Phyton (Horn, Austria)	**Vol. 50**	Fasc. 2	287–300	7. 2. 2011

Bioaccumulation Capacity for Pb, Cd and Zn from Polluted Soil in Selected Species of the *Brassicaceae* Family Growing in Different Vegetation Types

By

Boštjan GRABNER*), Cvetka RIBARIČ-LASNIK*), Nadja ROMIH*),

Hartwig W. PFEIFHOFER**) and Franc BATIČ***)

With 1 Figure

Received January 1, 2010

Accepted June 6, 2010

Key words: *Brassicaceae*, Zinc, Lead, Cadmium, Bioaccumulation, Biomonitoring.

Summary

GRABNER B., RIBARIČ-LASNIK C., ROMIH N., PFEIFHOFER H.W. & BATIČ F. 2011. Bioaccumulation capacity for Pb, Cd and Zn from polluted soil in selected species of the *Brassicaceae* family growing in different vegetation types. – Phyton (Horn, Austria) 50 (2): 287–300, with 1 figure.

To evaluate the response and the accumulation capacity concerning their possible use for biomonitoring of metal pollution, five species of the *Brassicaceae* family were chosen as characteristic representatives of common vegetation types: *Alliaria petiolata* – typical for forest edge vegetation; *Capsella bursa-pastoris* – typical for the arable land; *Diplotaxis tenuifolia* – typical for road margins; *Biscutella laevigata* – typical for closed, permanent grasslands, and *Cardamine enneaphyllos* – typical for the forest ground layer vegetation. Plants were collected at the beginning of flowering at three different locations in Slovenia: 1.) Vremščica Mountain (SW part of Slovenia, presumably unpolluted site); 2.) Celje (town in the middle of Slovenia, high contamination with metals due to the zinc industry), and 3.) Žerjav (Karavanke region of northern Slovenia with a century-old tradition of lead and zinc mining and

*) B. GRABNER, C. RIBARIČ-LASNIK, N. ROMIH, Institute of the Environment and Spatial Planning, Ipavčeva 18, 3000 Celje, Slovenia, e-mail: bostjan.grabner@iop.si

**) H. W. PFEIFHOFER, Institute of Plant Sciences, Karl-Franzens University of Graz, Schubertstrasse 51, A-8010 Graz, Austria.

***) F. BATIČ, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia.

smelting activity). Additionally two hybrids of oil seed rape (*Brassica napus* L. var. *napus*) were analyzed, crop plants, sown at differently polluted locations in Celje.

Metal pollution of the soil correlated with the concentration of metals in aboveground plant parts, offering to use these species as biomonitors of metal pollution in different types of natural and semi-natural vegetation. They can be used for monitoring short-term changes in heavy metal polluted arable land and urban areas as well as for monitoring long term heavy metal pollution of forests. None of the plant species of our investigation proved to be a feasible candidate for use in phytoremediation.

Zusammenfassung

Grabner B., Ribarič-Lasnik C., Romih N., Pfeifhofer H.W. & Batič F. 2011. Bioaccumulation capacity for Pb, Cd and Zn from polluted soil in selected species of the *Brassicaceae* family growing in different vegetation types. [Über die Fähigkeit ausgewählter Vertreter der *Brassicaceae* verschiedener Vegetationstypen, Blei, Cadmium und Zink aus schwermetallbelasteten Böden anzureichern.] – Phyton (Horn, Austria) 50 (2): 287–300, 1 Abbildung.

Um zu klären, ob sie für ein Biomonitoring von Schwermetallbelastungen geeignet sind, wurden fünf Arten der *Brassicaceae,* die in häufigen Vegetationstypen vorkommen, auf ihre Fähigkeit Metalle anzureichern untersucht: *Alliaria petiolata* – typisch für Waldränder; *Capsella bursa-pastoris* – typisch für Ackerland; *Diplotaxis tenuifolia* – typisch für Wegränder; *Biscutella laevigata* – typisch für Dauergrünland und *Cardamine enneaphyllos* – typisch für die Krautschicht von Wäldern. Die Pflanzen wurden zu Beginn der Blütezeit an drei verschiedenen Orten in Slowenien gesammelt: 1.) Am Berg Vremščica (im Südwesten Sloweniens, vermutlich ohne Umweltverschmutzung); 2.) in der Stadt Celje (Cilli; eine Stadt in Mittelslowenien mit hoher Schwermetallbelastung verursacht durch Zink verarbeitende Industrie) und 3.) in Žerjav in Koroška (Unterkärnten), der Karawankenregion Nordsloweniens, mit einer Bleischmelze und einem über Jahrhunderte bestehenden Blei- und Zinkbergwerk, das im Jahr 1994 stillgelegt wurde. Zusätzlich wurden zwei Rapssorten (*Brassica napus* L. var. *napus*) untersucht, die in unterschiedlich belasteten Flächen in Celje ausgesät worden waren.

