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UPTAKE AND LOCALIZATION OF CADMIUM BY *BISCUTELLA LAEVIGATA*, A CADMIUM HYPERACCUMULATOR

MARIA PIELICHOWSKA* AND MAŁGORZATA WIERZBICKA**

Environmental Plant Pollution Laboratory, University of Warsaw, ul. Miecznikowa 1, 02–096 Warsaw, Poland

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Biscutella laevigata has recently been recognized as a species able to accumulate large amounts of cadmium. The experiments reported in this paper were conducted on two geographically isolated populations of *B. laevigata* in Poland. Both populations grow on metalliferous soils: a lead-zinc (calamine) waste heap in Bolesław near Olkusz (189 mg Cd/kg d.m.) and limestone rock in the West Tatra Mts (1.4–6.1 mg Cd/kg d.m.). The two populations were compared after cultivating them in medium containing cadmium salt (2–120 mg/dm^3) for 3–30 days. Root-to-shoot transport of cadmium was higher in the waste-heap population than in the mountain population. In the waste-heap population, large amounts of cadmium were transported to the oldest leaves, reaching levels even twice those of the mountain population. This shows that the ability to hyperaccumulate metals may be a property of a population, not an entire species, and that the ability to accumulate cadmium in the oldest (withering) leaves may be a way the plant eliminates the toxic metal. Histochemical detection of cadmium (with dithizone) in tissues showed that it was taken up by the root hairs and then transported through vascular bundles to the leaves. The surface cells of the leaves, the epiderm and hairs accumulated particularly large amounts of cadmium. The leaves of the *B. laevigata* waste-heap population are much more thickly covered by hairs than those of the mountain population; we suggest that the ability to accumulate cadmium in leaf hairs may be a mechanism of detoxifying and hyperaccumulating cadmium in the shoots of that population.

Key words: *Biscutella laevigata,* cadmium, dithizone, detoxification of cadmium, hyperaccumulation.

INTRODUCTION

Because so many areas are degraded and polluted by heavy metals and other contaminants, species of plants able to grow under such conditions have been sought for years. These plants must have specific adaptations, that is, they must be metallophytes, pseudometallophytes, or hyperaccumulators (Wenzel and Jockwer, 1998; Anderson and Brooks, 1999). Numerous studies on the physiology of these plants aim to uncover the mechanisms that permit growth on polluted sites.

One interesting species occurring naturally on soils with elevated levels of heavy metals such as Zn, Cd, Pb, or Tl is *Biscutella laevigata* ssp. *gracilis*, a member of the Brassiceace family (Szafer, 1927; Rutkowski, 1998). Although it is a mountain species with a mainly alpine distribution, it occurs in Poland in two geographically isolated stands: in the West Tatra Mts and on a zinc-lead (calamine) waste heap in the vicinity of Olkusz in the Cracow-Częstochowa Uplands (Szafer, 1927; Dobrzańska, 1955; Grodzińska et al., 2000). Both on the waste heap and in the Tatra Mts, *B. laevigata* grows on soils containing elevated amounts of zinc, lead and cadmium in their surface layers. The waste-heap soil contains distinctly higher amounts of cadmium than the Tatra Mts soil (189 mg/kg d.m. vs. 1.4–6.1 mg/kg d.m.) (Godzik, 1993; Niemyska-Łukaszczuk, 1993; Miechówka, 2000; Szarek-Łukaszewska and Niklińska,

e-mail: *mpiel@biol.uw.edu.pl; **wierzbicka@biol.uw.edu.pl

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2002). Recently, *B. laevigata* has been recognized as a hyperaccumulator of heavy metals such as thallium, lead and cadmium. Very high amounts of these elements were found in the shoots of *B. laevigata* plants growing on metalliferous soils in the Alps: thallium > 1.4% d.m., lead > 1% d.m., and cadmium > 50 mg/kg d.m. (Wenzel and Jockwer, 1998; Anderson and Brooks, 1999).

This study assesses the ability of two *B. laevigata* populations occurring in Poland to take up cadmium, and analyzes its localization in plant tissues.

MATERIALS AND METHODS

MATERIAL

Seeds were collected from two populations of *B. laevigata*, one growing on a waste heap in Bolesław near Olkusz and the other growing in the Jaworzynka Valley in the West Tatra Mts (southern Poland).

