ROLE OF DIFFERENT TAXA AND CYTOTYPES IN HEAVY METALS ABSORPTION IN KNOTWEEDS (*FALLOPIA*)*

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The invasiveness of non-native plants is determined by several characteristics. One of them is the ability to grow in conditions that are limiting for other plant species. In the study we compared three highly invasive plant taxa of the genus: *Fallopia japonica* var. *japonica* ($2n = 88$), *F. sachalinensis* ($2n = 44$), and their hybrid *F.* × *bohemica* ($2n = 66$) regarding their growth ability at localities with high content of Cd, Pb, and Fe in the soil and regarding their ability to take up the heavy metals into their tissues. The plant material was collected from contaminated sites. Concentration of particular heavy metals was measured in rhizomes, roots, and leaves. The aim of the study is to analyze the response of plants to this pollution and ability to accumulate metal in different tissues. The taxa do not differ in metal uptake, but a marginal difference in uptake of Pb was found between ploidy levels. In all taxa and ploidy levels the heavy metals were accumulated significantly more in roots and rhizomes. High concentrations of Cd were found in roots and rhizomes of all taxa. This result suggests a specific ability of *Fallopia* to take up heavy metals and a great phytoremediation potential.

invasive plants; *Reynoutria*; hybridization; polyploidy; heavy metal uptake

INTRODUCTION

The spread of invasive species is determined by their ability to survive conditions in a secondary distribution area, spread and rapidly increase the population number and thus increase the propagule pressure (Blackburn et al., 2011). Invasive species usually inhabit a wide range of habitats (Kalusová et al., 2012) and outcompete the resident vegetation (e.g. Bímová et al., 2004) due to their competitive ability or high resource acquisition (Shea, Chesson, 2002). Some invasive species are able to occur in environments where the resident vegetation is limited by environmental factors, such as a high level of disturbance, salinity or pollution (e.g. Yang et al., 2007; Mateos-Naranjo et al., 2011).

Heavy metals pollution is a consequence of human activity that affects all parts of the ecosystem. Species that colonize polluted environments are tolerant to pollutants or have developed mechanisms resulting in their avoidance. In general, the avoidance can be related to limited uptake of heavy metals from soil, sequestration of toxic metals from protoplasm or their elimination (Punz, Sieghardt, 1993). Heavy metals tolerance is built up during genetic, physiological, and ecological evolution of plants growing in natural metalliferous areas (Ernst, 2006).

Invasive species such as *Spartina densiflora* or *Solidago canadensis* have developed physiological mechanisms to tolerate or avoid heavy metals and are able to germinate (Curado et al., 2010), grow, and spread in polluted soils (Nieva et al., 2001; Yang et al., 2007). Some authors found differences in tolerance on population (Nieva et al., 2001) or ecotype (Alvarez et al., 2009) levels that could evoke the appearance of rapid evolutionary changes in a secondary distribution area. However, results of some recent studies (Mateos-Naranjo et al., 2011) support the earlier views that invasive species, like other metal-tolerant plants (*Phragmites australis*, *Typha latifolia*, *Glyceria fluitans*), show no differences between populations or ecotypes in response to

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heavy metal pollution (McCabe et al., 2001; Mei et al., 2002; Ye et al., 2003; Matthew et al., 2004; Mateos-Naranjo et al., 2011). With regard to such biases, further investigation in this field is required to understand the mechanisms of plant response to soil contamination.