Die Metallbelastung der Böden korrelierte mit der Metallkonzentration in oberirdischen Pflanzenteilen, weshalb diese Arten als Monitorpflanzen für Metallbelastung in natürlichen und anthropogen beeinflussten Vegetationstypen eingesetzt werden könnten. Sie könnten sowohl für das Monitoring kurzfristiger Änderungen im Agrarland und auf urbanen Flächen als auch für ein Langzeitmonitoring der Schwermetallbelastung von Wäldern eingesetzt werden. Keine der untersuchten Pflanzenarten erwies sich als brauchbarer Kandidat für den Einsatz in der Phytoremediation.

Introduction

Soil contamination by metals is a serious environmental problem threatening human health and limiting plant productivity. Metals cause public health problems, entering the body in food or by inhalation of dust (Kos & Leštan 2003). A number of studies have also shown that contamination by metals reduces biodiversity in soil (Dahlin & al. 1997). In plants metals inhibit photosynthesis (Larson & al. 1998, Bazzaz & al. 1975, Singh & al. 1994), nitrate uptake (Devriese & al. 2001), respiration

(KESSELER & BRAND 1995) and most other physiological processes at different levels of metabolism.

Plants differ regarding their tolerance to high concentrations of metals and they have developed two strategies to cope with high concentrations of heavy metals in the soil. One is hyperaccumulation, including transport, chelation, and sequestration of metals in cell vacuoles by using protective mechanisms which diminish the harmful effects. According to BAKER & BROOKS 1989 hyperaccumulators are plants that can concentrate the pollutants in a minimum percentage which varies according to the pollutant involved (for example: more than 1000 mg/kg of dry mass for nickel, copper, cobalt, chromium or lead; or more than 10,000 mg/kg for zinc or manganese). The other strategy is exclusion, i.e. the avoidance of absorption and the prevention of translocation of heavy metals from the roots to the shoots (BAKER 1987). Hyperaccumulators are mostly plants with slow growth and low annual yield. The ideal plant for phytoremediation should, however, strongly accumulate heavy metals and produce biomass as much as possible (CHANEY & al. 1998).

Some plant species can also be used as biomonitors of short term pollution. Ideal as accumulative biomonitor are species where the concentration of the pollutant in the plant reflects external pollutant concentration in the soil. Some additional criteria for the selection of the plants for biomonitoring are: the species should be represented in large numbers all over the monitoring area; it should be easy to sample and to determine; and it should have a wide geographical range (WITTIG 1993).

In the past, more than 400 species of the genera *Alyssum, Arabidopsis, Petrisis*, and *Brassica* (ROOSENS & al. 2003) have been investigated for both phytoremediation and biomonitoring purposes.

Species of the *Brassicaceae* family, such as *Brassica juncea* (Indian Mustard) (BELIMOV & al. 2005, GHOSH & SINGH 2005), *Arabidopsis thaliana* (PATERSON & al. 2001) and *Thlaspi* caerulescens (ROOSENS & al. 2003) have been investigated for their capability to accumulate metals in their shoots for years. *Brassica napus* var. *napus* (GRISPEN & al. 2006) and *Biscutella laevigata* were also investigated as hyperaccummulators of zinc and cadmium (WIERZBICKA & PIELICHOWSKA 2004). ASKOY & al. 1998 also reported that *Capsella bursa-pastoris* (L.) could be a possible candidate for biomonitoring.

The aim of the study was to find new bioaccumulators of the *Brassicaceae* family in different habitats in order to improve environmental biomonitoring. Specimens of the selected *Brassicaceae* species were sampled in differently polluted regions of Slovenia. Vremščica Mountain (in SW Slovenia) was chosen as control site in a presumably unpolluted area. Polluted sites were chosen at two areas: Firstly, the municipality of Celje, where many reports (BATIČ 1984, DOMITROVIČ-UTRANJEK 1988, LOBNIK & al. 1989) indicate heavy metal contamination as a result of past activity of the zinc smelting and other industries in the area; and secondly, Žerjav in the

Upper Meža Valley, the Karavanke region of northern Slovenia with a century-old tradition of lead and zinc mining and lead smelting, leading to heavy pollution of soil (KUGONIČ & KOPUŠAR 2000).

Material and Methods

Experimental Design

Representative wild *Brassicaceae* species, growing abundantly in different habitats, were chosen: *Alliaria petiolata* Cav. & Grande, or garlic mustard, a biennial species of the forest edge; *Capsella bursa-pastoris* (L.) Med., or shepherd's-purse, an annual weedy species, representative of arable land; *Diplotaxis tenuifolia* (L.) DC., or wall-rocket, a ruderal perennial species, representative of road margins and lately also cultivated for salads; *Biscutella laevigata* L., or buckler mustard, a perennial representative of closed, permanent grasslands, and *Cardamine enneaphyllos* (L.) Crantz., or drooping bittercress, a perennial representative of forest ground layer vegetation. At Vremščica, garlic mustard, perennial wall-rocket, buckler mustard, drooping bittercress and shepherd's-purse were sampled. In Celje, garlic mustard, perennial wall-rocket and shepherd's-purse were sampled, and buckler mustard and drooping bittercress at Žerjav. Plants were collected at the beginning of the flowering season. At each locality five specimens of each plant species were sampled. Below- and above-ground parts were separately analyzed for metals.