The waste heap in Bolesław is in a hilly region with low-grade urbic antrosols in which 77% of the fractions are particles > 1 mm (Dobrzańska, 1955; Szarek-Łukaszewska and Niklińska, 2002). The surface layer is a few centimeters of raw, mostly undegraded humic sod, with pH in the range of 7.3–8.2. In the surface layer of the galmanic substrate, cadmium was found to occur in amounts ranging from 171 to 233 mg Cd/kg d.m, on average 189 mg Cd/kg d.m. (Dobrzańska, 1955; Godzik, 1993; Szarek-Łukaszewska and Niklińska, 2002). The waste heap has a specific microclimate in which wind exerts a very strong influence (Dobrzańska, 1955).

The Jaworzynka Valley lies at an altitude of 1100 m a.s.l. and is formed by Triassic dolomite rocks. The soils with vegetation are classified as humic rendzic leptosols (Godzik, 1993; Szarek-Łukaszewska and Niklińska, 2002). Cadmium has been found to occur in the surface layers of this soil in amounts ranging from 1.4 to 6.1 mg Cd/kg d.m. (Godzik, 1993; Szarek-Łukaszewska and Niklińska, 2002). Both the Jaworzynka Valley and the Bolesław waste heap are sites with high insolation.

CULTIVATION

The seeds were germinated in Petri dishes on damp filter paper. Seedlings were transferred to pots filled with garden peat and grown for 1.5–2.5 months, depending on the experiment. The plants were then removed from the pots, the peat was rinsed off, and they were transferred to liquid media for further cultivation in hydroponic culture. The plants were allowed to acclimate to Knop medium (Strebeyko, 1976) for 5 days, after which cadmium was administered in set concentrations ranging from 2 to 120 mg/dm^3 Cd^{2+} (from $CdCl_2$ in 1/2 Knop medium without PO_4^{3-} ions), depending on the experiment. The control plants were grown in 1/2 Knop medium without the addition of metal salts and PO_4^{3-} ions.

The mineral medium in which the plants were grown during the period of exposure to cadmium salt did not contain phosphate ions (to prevent precipitation of metal phosphates). The duration of incubation in cadmium salts ranged from 4 to 30 days, depending on the experiment, and with different metal concentrations. For detection of cadmium in plant cells and tissues, longer exposure of plants to a low concentration of metal seemed better than shorter exposure to a high concentration.

DETERMINATION OF CADMIUM IN PLANT PARTS

After incubation with cadmium, the plants were rinsed carefully and separated into aboveground parts (youngest leaves, mature leaves, withering oldest leaves) and underground parts (upper part of root not submerged in medium, lower part of root previously submerged in medium). The plant samples were then weighed, dried at 100°C, ground, and mineralized in a mixture of HNO_3 and H_2O_2 at 180°C and 800 W in Teflon containers. Element analysis employed atomic absorption spectrometry (AAS), the limit of detection for Cd was 0.032 μg/dm^3, and the reference material was Virginia tobacco leaves (CTA-VTL-2) from the Institute of Chemistry and Atomic Technology. Three samples of every plant part were used for cadmium detection.

CADMIUM LOCALIZATION

Cross- and tangential sections were made of the roots and leaves of plants incubated in cadmium-containing medium for 30 days. Cadmium was detected histochemically in these sections in cells and tissues of above- and belowground plant parts, using dithizone (diphenylthiocarbazone, 30 mg dissolved in 60 ml acetone and 20 ml distilled water; staining, 1.5 h). The procedure described earlier by Seregin and Ivanov (1997) was modified. After staining, the sections were rinsed in water and analyzed by light and stereoscopic microscopy. The presence of cadmium in tissues was detected in dark red to black complexes of cadmium with dithizone (Szmal and Lipiec, 1996; Seregin and Ivanov, 1997).