The *Fallopia* genus (*Fallopia japonica*, *Fallopia sachalinensis,* and *Fallopia × bohemica*) are among the most invasive aliens in both Europe and North America. The species from the genus *Fallopia* have intensive rhizomatous growth and massive vegetative regeneration abilities (Bímová et al., 2003) from stems and rhizomes. Brock and his colleagues found that a new plant can be created from a rhizome segment about 0.7 g in weight (Brock et al., 1995). Sexual reproduction is also present in the non-native range by hybridization, which can result in arising of new hybrid combinations that are better suited to the new environment (Bailey, 2003; Bailey et al., 2007). The success of the *Fallopia* genus is connected with its easy development in various environmental conditions. The taxa differ in habitat preferences in their native ranges. *F. japonica* is a plant of forest edges and river sides, but occurs in disturbed areas, man-made habitats or at roadsides, too (Bailey, 2003). The species is able to tolerate salinity (Richards et al., 2008) and the dwarf form (var. *compacta*) is a perennial pioneer species in the lava fields on Mt. Fuji (Adachi et al., 1996; Mariko et al., 2003). *F. sachalinensis* is a species of riverbank vegetation (Beerling et al., 1994) and the distribution of the hybrid is not well known in the primary distribution area. In the secondary distribution area all three taxa invade both natural and man-made habitats in Central Europe (Mandák et al., 2004). They are common along rivers, roads or motorways, dumps, deposits, and alongside railway tracks. In man-made habitats they often spread to waste dumps, spoil heaps or sludge pools. Generally, the taxa are able to grow in diverse soil types with various pH ranges and nutrient content (Beerling et al., 1994) and in habitats with different disturbance regimes (Bímová et al., 2004). However, it has yet to be properly examined whether pollution of soils by toxic metals interacts with their invasive abilities. The ability to grow in big cities was studied in *F. japonica* var. *japonica* and *F. × bohemica* (Soltysiak et al., 2011). Based on previous results we examined different types of soil contamination, different ploidy levels, and all three invasive taxa in this study. We conducted a meta-analysis of all (already published and newly obtained) data to (1) examine the differences between the three taxa in terms of their ability to survive in contaminated soils, (2) assess the influence of the ploidy level, and (3) find the differences in heavy metal uptake and accumulation, in particular in plant tissues. We tested the hypotheses whether the content of heavy metals is different (1) in particular taxa, (2) in particular ploidy levels within and between taxa, and (3) in above- and underground parts of plants.

MATERIAL AND METHODS

Study species

The study incorporated all three invasive taxa of the genus *Fallopia*: *Fallopia japonica* (Houtt.) Ronse Decraene var. *japonica*, *Fallopia sachalinensis* (F. Schmidt ex Maxim.) Ronse Decraene, and their hybrid *Fallopia × bohemica* (Chrtek et Chrtková) J.P. Bailey. The three taxa localities with suspicion of soil contamination were selected on the basis of published data (Kubík, Poláková, 2009) and the characteristics of the site (dump, roadside, etc.). The *Fallopia* genus contains several ploidy levels and different genetic variability within the taxa. *Fallopia japonica* (in this study only var. *japonica* was used) is octoploid ($2n = 88$), genetically uniform and reproduces in a purely vegetative way. In Europe there is only one female clone of *Fallopia japonica* (Mandák et al., 2005). For this reason, plant samples were collected from only one *Fallopia japonica* stand of each locality. *F. sachalinensis* occurs in three ploidy levels ($2n = 44, 66, 88$), but only tetraploid and octoploid plants were used in the study. The samples were collected in respect to the above mentioned variability, so just in stands of known chromosome numbers. The hybrid *F. × bohemica* shows the highest genetic and ploidy levels variability (Mandák et al., 2005). Hexaploid ($2n = 66$) and octoploid ($2n = 88$) plants were used in this study. The samples of the hybrid were taken from three different clumps at each locality and were analyzed separately. The genotype uniformity of samples was checked by the isosyme analysis using the method employed by Mandák et al. (2005). In all cases the uniformity of samples was affirmed and thus only one plant sample from each locality was used for heavy metal analysis.

Plant sampling

The selected sampling localities were situated in man-made habitats of Central Bohemia (in Prague and its vicinities) covered with ruderal vegetation. In total 46 samples were collected for all three taxa and ploidy levels together. The rest of samples was taken along the streets and in the parks of Wroclaw (2 samples for *F. japonica* and 2 samples for *F. × bohemica*) (Soltysiak et al., 2011). The list of localities is given in Appendix 1.