Two varieties of oilseed rape (*Brassica napus* L. var. *napus*), differing in height, tolerance of climate extremes, and yield, were also sown at two locations in Celje. The hybrid PR46 W31 is a winter crop with a high yield of grains and oil and a low content of glucosinolates. It is medium high and very stable under different ecological circumstances. The other hybrid, PR45 D01, is a hybrid with an implanted dwarf gene that reduces the possibilities of frost damage. The locations were chosen due to pollution of the soil according to the literature (ŽIBERT 2002). At each locality 10 plants were sampled, and below- and above-ground parts were separately analyzed for metals. Plant samples were air-dried for 4 days at 40 °C and ground to powder.

At the time of plant sampling, soil samples in the 0 to 30 cm depth range were taken for analysis at every spot where plants had been collected. The soil samples were air-dried for 4 days at 30 °C and ground to powder. The concentration of cadmium (Cd), zinc (Zn), and lead (Pb) of both soil and plant samples were determined using ICP mass spectrometry (EPOV & al. 2007).

Metal Accumulation in Roots and Shoots

In order to assess metal accumulation traits, the content of Pb, Zn and Cd were quantified in shoots and roots, and the ratios of shoot to root concentration (S/R) were calculated. We also estimated the mobility of metals in the soil-plant system by the bioaccumulation factor (BAF), calculated as the coefficient between the concentration in the shoot (mg/kg DM) and the concentration in the soil (mg/kg DM) (KACHENKO & SINGH 2006).

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{concentration in shoot}}{\text{concentration in soil}}$$

The ratios of shoot to root concentration (S/R) and BAF enable us to compare the uptake of metals by different species collected at differently polluted soils and to estimate their potential use for bioremediation or biomonitoring.

Results and Discussion

Soil Analysis

The Vremščica Mountain was chosen as an unpolluted site because there are no major local sources of metal pollutants in the vicinity. To our surprise (Tables 1–3), the levels of Zn, Pb and Cd were above the allowed immission values according to Slovenian legislation (ANONYMOUS 1996). The Zn concentration in the soil exceeded the threshold value of 200 mg/kg DM at the sampling site of *Capsella bursa pastoris* (220 mg/kg DM), the Pb concentration in the soil exceeded the limit value of 85 mg/kg DM at the sampling site of *Alliaria petiolata* (94.8 mg/kg DM), while the Cd concentration in the soil exceeded the limit value (1 mg/kg DM) at two localities, at the sampling site of *Diplotaxis tenuifolia* (1.1 mg/kg DM) and at the sampling site of *Biscutella laevigata* (1.1 mg/kg DM). At other three plant sampling localities in this area the Cd content exceeded the warning value of 2 mg/kg DM, namely at the sampling site of *A. petiolata* (2.3 mg/kg DM), at the sampling site of *Cardamine enneaphyllos* (2.4 mg/kg DM) and at a sampling site of *C. bursa pastoris* (2.4 mg/kg DM). In the literature there is no data of previous analysis of the soil on Vremščica, but the study made by JERAN & al. 1996, showed high deposition of Zn and Cd in lichens in this area as a result of transport from the industrial region of NW-Italy. That might also be a reason for elevated values of metals in the soil.

The Municipality Celje and upper Meža valley were chosen as localities with highly elevated content of metals in soils due to long term industrial activity in Celje and ore mining and smelting in the upper Meža valley (BATIČ 1984, DOMITROVIČ-UTRANJEK 1988, LOBNIK & al. 1989, (KUGONIČ & KOPUŠAR 2000). An exception was the sampling site of *B. laevigata* (Table 2) in the upper Meža valley, where the Zn content of the soil was the lowest among all the sites investigated (63 mg/kg DM), but the content of Pb and Cd was very high at this locality and exceeded warning values. The reason for this discrepancy is difficult to explain and it might be ascribed to soil heterogeneity at this specific site directly above the entrance to the lead and zinc mine in Žerjav where soils were completely eroded during the mining activity and no other plants grew there. At present, after mining activity ceased, the metal content of the soil is lower than in more distant areas, for example at the site about 2 km away from the mine entrance, where *Cardamine enneaphyllos* (a representative of forest ground layer vegetation) was sampled in the forest. At this site the content of Pb exceeds the critical immission value 20 times, that of Zn 2 times and that of Cd 10 times. At this site the forest vegetation prevents erosion, thus the heavy metals were accumulated over the years of mining and smelting activities.

Lead (Pb)

The uptake of Pb by all species of our study was low with the highest levels being detected in *Biscutella laevigata* (Table 1). According to ADRIANO 2001 hyperaccumulators can amass up to 0.1% Pb (1000 mg/kg DM) in their above-ground parts. WENZEL & JOCKWER 1999 suggested that *B. laevigata* could be a candidate for a hyperaccumulator of lead, accumulating up to 1090 mg/kg DM Pb in shoots on a site that had 4010 mg/kg DM Pb. *Biscutella laevigata* specimens of our study grew on locations containing 179.8 mg/kg DM Pb and accumulated 15.3 mg/kg DM Pb in aboveground parts, which is considerably lower as compared to the study of WENZEL & JOCKWER 1999, even if the different soil pollution is taken into account. Also S/R and BAF are low, suggesting that this species cannot accumulate such high concentrations of lead even on highly polluted soil. Using the BAF we can estimate and evaluate the ability to accumulate Pb in upper plant parts of the species and the potentially safe usage. According to our results the bioavailability of Pb is low at all locations and in all species included in the study.