TABLE 1. Concentration of cadmium (mg/kg d.w.) in organs of *B. laevigata* from waste-heap and mountain populations. Plants were treated for 10 days with cadmium (4 mg/dm^3 d.w. Cd from $CdCl_2$) added to mineral medium. Standard deviation is given in parentheses

Plant organ	Waste-heap population		Mountain population	
	Control	Cadmium	Control	Cadmium
Lower part of root (submerged in medium)	9.5 (6.6)	1986 (949)	2.0 (0.8)	2520 (235)
Upper part of root (above medium)	2.3 (0.1)	1738 (1142)	5.7 (2.5)	516 (444)
Oldest leaves (withering)	2.3 (0.6)	242 (110)	1.8 (0.5)	51 (39)
Mature leaves	2.4 (1.1)	198 (141)	1.6 (0.9)	142 (32)
Youngest leaves	4.8 (3.0)	207 (156)	4.5 (1.7)	144 (94)

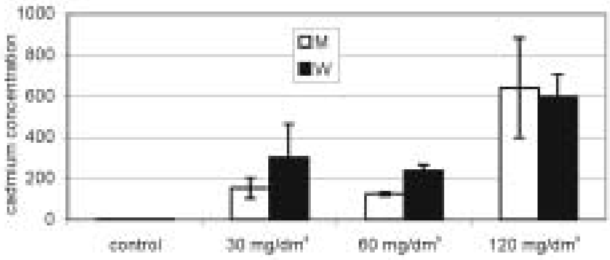


Fig. 1. Concentration of cadmium (mg/kg d.w.) in the oldest (withering) leaves of *B. laevigata* from the waste heap and mountain populations after 96 h incubation in solutions of cadmium salt ($CdCl_2$) at 30, 60 and 120 mg/dm^3 Cd concentrations. M – mountain population; W – waste-heap population.

RESULTS

CADMIUM CONCENTRATION IN PLANT ROOTS

The concentrations of the cadmium salt used to treat the plants were selected not to be phytotoxic. Incubation in 4 mg/dm^3 cadmium chloride for 10 days was well tolerated by the plants. Only slight inhibition of biomass accumulation was found. The weight of the shoots of cadmium-treated plants was 88–111% of the controls, and the weight of roots was 89–94% of the controls.

After 10 days of exposure to cadmium (4 mg/dm^3), the largest amounts (2000–2500 mg/kg d.w. Cd) were found in the lower, submerged part of the roots of both studied populations (Tab. 1). The upper, non-submerged part of the roots of the waste-heap population contained 3 times more cadmium (average 1738 mg/kg d.w.) than the corresponding specimens from the mountain population (average 516 mg/kg d.w.). This result suggests more root-to-shoot transport of cadmium in the waste-heap population than in the mountain population.

CADMIUM CONCENTRATION IN LEAVES

During 10 days of treatment with cadmium (4 mg/dm^3), it was transported from roots to shoots (Tab. 1). In the waste-heap population, 17% of the cadmium taken up by the plants was transported to the leaves, 11% in the mountain population. This result is in agreement with the data for roots, and points to more intensive of root-to-shoot transport of cadmium in the waste-heap population than in the mountain population. In the waste-heap population the largest amounts of cadmium were accumulated in the oldest (withering) leaves, 242 mg/kg d.w.; the mountain population had only 51 mg/kg d.w. cadmium in those leaves (Tab. 1).

Further experiments were conducted to ascertain whether the waste-heap population of *B. laevigata* is able to intensively accumulate cadmium in withering leaves after only short incubation (to eliminate the factor of leaf aging). Three cadmium concentrations were used: 30, 60 and 120 mg/dm^3 Cd. Incubation for 96 h with 120 mg/dm^3 cadmium proved toxic to the plants. The results are presented in Figure 1.

It was found that increased accumulation of the metal in the oldest leaves of the waste-heap population occurred at the two lower doses of cadmium used and was double that of mountain population. This relationship was not found at the highest (lethal) concentration (Fig. 1). This result points to a significant difference in root-to-shoot translocation of cadmium, depending on the age of the leaf, the population of *B. laevigata*, and the cadmium dose.

CADMIUM LOCALIZATION IN TISSUES

The use of a histochemical method of locating cadmium in the tissues and cells of *B. laevigata* made it possible to trace the path of cadmium in these plants.

The largest amounts of cadmium were found in the lower part of the root (Figs. 2, 3), the part that had been submerged in the cadmium-spiked medium. Cadmium was found on the surface of the root despite careful rinsing of the plants after incubation, as well as in internal root tissues (Figs. 2, 3). It was present in root apical meristems (Fig. 2) and the higher parts of the roots (Fig. 3). The presence of cadmium in the root hairs (Fig. 4) points to the route by which it is taken up by plants (as with other trace and macroelements). Cadmium was transported to the aboveground parts of the plant mainly through vascular bundles. This is indicated by its presence in the vascular bundles of petioles and leaves (Figs. 5, 6). Cadmium precipitates were also found in parenchyma surrounding the bundles, as well as in that of leaves (Fig. 6). Cadmium deposits were present in leaf epiderm cells (Figs. 7, 8) and in the hairs thickly covering the leaf surface (Figs. 9, 10). The largest amounts of cadmium in leaves were found in the vascular bundles, epidermis and hairs.