Fifty plant samples were analyzed in total: *F. japonica* ($2n = 88$), 10 samples; *F × bohemica* ($2n = 66$), 8 samples; *F × bohemica* ($2n = 88$), 2 samples; *F. sachalinensis* ($2n = 44$), 3 samples;

Appendix 1. List of localities

No.of sample	Sampling area		Species	Ploidy level
1	Roadside (Wrocław, Działkowa street)	PL	J	88
2	Undeveloped area (Wrocław,Tramwajowa street)	PL	J	88
3	Roadside (Praha, Rozvadovská spojka street), site 1	CZ	J	88
4	Roadside (Prague, Dejvicka street)	CZ	J	88
5	Car Prak (Prague, Kamýcká street)	CZ	J	88
6	Roadside (Prague, Podbaba street)	CZ	J	88
7	Car Park Hotel (Benešov, highway to České Budějovice), site 2	CZ	J	88
8	Roadside (between Prague and Horoměřice village), site 1	CZ	J	88
9	Roadside (between Prague and Horoměřice village), site 2	CZ	J	88
10	Roadside (Praha, Rozvadovská spojka street), site 2	CZ	J	88
11	Car Park Hotel (Benešov, highway to České Budějovice), site 1	CZ	B	66
12	Car Park Hotel (Benešov, highway to České Budějovice), site 2	CZ	B	88
13	Roadside (Prague, Holešovičkách street), site 1	CZ	B	66
14	Car park (Wrocław,Paczkowska street), site 1	PL	B	66
15	Undeveloped area (Wrocław,Orzechowa street)	PL	B	66
16	Roadside (Mělník, road N. 9), site 2	CZ	B	88
17	Roadside (Praha, Rozvadovská spojka street), site 1	CZ	B	66
18	Roadside (between Prague and Horoměřice village), site 1	CZ	B	66
19	Roadside (between Prague and Horoměřice village), site 2	CZ	B	66
20	Roadside (Praha, Rozvadovská spojka street), site 2	CZ	B	66
21	Roadside (Mělník, road N. 9)	CZ	S	44
22	Car Park Hotel (Benešov, highway to České Budějovice)	CZ	S	44
23	Roadside (Mělník, road N. 9), site 2	CZ	S	44
24	Roadside (Mělník - Vehlovice, road N. 9)	CZ	S	88
25	Car Park Hotel (Benešov, highway to České Budějovice), site 2	CZ	S	88

F. sachalinensis ($2n = 88$), 2 samples. The unbalanced design was taken due to different frequency of presence of taxa and ploidy levels within taxa (M a n d á k et al., 2004).

Soil sampling

Samples of subsurface soil reaching to the depth of 15 cm were collected, placed individually into plastic bags, and subsequently transported to the laboratory. Heavy metal content was determined. The number of the soil samples differed with respect to taxa. In *F. japonica* and *F. sachalinensis* localities only one soil sample was collected in the stand. In the hybrid, 3 soil samples per the stand were collected, i.e. 9 samples from the locality. This irregular design was adopted due to high genotype variability in the hybrid (see above). In the case of genetically non-various stands of the hybrid only one randomly chosen sample of soil was used in the final analysis. Consequently, for each plant sample one soil sample was used to compare the amount of heavy metals in soil and plant tissues.

Heavy metal concentration analysis

The method employed by S o l t y s i a k et al. (2011) was followed. Samples of soil, roots, rhizomes, and leaves collected at natural localities were air-dried, dried until constant weight at 105°C, and digested in HNO_3. An atomic absorption spectroscopy (AAS) was used to determine Cd, Pb, and Fe concentrations. All samples were treated separately as individual parts of the plants can accumulate metals differently (stems were not analyzed).