Cardamine enneaphyllos, containing 691.6 mg/kg DM Pb in roots and 19.7 mg/kg DM in above-ground parts, surpassed *B. laevigata* in accumulation of Pb. However, the content of lead in soil of the sampling site of *C. enneaphyllos* was extremely high, more than 10,000 mg/kg DM Pb, i.e. 55 times more than at the site where *B. laevigata* was sampled. The lowest accumulation capacity was found in *Alliaria petiolata* which, at heavily polluted sites in Celje (2861 mg/kg DM Pb) accumulated only 2.9 mg/kg DM Pb in roots and 5.6 mg/kg DM in above-ground parts (S/R ratio 1.91). For comparison, from soils that were 16 times less polluted *B. laevigata* accumulated 10 times more Pb in roots and 2.7 times more in above-ground plant parts.

Based on BAFs, the plant species of our study successfully prevented uptake of lead from the soil into above-ground plant parts. Even *A. petiolata*, whose S/R was above 1 at both locations, had a very low BAF (0.0020 in Celje). Thus we conclude that *A. petiolata* had an efficient mechanism to avoid lead uptake from the polluted soil, but it had a weak avoidance mechanism against intra-plant translocation, once Pb had been taken up. Considering its fast growth rate and high biomass production, *A. petiolata* would be a potentially good candidate for phytoremediation, but unfortunately it accumulates too little metals in its above-ground parts.

Zinc (Zn)

Another species of the *Brassicaceae* family, *Thlaspi praecox* Wulf, was found to be a hyperaccumulator of metals. It accumulated up to 1.5 % of Zn in shoots (dry mass), as reported by REGVAR & al. 2006. Species analyzed in this study did not accumulate such high concentrations of Zn

Table 1. Lead concentration in soil samples from Vremcica, municipality Celje and the upper Mea valley as well as content of Pb in shoots and roots, the ratio of shoot to root concentration (S/R) and bioaccumulation factor (BAF) of selected plant species from the Brassicaceae family.

Plant species	Soil (mg/kg)	Roots (mg/kg)	Shoots (mg/kg)	S/R	BAF
Low metal sites					
Vremščica					
Capsella bursa-pastoris	20.10	7.33	0.29	0.04	0.0144
Diplotaxis tenuifolia	32.40	5.26	0.68	0.13	0.0210
Biscutella laevigata	58.30	1.81	0.34	0.19	0.0058
Cardamine enneaphyllos	78.60	1.39	0.76	0.55	0.0097
Alliaria petiolata	94.80	0.18	0.52	2.89	0.0055
Medlog					
Brassica napus L. var. *napus* PR46 W31	36.10	2.06	1.03	0.50	0.0285
Brassica napus L. var. *napus* PR45 D01	37.40	2.09	1.66	0.79	0.0444
High metal sites					
Celje					
Diplotaxis tenuifolia	129.60	36.49	12.36	0.34	0.0954
Capsella bursa-pastoris	2.096.00	21.21	8.33	0.39	0.0040
Alliaria petiolata	2.861.00	2.94	5.62	1.91	0.0020
Žerjav					
Biscutella laevigata	179.80	210.04	15.27	0.07	0.0849
Cardamine enneaphyllos	10.000.00	691.55	19.71	0.03	0.0020
Škofja vas					
Brassica napus L. var. *napus* PR46 W31	205.30	9.87	1.77	0.18	0.0086
Brassica napus L. var. *napus* PR45 D01	239.70	16.18	2.67	0.17	0.0111

(Table 2). The highest concentration of Zn in shoots was detected in *Alliaria petiolata* growing on a heavy polluted soil in Celje with a Zn content of 10,000 mg/kg DM. Above-ground plant parts of this species contained 392.9 mg/kg DM Zn and also had a high S/R ratio (1.33). A similar intensive uptake of Zn was observed also in *Diplotaxis tenuifolia* which contained 261.6 mg/kg DM Zn in shoots. However, these plants were growing on a considerably less polluted soil (1,058 mg/kg DM Zn).

Capsella bursa-pastoris had the smallest uptake rate of Zn at the sampling site in Celje where it accumulated from much more polluted soils with 4,649 mg/kg DM Zn only 24.2 mg/kg DM Zn into its above-ground parts. This is similar to the Zn content found in *A. petiolata* at the Vremcica site, where the soil concentration of Zn was 45 times lower.

Alliaria petiolata had an S/R ratio higher than 1 at both locations (Fig. 1). Considering the fact that Zn is an essential micronutrient, our results

Table 2. Zinc concentration in soil samples from Vremščica, municipality Celje and the upper Meža valley as well as content of Zn in shoots and roots, the ratio of shoot to root concentration (S/R) and bioaccumulation factor (BAF) of selected plant species from the *Brassicaceae* family.