No qualitative but only quantitative differences were found in the distribution of cadmium in the leaves of the waste-heap and mountain populations of *B. laevigata.*

DISCUSSION

As mentioned earlier, *B. laevigata* occurs both in the Alps and in Poland on soils rich in heavy-metals; hence this species may possess specific mechanisms of tolerance to these elements (Wenzel and Jockwer, 1998; Miechówka, 2000; Mesjasz-Przybyłowicz et al., 2001; Szarek-Łukaszewska andNiklińska, 2002).

In earlier studies we found that the waste-heap population of *B. laevigata* is characterized by elevated tolerance to Cd, Zn and Pb. We also found genetically established morphological differences between the two populations. The waste-heap population has lighter-colored leaves and higher leaf hair density. The mountain population, on the other hand, has dark green leaves with a small number of hairs. Its leaves are thicker than those in the waste-heap population (Wierzbicka and Pielichowska, 2004).

The study described here on the growth of plants in cadmium-spiked liquid media shows when fully available the metal was taken up by the plants in large amounts. Its translocation to shoots was observed. Waste-heap plants transported more cadmium to their shoots than the mountain population did.

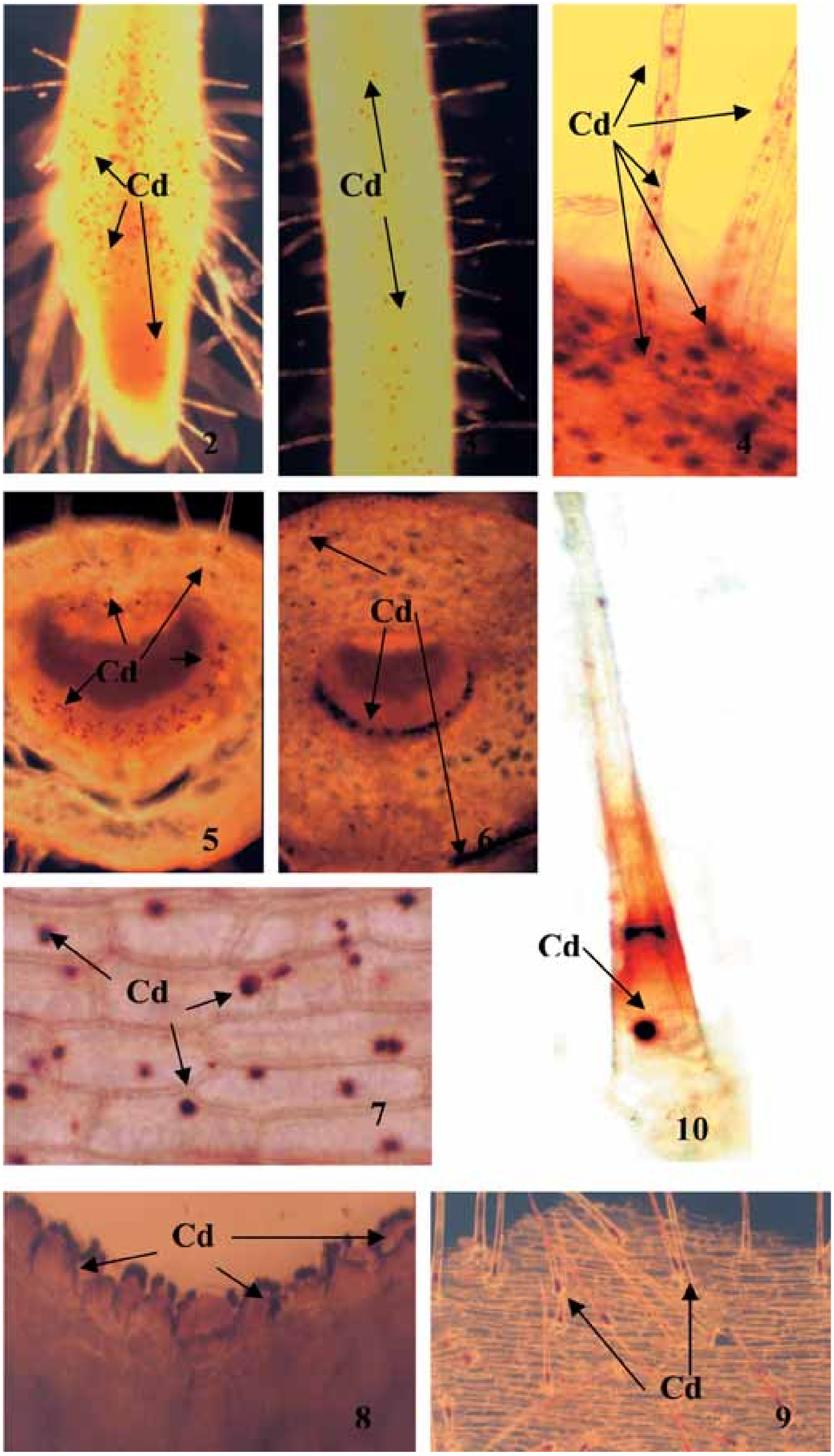
Earlier field studies (Szarek-Łukaszewska and Niklińska, 2002) on cadmium content in *B. laevigata* plants under natural conditions reported much lower amounts than given here (Tab. 2). The amounts of cadmium detected in Alpine populations of *B. laevigata* (Wenzel and Jockwer, 1998) were also much lower than those reported here (Tab. 2). These differences are probably due to the full availability of cadmium in hydroponic culture.