Statistical analysis

The differences in heavy metal content between the taxa, ploidy levels, and under- and above-ground plant parts were estimated using the analysis of covariance. The data were first tested for normality with the Shapiro-Wilcoxon test and for homogeneity of variances with the Brown-Forsythe test. Data were approximated by normal distribution. For each explanatory variable a separate test was conducted because

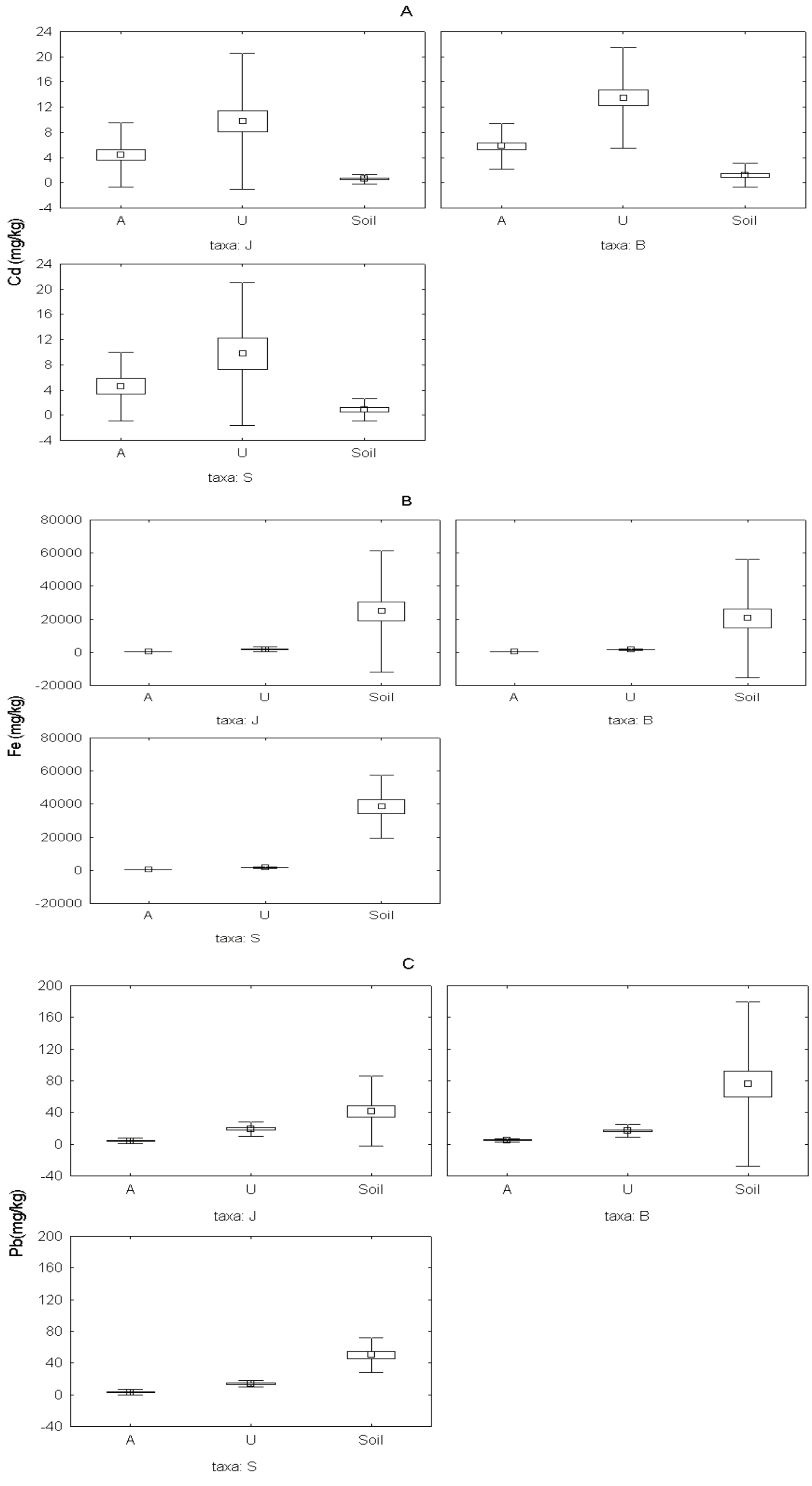

Fig. 1. Metal content (A – Cd, B – Fe, C – Pb) in three invasive Fallopia taxa. Differences between contents in above (A) and under (U) ground parts of plant and soil are shown. J – F. japonica var. japonica, B – F. ×bohemica, S – F. sachalinensis. Middle point – mean, box – SE, whiskers – SD.