Plant species	Soil (mg/kg)	Roots (mg/kg)	Shoots (mg/kg)	S/R	BAF
Low metal sites					
Žerjav					
Biscutella laevigata	63.00	52.40	24.20	0.46	0.38
Vremščica					
Diplotaxis tenuifolia	78.00	68.00	37.80	0.56	0.48
Biscutella laevigata	93.00	25.60	44.50	1.74	0.48
Cardamine enneaphyllos	118.00	15.90	35.10	2.21	0.30
Alliaria petiolata	147.00	20.10	25.70	1.28	0.17
Capsella bursa-pastoris	220.00	52.70	24.50	0.46	0.11
Medlog					
Brassica napus L. var. *napus PR 45D01*	106.00	31.60	11.44	0.36	0.11
Brassica napus L. var. *napus PR46 W31*	115.00	40.48	12.54	0.31	0.11
High metal sites					
Celje					
Diplotaxis tenuifolia	612.00	490.00	151.60	0.31	0.25
Capsella bursa-pastoris	4.649.00	208.50	24.20	0.12	0.01
Alliaria petiolata	10.000.00	294.70	392.90	1.33	0.04
Žerjav					
Cardamine enneaphyllos	1.058.00	70.10	261.60	3.73	0.25
Škofja vas					
Brassica napus L. var. *napus PR 45D01*	1.310.00	184.77	52.83	0.29	0.04
Brassica napus L. var. *napus PR46 W31*	1.356.00	113.87	53.93	0.47	0.04

explain higher BAFs for Zn than for Pb (Tables 1 and 2). They also proved, similar to the results obtained with Pb pollution, that plants had no efficient mechanism to avoid the intra-plant movement of Zn from roots to shoots. The low BAF (0.04) reveals that further uptake and enrichment of Zn was successfully prevented by the plant roots.

Cadmium (Cd)

Cadmium is a highly toxic element without any function in vascular plants. The known hyperaccumulator of Cd (Marschner 1995), *Thlaspi caerulescens*, can accumulate Cd up to 100 mg/kg of shoot dry biomass. In our study, however, the concentration of Cd in roots and shoots remained low, even on locations with very high levels of Cd in the soil (Table 3). We

T a b l e 3. Cadmium concentration in soil samples from Vremščica, municipality Celje and the upper Meža valley as well as content of Cd in shoots and roots, the ratio of shoot to root concentration (S/R) and bioaccumulation factor (BAF) of selected plant species from the *Brassicaceae* family.

Plant species	Soil (mg/kg)	Roots (mg/kg)	Shoots (mg/kg)	S/R	BAF
Low metal sites					
Vremščica					
Diplotaxis tenuifolia	1.10	4.66	0.85	0.18	0.77
Biscutella laevigata	1.10	0.60	0.73	1.22	0.66
Alliaria petiolata	2.30	0.70	0.75	1.07	0.33
Capsella bursa-pastoris	2.40	4.69	1.04	0.22	0.43
Cardamine enneaphyllos	2.40	0.33	0.83	2.52	0.35
Medlog					
Brassica napus L. var. napus PR 45D01	1.20	0.44	0.71	1.61	0.59
Brassica napus L. var. napus PR46 W31	1.40	0.66	0.85	1.29	0.61
High metal sites					
Celje					
Diplotaxistenuifolia	3.90	6.22	5.24	0.84	1.34
Alliaria petiolata	26.00	3.59	2.11	0.59	0.08
Capsella bursa-pastoris	38.40	5.48	4.38	0.80	0.11
Žerjav					
Biscutella laevigata	4.50	7.39	2.64	0.36	0.59
Cardamine enneaphyllos	67.00	2.13	5.41	2.54	0.08
Škofja vas					
Brassica napus L. var. napus PR46 W31	9.70	1.26	0.84	0.67	0.09
Brassica napus L. var. napus PR 45D01	10.90	0.85	0.91	1.07	0.08

detected the highest uptake of Cd in *Diplotaxis tenuifolia*. In Celje, where the soil concentration of Cd was elevated (3.9 mg/kg DM), this plant species contained 6.2 mg/kg DM Cd in roots and 5.2 mg/kg DM Cd in above-ground plant parts. *Biscutella laevigata* at the site Žerjav accumulated 7.4 mg/kg DM Cd in roots and 2.6 mg/kg DM Cd in above-ground parts growing on soils with 4.5 mg/kg DM Cd. *Alliaria petiolata* and *Capsella bursa-pastoris* took up similar amounts of Cd at the site in Celje, but the sampling site there was much higher polluted with Cd. Comparing the ability of excluding Cd from shoots, only *Cardamine enneaphylos* and *Brassica napus* PR45 D01 had S/R ratio above 1 on highly polluted soils (Fig. 1).