This comparison shows that this species, and its waste-heap population in particular, has considerable cadmium-accruing potential and can transport this metal into shoots; this ability is dependent on the availability of cadmium and also on the population.

On the basis of earlier studies, *B. laevigata* was classified as a cadmium hyperaccumulator because the plants growing in the Alps were found to store this metal in leaves in the amount of 78.3 mg/kg d.w. (Wenzel and Jockwer, 1998). Studies in Poland on a calamine waste heap rich in heavy metals did not show such high amounts of cadmium in *B. laevigata* shoots as in the Alpine plants (Szarek-Łukaszewska and Niklińska, 2002).

On the other hand, our studies conducted on two geographically isolated populations of this species occurring in Poland at a calamine waste heap near Olkusz and in the West Tatra Mts, described from those two stands for over 130 years, showed that this species is able to transport significant amounts of cadmium to its aboveground parts. This trait is correlated with the amount of cadmium in the soil from the site providing the plant seeds used in the experiment. Greater transport to shoots was observed in plants from the waste heap, where there is ~189 mg Cd/kg d.w. in the soil and where its availability is 2.3% (Szarek-Łukaszewska and

Figs. 2–10. Visualization of cadmium by dithizone complex (diphenylthiocarbazone complex) in tissues and cells of *B. laevigata* roots and shoots. Incubation 30 days at 3 mg/dm^3 Cd (M – mountain population; W – waste-heap population). **Figs. 2–3.** Cadmium in root apex and root hair zone; population W. × 250. **Fig. 4.** Cadmium in root hairs; population M. × 1000. **Fig. 5.** Cadmium in vascular bundle (phloem) of leaf, parenchyma cell, and leaf hair, cross section; population W. × 250. **Fig. 6.** Cadmium in cells surrounding vascular bundle of leaf and parenchyma cells of leaf, cross section; population M. × 250. **Fig. 7.** Cadmium on surface of leaf epiderm cells, cross section; population M. × 500. **Fig. 8.** Cadmium in epiderm of leaf, leaf surface; population M. × 500. **Fig. 9.** Cadmium in hairs thickly covering leaf surface, tangential section with leaf surface; population W. × 250. **Fig. 10.** Cadmium in hairs on leaf surface; population W. × 1000.


Cd
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4
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Cd
9

TABLE 2. Concentration of cadmium in the aboveground parts of *B. laevigata* from the Alps (Wenzel and Jockwer, 1998), the waste heap in Bolesław near Olkusz (Szarek-Łukaszewska and Niklińska, 2002), the Tatra-Mountains, and waste-heap populations in hydroponic culture treated with cadmium (30 mg/dm^3) for 4 days

Natural populations		Hydroponic culture	
Alpine population	Waste-heap population from Bolesław near Olkusz	Waste-heap population	Mountain population
78.3 mg /kg d.w.	Up to max. 20 mg /kg d.w.	Up to max. 647 mg /kg d.w.	Up to max. 338 mg /kg d.w.

