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REGULAR ARTICLE

Metal uptake by xerothermic plants introduced into Zn-Pb industrial wastes

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Abstract The dusty surfaces of post-flotation wastes contain high concentrations of toxic compounds and spread widely if appropriate vegetation is not introduced. It has been previously established that effective restoration of such waste areas are best met by xerothermic, mycorrhiza-assisted plants (Turnau et al. Plant and Soil 305:267–280, 2008). The aim of the current study was to improve phytostabilisation practices by gaining insight into the elements uptake in plants after their change of habitat. Total Reflection X-ray Fluorescence (TXRF) was employed to evaluate element concentration in the leaves of 23 plant species growing in the wild and on Zn-Pb waste. Higher levels of heavy metals (Zn, Y, As, Pb, Cu) in plants from tailings were usually accompanied by increased Ca concentration, suggesting a possible role of this element in detoxification mechanisms. Also, when compared to grassland specimens, plants from the tailings, exhibited potassium-deficiency. Thus, K-supplementation of the waste substrata should be considered to improve plant growth. Among all the introduced plants, three grass species (*Melica transsilvanica*, *Bromus inermis*, *Elymus hispidus*) and one legume (*Anthylis vulneraria*) were the most suitable for phytostabilisation. Heavy metal-accumulating properties of *Verbascum thapsus* need further investigation.

Keywords Phytoremediation · Heavy metals · TXRF · Mycorrhizal plants · Applied symbiosis

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Introduction

Intensive industrial mining has greatly contributed to environmental pollution in southern Poland. Post-flotation wastes originating from this particular kind of extraction are an unquestionable source of various biological hazards. As the dusty surfaces of the waste are rich in toxic compounds and spread readily to surrounding areas, most remediation practices focus on preventing erosion. Unfortunately, the deposited substratum, in addition to being toxic, is also hard to stabilise due to the poor water holding capacity and steep slopes. Furthermore, lack of nutrients and organic matter in the soils causes an assortment of difficulties when undertaking phytostabilisation (Strzyszcz 2003; Turnau et al. 2006; Mendez and Maier 2008; Dickinson et al. 2009; Robinson et al. 2009). These problems are usually solved by covering the waste surface with a layer of relatively healthy soil transported from another area and subsequent introduction of trees (e.g. *Populus* spp., *Pinus sylvestris*) and grasses (e.g. *Lolium perenne*). Although most of the plant species used are known to form mycorrhiza (or other symbiotic associations), no fungal inoculation is necessary. After a few years, especially if the area is not being irrigated, the soil cover is destroyed and the vegetation is lost—leaving bare industrial waste. Our previous research on plants and their symbiotic associations occurring spontaneously on the Zn-Pb waste in Chrzanów revealed that the most successful colonizers of such sites are species which originate from xerothermic grasslands (reviewed by Turnau et al. 2006). In 2004, 23 plant species, pre-cultivated in the presence of mycorrhizal inoculum, were introduced on experimental plots (Turnau et al. 2008). The introduced plants survived and to the naked eye, they did not differ much from individuals growing in the wild. The one exception was that many of the plant species growing on the waste reproduced mostly vegetatively. The selection of plants suitable for phytostabilisation must be carried out with great caution; e.g. the use of naturally occurring metal hyperaccumulators may lead to the introduction of toxic metals into the food chain. Although hyperaccumulating plant species can be useful in other modes of remediation (i.e. phytoextraction), in this particular case it is more economically and environmentally feasible simply to stabilise the substratum and to keep the metals in place. Unfortunately the amount of data concerning the extent to which elements are taken up by plants that are introduced into phytostabilised tailings is limited mostly to woody plants (Dominguez et al. 2008, 2010; Migeon et al. 2009). Our main aims in the present study were: (i) to discover and verify which plant species from xerothermic grasslands do not accumulate large amounts of toxic elements in shoots while growing on industrial wastes, and (ii) to reveal interactions between heavy metals and other elements that are altered following the change of habitats.

Methods

Experimental plots were established in autumn 2004 on the industrial waste of the ZG Trzebionka Mining Company located near Chrzanów (southern Poland, 30 km west of Kraków, N 50° 09′, E 19° 25′). The selected area was almost completely devoid of vegetation due to an accidental spill of sedimental pulp. The chemical composition of the tailing material is unfavourable for plant growth, as the carbonate content exceeds 75%. High concentrations of Ca^{2+} and SO_4^{2-} ions together with low concentrations of Na^+, K^+, Mg^{2+}, Cl^- and HCO_3^- ions are also typical for this site. Original tailing material contains no organic matter and is P- and N-deficient. The pH of the waste substratum is alkaline (pH 7.4), and it contains up to 15,000 mgkg^{-1} of Zn, 7,000 mgkg^{-1} Pb, 22,000 mgkg^{-1} Fe, 24,000 mgkg^{-1} Al_2O_3, 80 mg kg^{-1} Cd, 500 mgkg^{-1} As, and 200 mgkg^{-1} Cu (Orłowska et al. 2005).