Table 1. Results of ANCOVA for taxa, ploidy level and above-/underground part of plant representing explanatory variables for three heavy metals (Cd, Fe, Pb) concentration (mg/Kg). the concentration of particular metal in soil was used as covariate.

Heavy metal	Cd				Fe				Pb			
	F	DF	p		F	DF	p		F	DF	p	
Taxa	0.91	2, 46	0.42	ns	0.061	2, 46	0.94	ns	0.67	2, 46	0.51	ns
Ploidy level	0.67	2, 46	0.51	ns	0.021	2, 46	0.98	ns	3.96	2, 46	0.026	*
Plant part	33.23	1, 47	<0.0001	***	154.45	1, 47	$<10^{-7}$	***	137.67	1, 47	$0<10^{-7}$	***

Table 2. Heavy metal concentration in soil, aboveground and underground part of plant. Values are mean ± SE of 25 replicates across taxa and ploidy levels.

Heavy metal concentration (mg/kg)	Cd	Fe	Pb
Soil	0.90 ± 0.109	25836.56 ± 245.91	57.24 ± 5.36
Aboveground part of plant	5.01 ± 0.463	320.00 ± 15.60	4.34 ± 0.32
Underground part of plant	11.25 ± 1.013	1684.00 ± 107.71	17.33 ± 0.87

of the unbalanced design of the study. Significant test results were followed by a HSD test for an unequal number of samples. The data were computed using STATISTICA software (Version 9.0, 2012).

RESULTS

The analysis of metal concentrations in plant tissues revealed few differences between plant parts and taxa, even though the concentrations of a particular heavy metal magnitude showed a high variability among samples. The high variability was found both in the soil and plant samples (Fig. 1). Metal concentrations of soils were on the average much higher than the natural ranges according to Allen (1989): 0.03–0.3 $\mu g \cdot g^{-1}$ ($mg \cdot kg^{-1}$) for Cd, 2–20 $\mu g \cdot g^{-1}$ for Pb, and 50–1000 $\mu g \cdot g^{-1}$ for Fe.

In the majority of plant samples a higher than natural metals content was found. Normal values accepted for plants were: 0. 01–0.30 $\mu g \cdot g^{-1}$ ($mg \cdot kg^{-1}$) for Cd, 40–5000 $\mu g \cdot g^{-1}$ for Fe, and 0.05–3.0 $\mu g \cdot g^{-1}$ for Pb (Allen, 1989). The metal content was significantly higher in underground parts of the plants (roots and rhizomes) in all taxa and ploidy levels (Table 1), but the taxa did not respond differently to metal exposure. Between the ploidy levels a significant difference was found only in Pb concentrations. Pb concentrations in tetraploid plants were significantly lower than in hexaploid and octoploid plants (means ± SE: $2n$ =44 – 8.041 ± 1.22, $2n$ = 66 – 12.15 ± 0.74, and $2n$ = 88 – 10.68 ± 0.56; Unequal N HSD test: between MSE = 8.87, DF = 43, P = 0.027). The result could be blurred by a significant influence of covariate, thus different concentrations of Pb in soils.

Mean concentrations of metals were different in above-ground and underground tissues in the case of all the studied heavy metals (Table 2). A high uptake and significant accumulation of Cd in underground parts was found in all taxa and ploidy levels. The mean concentration of Cd is 10 times higher in plant underground tissues than in soils and 2 times higher in comparison with its concentration in leaves (Table 2, Fig. 1).

DISCUSSION

Urban soils are often characterized by high concentrations of heavy metals that create difficult conditions for most plants growing there. Therefore, an adaptation to stress caused by heavy metals can facilitate invasion of plants in anthropogenic areas (Yang et al., 2007). Japanese knotweed was noted on soils contaminated by heavy metals both in its secondary and native range of distribution. Nishizono et al. (1989) reported *Polygonum cuspidatum* (*Fallopia japonica*) from heavy metals (Cu, Zn, and Cd) polluted areas of Japan. In the Czech Republic it was observed on spoil heaps and near sludge pools with extremely high levels of anthropogenic pollution (personal observations). The investigation has shown that the species is able to grow and prosper on contaminated soils in Central Europe. Knotweed taxa have spread on soils with higher than natural content of Cd, Fe, and Pb. The taxa are growing vigorously under such conditions and are even able to accumulate heavy metals in their tissues.