In most investigated species the BAF of Cd is in the same order of magnitude as the BAF of Zn. The exception is *Diplotaxis tenuifolia* in Celje (BAF = 1.34) and on Vremcica (BAF = 0.77), suggesting that it could accumulate Cd; however, this species needs to be tested on more locations with highly Cd

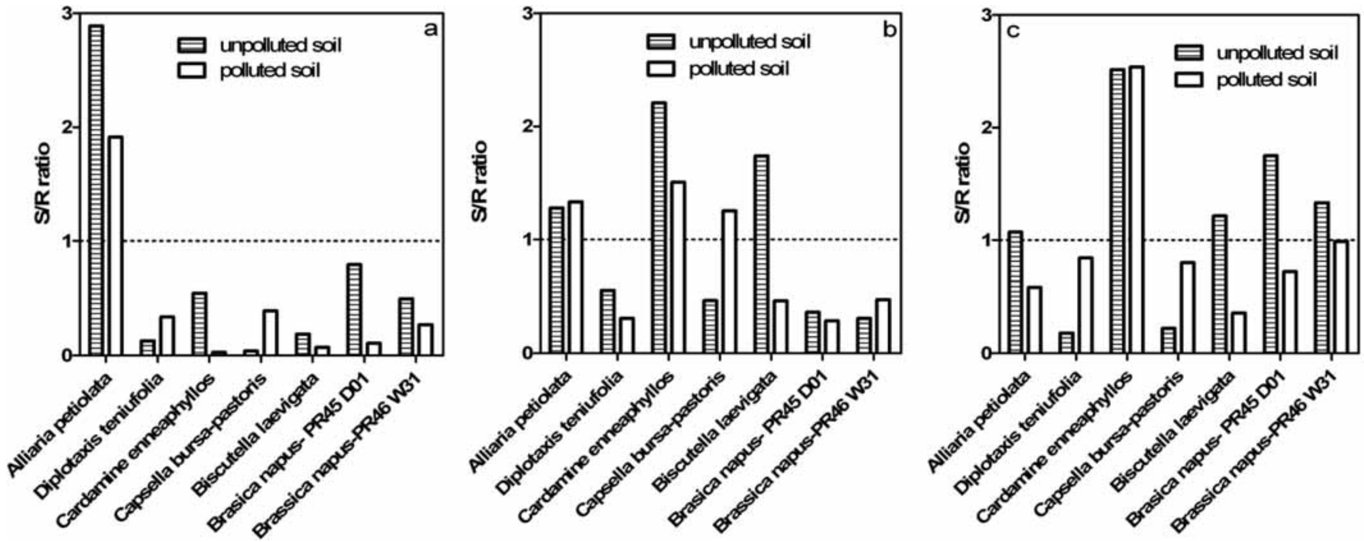

Fig. 1. Shoot/root ratio (S/R) in the chosen plant species of the *Brasssicaceae* family on unpolluted and polluted soil. (a) S/R ratio of Pb in plants. (b) S/R ratio of Zn in plants. (c) S/R ratio of Cd in plants.

polluted soils. Nevertheless, a relationship between the soil concentration of Cd and the Cd content in *D. tenuifolia* shoots was shown. Plants sampled on a soil with a threefold Cd concentration accumulated more Cd in the shoot compared with those sampled on less polluted soil (Table 3).

Conclusions

The plant species in our study belonged to different life history types. Some of them were annual (*Capsella bursa-pastoris, Brassica napus*), others biennial (*Alliaria petiolata*) or perennial (*Diplotaxis tenuifolia, Biscutella laevigata and Cardamine enneaphyllos*) herbs. Apart from metal concentrations in soils, the bioaccumulation capacity is also influenced by plant characteristics such as life forms and their associated functional traits, e.g. duration of vegetation period and dynamics of growth (WITTIG 1993). Higher uptake was associated with perennial species, such as the uptake of Zn and Pb by *C. enneaphyllos* at the Žerjav site, and a high uptake of Cd by *D. tenuifolia* and *B. laevigata*.

Some of the species we used in our research are edible as wild or cultivated salad plants (*D. tenuifolia, A. petiolata, and C. bursa-pastoris*). For example, *Diplotaxis tenuifolia* has lately been widely cultivated and served as salad in Italian-like kitchens. Even though MCLAUGHLIN & al. 1995 reported that fertilizer Cd concentrations had little influence on Cd concentrations in tubers of commercially grown potato (*Solanum tuberosum* L.) crops, he also concluded that residual Cd in the soil was a major contributor to the Cd uptake by the crops on these soils. That is why contamination by metals, especially Cd, caused by mineral fertilizer should be controlled individually for every plant species. According to Slovenian legislation considering soil pollution (ANONYMOUS 1996), soils in which heavy metal content exceeds critical values (Table 4), are not allowed for food production and soil that is under critical value, but still polluted, is not recommended for vegetable production. As for medium polluted soil we must refer to another law (ANONYMOUS 1983) which states that the concentration of Pb should not exceed 3 mg/kg and the concentration of Cd should not exceed 0.3 mg/kg in dry vegetables (Table 4). Our investigation showed that on medium polluted soil the concentration of Pb in shoots of *D. tenuifolia* exceeds the allowed level, while in *A. petiolata* it does not. Consequently, *A. petiolata* could be grown on medium polluted soil and used as edible plant, while *D. tenuifolia* could not. For Cd the situation proves to be quite different. The allowed concentration in shoots (Table 4) was exceeded in all plant species at all locations (Table 3). To grow any of the plants from our research for food production in medium Cd polluted soil is not advisable.