Seeds of xerothermic plants were collected from dry calcareous grasslands (*Festuco-Brometea* class) located in Southern Poland. The species diversity and characteristics of the soil are given e.g. by Dzwonko and Loster (1998) and Bąba (2004). In the present study, seeds of the following plant species were collected: *Hieracium pilosella* L., *Festuca sulcata* (Hack.) Nyman, *Dianthus carthusianorum* L., *Veronica spicata* L., *Echium vulgare* L., *Fragaria viridis* Duchesne, *Elymus hispidus* (Opiz) Melderis (syn. *Agropyron intermedium* (Host) P. Beauv.) in Skołczanka (N 50° 00′ 48. 55″ E 19° 49′ 16. 21″); *Astragalus cicer* L., *Silene vulgaris* (Moench) Garcke in Krzemionki (N 50° 01′ 52. 03″ E 19° 57′ 28. 41″); *Seseli libanotis* (L.) W. D. J. Koch (syn. *Libanotis montana* Crantz) in Glanów (N 50° 18′ 47. 96″ E 19° 48′ 30. 54″); *Verbascum thapsus* L. and *Melica transsilvanica* Schur in Bukowska Wola (N 50° 20′ 55. 41″ E 20° 04′ 43. 95″); *Carex caryophyllea* Latourr. in Sławice Duchowne (N 50° 18′ 59. 96″ E 20° 04′ 10. 70″); *Bromus inermis* Leyss. in Chrzanów – Bertowa Góra (N 50° 08′ 55. 24″ E 19° 25′ 17. 00″); *Anthyllis vulneraria* L., *Plantago media* L., *Thymus pulegioides* L., *Brachypodium pinnatum* (L.) P. Beauv., *Potentilla cinnerea* Chaix ex Vill. (syn. *P. arenaria* Borkh.), *Inula ensifolia* L., *Cirsium pannonicum* (L.f.) Link, *Stachys officinalis* (L.) Trevis. in Kalina Lisiniec (N 50°33′94″ E20°17′ 97″). Plant epithets and authors used are in accordance with Flora Europaea (http://rbg-web2.rbge.org.uk/FE/fe.html). Among the plants mentioned, only *S. vulgaris* and *C. caryophyllea* turned out to be devoid of arbuscular mycorrhiza. Several species (A. *vulneraria, T. pulegioides, S. officinalis, M. transsilvatica, F. sulcata*) studied in the present paper were not investigated in our previous research (Turnau et al. 2008) because they were introduced into the tailings later. Moreover, several species that were included in previous studies were omitted here due to poor growth at the time of sampling.

The germinated seedlings were grown in non-sterile grassland soil mixed 4:1 v/v with industrial waste substratum. After 2 months, they were planted on the waste in rows (15 cm apart) whereupon they kept growing for almost 3 years. During this time, the vitality of the plants and their mycorrhizal status were monitored (Turnau et al. 2008). In October, 2007 green upper part of the leaves ($N=5$) were collected to measure metal concentrations; roots were left to allow further growth. In cases where brown, dead leaves were present, separate samples were collected. The leaves of the same species were also collected at the place of seed origin (as listed above).

The analysis of metal concentration in plant material was done using Total Reflection X-ray Fluorescence (TXRF module produced in Technical University of Vienna; Hołyńska et al. 1998). Plant samples were carefully washed in distilled water before analysis. Substratum samples, both from industrial wastes and xerothermic grasslands, were also collected for metal concentration analysis with the use of TXRF. Two different extraction preparation protocols were used (Hołyńska et al. 1998). According to the first protocol, samples were digested in Parr acid digestion bombs. 8 ml of nitric acid of Suprapur quality was used for approximately 500 mg of sample. The samples were incubated at 185°C for 8 h. The solution was colourless; minute amounts of SiO_2 residue were found. To analyze water-soluble elements, 10 g of soil was mixed with 100 ml of deionized water and shaken for 24 h. The supernatant was collected and used for analysis. 2 µl of sample were applied onto the clean quartz reflector and measured with a TXRF spectrometer equipped with a Mo X-ray tube. Quantitative analysis was performed using an internal standard (Se) with final concentration approximately 600 ppm for the soil and between 200 and 600 ppm for plant samples depending on substratum (see supplementary material). The exact amount and volume added varied depending on the exact weight of the sample. The original concentration of the standard solution was 1,000 ppm. The volume of added solution (v, in µl) was calculated with the use of the following formula: $v = f\ m\ b^{-1}$, where f and b were final and beginning concentrations respectively (in ppm) and m, the weight of the sample (in mg). The software Axil was used for calculations of fitting spectra and quantitative analysis. The detection limits for the plant samples were as follows (in mg/kg): K: 29; Ca: 18.7; Ti: 9.4; Cr: 5.2; Mn: 5.1; Fe: 3.2; Ni: 2.8; Cu: 2.2; Zn: 1.8; As: 1.6; Se: 1.2; Rb: 1.7; Sr: 1.7; Y: 1.9; Pb: 2.8.