Niklińska, 2002). Lower transport was observed in Tatra Mts plants, where the soil of the Jaworzynka Valley contains ~1.4–6.1 mg Cd/kg d.w. (Godzik, 1993; Szarek-Łukaszewska and Niklińska, 2002).

This dependence was particularly marked in the difference in the two populations' ability to accumulate cadmium in the oldest leaves. The oldest leaves of the waste-heap population contained even twice as much cadmium as those in the mountain population of *B. laevigata*. This species' ability to accumulate significant amounts of heavy metals in dry leaves was observed and described in earlier reports (Godzik, 1993; Siedlecka et al., 2001).

The data presented here suggest significant differences in the transport of cadmium to leaves differing in age, suggesting that internal transport in the aerial parts of these plants is differentiated. This may be one of the plant's mechanisms of detoxifying metals, based on depositing toxic metals in the cells and tissues of old leaves and then eliminating the metals by shedding these leaves at the end of the growth season. As found in other species, accumulation of heavy metals in older (faded, dry) leaves involves the formation of metal-phenol complexes that are transported from the roots to aging leaves, which then are shed (Ernst et al., 1992). Adoption of such an explanation for *B. laevigata* requires further study, however.

Our results point to another interesting relationship, described here for the first time. The ability to accumulate cadmium in leaves depends on the *B. laevigata* population. Interestingly, this is not only a property of *B. laevigata*: in another study we found that *Armeria maritima* spp. *hallerii* populations exhibit a similar dependence in relation to Pb, Zn, and Cd (Szarek-Łukaszewska et al., 2003).

Our results indicate that both *B. laevigata* populations occurring in Poland, the waste-heap population in particular, have the potential to hyperaccumulate cadmium. This is indicated by the high concentration of it found in shoots (over 100 mg, in keeping with the definition of a Cd hyperaccumulator by Baker and Brooks, 1989). The difference between the waste-heap and mountain populations of *B. laevigata* in their ability to accumulate cadmium in leaves may result from a difference in the intensity of cadmium transport through cell membranes. As shown earlier by other authors, differences on the molecular level in plants hyperaccumulating zinc or cadmium are based on factors including the presence of specific membrane transporters (Hall, 2002; Małkowski and Kurtyka, 2003).

Our earlier studies also point to major differences in identification of a species as a hyperaccumulator. *B. laevigata* has been reported to be a thallium hyperaccumulator (Anderson and Brooks, 1999); studies on Alpine populations showed it to accumulate > 1.4% kg d.w. thallium. Our studies, however, point to quite the opposite (Wierzbicka et al., 2004). The waste-heap population from Bolesław near Olkusz exhibited an exceptional ability to prevent the accumulation of thallium in shoots. The soil of the waste heap was found to contain up to 78 mg Tl/kg d.w. and up to 321 mg Tl/kg d.w. was found in the tissues of other species growing on this site; only *B. laevigata* plants did not contain thallium in their tissues (Wierzbicka et al., 2004). These results also point to very large differences in the ability to hyperaccumulate metals between different populations of the same species. This means that the ability to hyperaccumulate metals should be attributed to a specific population, not to the entire species.

Another important observation pertains to the role of leaf hairs in detoxifying cadmium. The hairs on the leaves of *B. laevigata* protect against excessive evaporation of water. They also play an important role by accumulating and then probably eliminating the excess cadmium ions that were taken up. The waste-heap plants possess this ability in particular, since this population has a genetically established trait of greater leaf hair density than the Tatra Mts population (Wierzbicka and Pielichowska, 2004). The ability to accumulate zinc and cadmium was also found in the basal part of leaf hairs of *Arabidopsis hallerii* (Kupper et al., 2000); in that paper, these cells (in addition to parenchyma) were suggested to play an important role in hyperaccumulation of metals.

Both restriction of metal hyperaccumulation to a specific population and detoxification of cadmium through leaf hairs make *B. laevigata* a particularly valuable species for the study of metal hyperaccumulation mechanisms, which is of particular importance in the context of the potential use of this plant in soil phytoremediation.

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