Higher plants are characterized by various abilities to distribute and accumulate heavy metals. They are able to accumulate toxic metals in roots and rhizomes and restrict metal transfer into the above-ground parts (metal excluders), or they can gather heavy metals in above-ground parts, particularly in leaves (metal accumulators). Some species are heavy metal indicators and accumulate metals in shoots with the amount reflecting their content in soil (Punz, Sieghardt, 1993; M e m o n et al., 2001). Heavy metals (Cu, Cd, and Zn) accumulation by *Fallopia japonica* is known from its native range (N i s h i z o n o et al., 1989). The metal tolerance in *Fallopia* taxa could be caused by metabolic processes in rhizomes and roots (Cu–binding proteins presence) (K u b o t a et al., 1988). The results of the study are consistent with those of the previous studies from the native distribution area (K u b o t a et al., 1988) and with our own previous research (S o l t y s i a k et al., 2011). The concentration of all the studied heavy metals (Cd, Fe, Pb) was significantly higher in underground parts of plants in comparison to leaves. It is known in high plants that metal-tolerant races show clearly greater root uptake of metals than non-tolerant ones (B a k e r, W a l k e r, 1990). However, the concentration of metals recorded in underground parts of all *Fallopia* taxa indicated that all these plants could have a great capacity for accumulating heavy metals in roots and rhizomes, even in concentrations above the toxic threshold for plants (A l l e n, 1989; K a b a t a - P e n d i a s, P e n d i a s, 2001). Similarly, a high heavy metal concentration ability was found in other invasive plants, e.g. in *Spartina* populations of the south coast of Spain (M a t e o s - N a r a n j o et al., 2011). In our previous studies we found a high ability of *Fallopia* taxa to concentrate Cr even in cases when Cr contents in soils were similar to natural contents (S o l t y s i a k et al., 2011). Also the extremely high amount of Cd in all plant tissues suggests that *Fallopia* taxa could be taken as metal hyperaccumulators and their phytoremediation ability (following A s h r a f et al., 2010) may be considered.

S u k o p p, S t a r f i n g e r (1995) reported that *Fallopia sachalinensis* can accumulate heavy metals in leaves and stems. From the results of the presented study the role of the taxa and ploidy level in the heavy metal uptake is not clear. Statistically significant differences were found in the concentration of Pb and the tetraploid plants showed lower ability to accumulate Pb than the hexaploid and octoploid plants. If we accept the difference as a meaningful result, we can conclude that different evolutionary origin of the particular taxa/cytotypes (S u d a et al., 2010) may be reflected in the physiological characteristics of plants, which could be revealed by the ability to concentrate heavy metals. These abilities could also be genotype specific, which could be indicated by a high variability in metal concentrations in the whole sample pool. The higher ability to uptake or accumulate heavy metals by the hybrid *F.* × *bohemica* was not confirmed.

CONCLUSION

The presented study was conducted on seminatural habitats of soils polluted by heavy metals in Central Europe. It shows a high ability of knotweeds to grow on such stands. Thus, the data presented suggest that if the metal tolerance is considered to be manifested as a physiological trait (M a t e o s - N a r a n j o et al., 2011), all studied members of the *Fallopia* genus show a high level of tolerance and ability to grow in polluted soils. The metal accumulation is high in underground tissues and is best visible on extremely high concentrations of Cd. The particular taxa do not show different accumulation ability despite high differences between genotypes and marginal differences between ploidy levels. In this context, the general use of knotweed as a phytoremediator for selected pollutants (Cr, Cd) should be discussed. Members of the *Fallopia* genus are highly invasive and even a small piece of rhizome can be a source of their invasion to new areas (B í m o v á et al., 2004). High invasiveness in spite of significantly higher ability to accumulate heavy metals in roots and rhizomes exclude these species from the family of useful and safe phytoremediators.

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