggested that in the absence of the controlling influence of the endodermis, the metals are able to enter via the transpiration stream, and their accumulation reflects concentration by evaporation of water from the plants. However, the results suggest some interactions between metals, and the chronology of accumulation is not easy to explain, particularly when the highest concentrations were reached before the end of the experiment. Metal concentrations in leaves were neither directly proportional to the concentrations in solutions, nor to the period of accumulation. The highest concentrations were obtained at different times for the elements used (Pb – 3rd day, Ni – 5th day, Zn – 6th day, Cd – 7th day), thereafter decreasing during the rest of the experiment.

This ability of *B. coddii* could be utilized for phytoextraction/phytofiltration of metals from wastewater contaminated by heavy metals, by immersion of the cut ends of shoots in metal-enriched flotation pulp or in other industrial settling ponds. With respect to phytofiltration, by which plants are used to clean aqueous environments, two terms were defined: rhizofiltration, when rooted plants are used (Dushenkov et al., 1995); and blastofiltration, when young seedlings are used (Raskin et al., 1997). In this context we propose a new term: caulofiltration (Latin *caulis* = shoot), when excised plant shoots are used to clean wastewater.

Berkheya coddii can produce high biomass, reaching 22 $t \cdot ha^{-1}$ dry mass after moderate fertilization. For comparison, the crop plant *Zea mays* produces 30 $t \cdot ha^{-1}$ (Robinson et al., 1997). Excision of shoots during vegetative growth more than doubled the amount of nickel in newly grown plants (Robinson et al., 1999). *B. coddii* accumulates higher amounts of Ni in leaves than in shoots. From our calculation of the biomass of the vegetative shoots of *B. coddii*, the relation between leaves and stems is 57/43 (dry mass). Without data on the concentration of metals in stems (except for Ni), only the potential phytoextraction capability of the leaves can be assessed. Thus estimated, 60% of the 22 t of biomass are leaves (13 t), which are able to accumulate the following amounts of metal from water solution after 7 days of immersion: up to 53 kg Zn, 116 kg Cd, 9 kg Pb or 78 kg Ni. If the time of immersion in water solution were optimized for the highest metal accumulation, it could even reach values of 79 kg Zn, 52 kg Pb or 220 kg Ni. In this calculation, the amount of nickel present in control plants was subtracted. The total amount of Ni from the soil and water solution could reach a value of 410 kg without this subtraction. Thus, *B. coddii* could accumulate Ni from the soil, and in addition the excised shoots could be used to clean water contaminated with heavy metals in effluent disposal sites of industrial areas.

Phytoremediation is an emerging and promising technology. There is still a significant need for both fundamental and applied research to fully exploit the physiology and metabolic biodiversity of plants. Studies are currently being conducted on the element distribution in *B. coddii,* to gain a better understanding of the mechanisms of hyperaccumulation in this plant species (Mesjasz-Przybylowicz et al., 2001; Budka et al., 2004; Mesjasz-Przybylowicz et al., 2003; Robinson et al., 2003). Mycorrhizal symbiosis recently was reported for the first time in four South African hyperaccumulating plants (*B. coddii* and three other species). Pilot studies have shown that mycorrhization in *B. coddii* increased the biomass of the plant and the Ni content of the shoots (Turnau and Mesjasz-Przybylowicz, 2003). This species is considered useful for phytoremediation purposes in other countries, but the danger that it can become an invasive weed outside its natural habitat needs to be considered. Of importance is the finding that a phytophagous insect, *Chrysolina pardalina* F., is a potential agent for control of *B. coddii*. *C. pardalina* is a monophagic species feeding exclusively on *B. coddii* and capable of completing its entire life cycle for several generations using leaves of this species (Mesjasz-Przybylowicz, 1999; Mesjasz-Przybylowicz and Przybylowicz, 2001; Augustyniak et al., 2002; Mesjasz-Przybylowicz et al., 2002).

ACKNOWLEDGEMENTS

The results in this paper were presented at the 'Biodiversity and ecotoxicology of industrial areas in reference to their bio-reclamation' International Conference held in Katowice on June 5–6, 2003. This publication was supported by the Provincial Fund for Environmental Protection and Water Management in Katowice.

These studies are part of the Joint South African and Polish project 'Ecophysiological aspects of *Chrysolina pardalina* (Chrysomelidae) development as a potential phytophagous insect monitoring distribution of *Berkheya coddii* (Asteraceae), a useful Ni-hyperaccumulator' and KBN project no. 6P04G00721, supported by South African National Research Foundation and by the State Committee for Scientific Research (KBN) in Poland. We thank the Mpumalanga Parks Boards and SAPPI Forestry for kind permission to access the sites and for all assistance. Christian Koeberl's research is supported by the Austrian Fonds zur Förderung der wissenschaftlichen Forschung. Reimold's work is funded by the National Research Foundation of South Africa and the Research Office of the University of the Witwatersrand. We are greatly indebted to John Proctor and Eugeniusz Małkowski for fruitful discussion and to Alban Barnabas for linguistic correction of the manuscript.

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