Oilseed rape is not only used for oil production, but also for feeding animals. In order to assess the possible use of oilseed rape, grown on pol-

Table 4. Limit values for heavy metals in soil*, foodstuff** and animal feed*** by Slovenian legislation.

	Pb (mg/kg)	Zn (mg/kg)	Cd (mg/kg)
* Warning value	≥85	≥200	≥1
Alert threshold	≥100	≥300	≥2
Critical value	≥530	≥720	≥12
** Permitted quantity of toxic substances in dry vegetables	3	/	0.3
*** Maximum content in animal feed	30	/	1

*** Decree on the limit values, alert thresholds and critical levels of dangerous substances into the soil

*** Rules on the permissible quantities of pesticides and other toxic substances, hormones, antibiotics and microtoxins, in foodstuffs

*** Rules on feed safety criteria

luted soil, we have to consider a third Slovenian law (Anonymous 2006) stating that animal fodder should not contain more than 30 mg/kg Pb and 1 mg/kg Cd (Table 4). In our study we grew oilseed rape on medium polluted soil where Pb concentration in the soil did not exceed critical values (Table 4). The concentration in shoots did not exceed allowed concentration of Pb and Cd at any location either. As a result *B. napus* could be grown as animal fodder on medium polluted soil.

According to Adriano 2001, hyperaccumulators have the ability of accumulating up to 0.001 % Cd, 0.1 % Pb and 1 % Zn dry mass in their upper parts. None of the species included in our study could be categorized as a hyperaccumulator and thus they cannot be used for phytoremediation purposes. This holds true for *B. napus, A. petiolata* and *D. tenuifolia* which otherwise would be suitable, because they can produce high biomass and are easy to grow. The results show a correlation between the pollution of the soil and the concentration of the metals in the above-ground plant parts. Consequently the selected plant species can be used as biomonitors of heavy metals in different habitat types of natural and semi-natural vegetation. Especially short-term changes in heavy metal pollution of cultivated arable land (*C. bursa-pastoris, D. tenuifolia*) and urban areas (*A. petiolata, D. tenuifolia*) could be monitored.

Acknowledgments

The operational part was financed by the European Union, European Social Fund.

References

Adriano D. C. 2001. Trace elements in terrestrial environments: Biochemistry, bioavailability, and risk of metals. – Springer-Verlag, New York, Berlin, Heidelberg.

ANONYMOUS 1983. Rules on the permissible quantities of pesticides and other toxic substances, hormones, antibiotics and microtoxins in foodstuffs. 1983. – Official Journal of SFRJ. No.59-740/1983, p. 1634.

ANONYMOUS 1996. Decree on the limit values, alert thresholds and critical levels of dangerous substances into the soil. – Official Journal of Republic of Slovenia No.68/1996. p. 7–8.

ANONYMOUS 2006. Rules on feed safety criteria. 2006. Official Journal of Republic of Slovenia No. 101–4361/2006, p. 1405.

ASKOY A., HALE W. H. G. & DIXON J. M. 1998. *Capsella bursa-pastoris* (L.) Medic. as a biomonitor of heavy metals. – Sci. Total Environ. 226: 177–186.

BAKER A. J. M. 1987. Metal tolerance. – New Phytol. 106 (Suppl.): 93–111.

BAKER A. J. M. & BROOKS R. R. 1989. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. – Biorecovery 1: 81–126.

BATIČ F. 1984. Determination of air pollution by epiphytic lichens and card of Slovenian lichens as a result of work. – In: Gosar M. (ed.), Research of air pollution in Slovenia 2, p.76 – Ljubljana.

BAZZAZ F. A., CARLSON R. W. & ROLFE G. L. 1975. The inhibition of corn and sunflower photosynthesis by lead. – Physiol. Plant. 34: 326–329.

BELIMOV A. A., HONTZEAS N., SAFRONOVA V.I., DEMCHINSKAYA S.V., PILUZZA G., BULLITTA S. & GLICK B.R. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.). – Soil Biol. Biochem. 37: 241–250.

CHANEY R. L., LI Y. M., ANGLE J. S., BREWER E. P., SAUNDERS J. P. & BAKER A. J. M. 1998. Converting metal hyperaccumulator wild plants into commercial phytoremediation systems: Approaches and progress. – In: BANUELOS G.S. & TERRY N. (Eds.), Proceedings of the fourth international Conference on Biochemistry of trace elements. – Berkley, CA, pp. 623–624.

DAHLIN S., WITTER E., MARTENSSON A., TURNER A. & BAATH A. 1997. Where is the limit? Changes in the microbiological properties of agricultural soils at low levels of metal contamination. – Soil. Biol. Biochem. 29: 1405–1415.

DEVRIESE M., TSAKALOUDI V., GARBAYO I., LEON R., VILCHEZ C. & VIAGRA J. 2001. Effect of heavy metals on nitrate assimilation in the eukaryotic microalgae *Chlamydomonas reinhardtii*. – Plant Physiol. Biochem. 39: 443–448.

DOMITROVIČ-UTRANJEK D. 1988. Ecology balance of the region Celje – II. phase. A Miscellany of Celje: 23–65.

EPOV V.N., DOUGLAS R. E., ZHENG J., DONARD O. F. X & YAMADA M. 2007. Rapid fingerprinting of ^{239}Pu and ^{240}Pu in environmental samples with high U levels using on-line ion chromatography coupled with high-sensitivity quadruple ICP-MS detection. J. Anal. At. Spectrom. 22: 1131–1137.

GHOSH M. & SINGH S. P. 2005. A comparative study of cadmium phytoextraction by accumulator and weed species. – Environ. Pollut. 133: 365–371

GRISPEN V. M. J., NELISSEN H. J. M. & VERKLEIJ J. A. C. 2006. Phytoextraction with *Brassica napus* L.: A tool for sustainable management of heavy metal contaminated soils. – Environ. Pollut. 144: 77–83.

JERAN Z., JAČIMOVIČ R., BATIČ F. & SMODIŠ B. 1996. Atmospheric heavy metal pollution in Slovenia derived from results for epiphytic lichens. – Fresenius J. Anal. Chem. 354: 681–687.

KACHENKO A. & SINGH B. 2006. Heavy metal contamination of home grown vegetables near metal smelters in NSW. SuperSoil 2004: 3rd Australian New Zealand Soils Conference, 5–9 December 2004, University of Sydney, Australia.

KESSELER A. & BRAND M. D. 1995. The mechanism oft he stimulation of state 4 respiration by cadmium in potato tuber (*Solanum tuberosum*) mitochondria. – Plant Physiol. Biochem. 33: 519–528.

KOS B. & LEŠTAN D. 2003. Induced phytoextraction/soil washing of lead using biodegradable chelate and permeable barriers. – Environ. Sci. Technol. 37: 624–629.

KUGONIČ N. & KOPUŠAR N. 2000. Garden soil and vegetable pollution assessment of the Upper Meža Valley (Slovenia). – Phyton 40: 117–121.

LARSSON E. H., BORNMANN J. F. & ASP H. 1998. Influence of UV-B radiation and Cd^{2+} on chlorophyll fluorescence, growth and nutrient content in *Brassica napus*. – J. Exp. Bot. 49: 1031–1039.

LOBNIK F., HRUSTEL M., ZUPAN M., VRŠČAJ B., RUPREHT J., ŠPORAR M.C., HODNIK A., TROBIŠ-LEDNIK M., VIDIC N., PUS T., VIRANT D., KOČEVAR H., HUDNIK V., VUČKO H., BIZJAK M., GRGIČ I., MEDVED M., LAPAJNE S., BRUMEN S., ŽERJAL E., VONČINA E., ŠTAJNBAHER D. & ŠTANCER A. 1989. The thematic map of soil pollution in the Municipality of Celje. – Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair of agronomy, crops production and ecology: 159.

MARSCHNER 1995. Mineral nutrition of higher plants. 2nd edition. – Academic Press, London, UK.

MCLAUGHLIN M. J., MAIER N. A., FREEMAN K., TILLER K., G., WILLIAMS C. M. J. & SMART M. K. 1995. Effect of potassic and phosphatic fertilizer type, phosphatic fertilizer cadmium content, and additions of zinc on cadmium uptake by commercial potato crops. – Fert. Res. 40: 63–70.

PATERSON A. H., LAN T., AMASINO R., OSBORN T. C. & QUIROS C. 2001. Brassica genomics: a complement to and early beneficiary of the Arabidopsis sequence. – Genome Biol. 2: 1011.1 – 1011.4.

REGVAR M., VOGEL-MIKUS K., KUGONIC N., TURK B., BATIČ F. 2006. Vegetational and mycorrhizal successions at a metal polluted site: Indications for the direction of phytostabilisation? – Environ. Pollut. 144: 976–984.

ROOSENS N., VERBRUGGEN N., MEERTS P., XIMENEZ-EMBUN P. & SMITH J. 2003. Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from Western Europe. – Plant Cell Environ. 10: 1657–1672.

SINGH R. P., MAHESWARI R. & SINHA S. K. 1994. Recovery of lead caused decreased in biomass accumulation of mung bean (*Vigna radiata*, L.) seedlings of K_2HPO_4 in $CaCl_4$. – Indian J. Exp. Biology. 32: 507–510.

WENZEL W. W. & JOCKWER F. 1999. Accumulation of heavy metals in plants grown on mineralized soils of the Austrian Alps. – Environ. Pollut. 104: 145–155.

WIERZBICA M. & PIELICHOWSKA M. 2004. Adaptation of *Biscutella laevigata* L., a metal hyperaccumulator, to growth on a zinc-lead waste heap in southern Poland I: Differences between waste-heap and mountain populations. – Chemosphere 54: 1663–1674.

WITTIG R. 1993. General aspects of biomonitoring heavy metals by plants. V: Plants as biomonitors: Indicators for heavy metals in the terrestrial environment. – In: MARKERT B. (Ed.) Weinheim, VCH Verlagsgesellschaft, pp. 1–27.

ŽIBERT G. 2002. Geochemical properties of soil and attic dust in Celje. – Graduation Thesis. Ljubljana, Faculty of Natural Sciences and Engineering, Department of Geology, p. 62.