The data on element concentration in substrata and plant tissues growing in polluted and unpolluted environments were compared using nonparametric Kruskall-Walis tests with the inclusion of Bonferroni correction. Data after logarithmic transformation were also analysed by principal component analysis (PCA) using correlation matrix in the CANOCO v. 4.5 software (Lepš and Šmilauer 2003) to explain the variability in element concentration in the substratum and plants.

The degree of linear relationship between two metal concentrations in leaves were calculated using the Pearson correlation including Bonferroni correction.

Results

Metal content in the waste substratum and xerothermic soil

Figure 1 shows two groups of points indicating individual substratum samples of similar metal concentration. The first axis divides the samples into those from the tailings and those from the xerothermic grasslands, and it explains 52.3% of metal composition variance. The tailing group differs from the other group by having significantly higher total concentration of Pb (20 times higher than the mean for all the

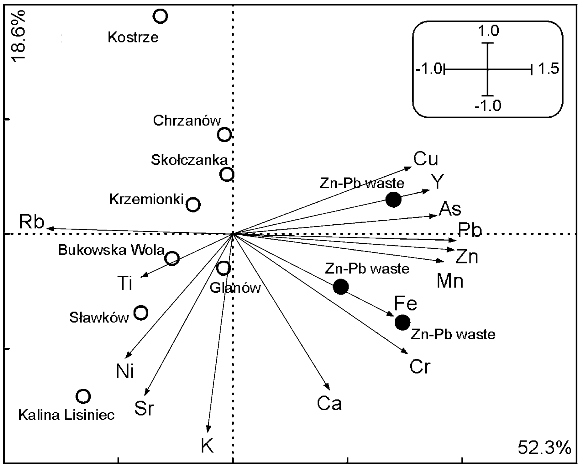


Fig. 1 Principal Component Analysis (PCA) ordination diagrams grouping sites with respect to chemical properties of substratum; numbers above the axis show percent of explained variance; black circle—Zn-Pb waste; open circle—sites of xerothermic grasslands

sites of xerothermic grasslands), Zn (50), As (11), Y (3), Cu (200), Mn (4), Cr (3), Fe (2) and Ca (4). (Fig. 1, Table 1). Only the mean concentration of Rb and Sr were higher in xerothermic soils. Although PCA analysis did not show differences in metal content in soils among localities of xerothermic grasslands, there were, nevertheless, some differences in the individual elements. The highest Pb content was found in Chrzanów and Glanów, As in Skołczanka, Zn in Chrzanów, Ni in Kalina Lisiniec, Cr in Bukowska Wola and Fe in Bukowska Wola and Chrzanów. Large differences were also found in macronutrients such as Ca and K. The highest content of Ca was found in Kalina Lisiniec (only slightly lower than Ca content in the tailings) while several fold lower Ca content was found in Kostrze and Bukowska Wola. The differences were not that large in the case of K, but soil K content was much lower than on the industrial wastes in several localities, such as Kostrze, Chrzanów and Skołczanka.

The water-extractable metal content turned out to be less variable (Table 2). Significantly higher availability of Zn, As and Pb were found in the waste substrata while other elements, including Fe, were more available in xerothermic soils (Table 2). This was particularly pronounced in the case of K and Ca which exhibited 22 and over 7-fold difference in availability in grassland soil when compared to the waste substrata.

Metal content in plant shoots

Metal concentrations in plants collected from xerothermic grasslands differed from those collected from the tailings. Plants growing on the tailings generally had greater concentrations of heavy metals (Zn, Y, As, Pb and Fe) and lower concentrations of the K than plants from xerothermic grasslands (Figs. 2 and 3).

Among all elements detected, Ca prevailed in the plant material. Its concentration ranged from 0.4% in *F. sulcata* to over 7% in *P. media*. Over half of the plant species contained more Ca when growing on the waste, and on average its amount was 12% higher for plants from the polluted area (Fig. 3). In the case of grasslands, Ca concentration was strongly correlated with Sr (r=0.94). Although the correlation remained statistically significant in plants from the Zn-Pb tailings, the r value decreased to 0.63 and, at the same time, other elements such as Mn, Zn, As, Y, Pb, Cr, Zn became significantly correlated with Ca (Table 3).

A similar situation was observed in the case of K and Rb (Table 3). These elements were more strongly correlated at the xerothermic grasslands than at the tailing. Half of the plant species from the tailings had significantly higher Rb concentration than those from the grasslands (Fig. 3). Significantly lower concentrations on the tailing were found only in *A. cicer*, *H. pilosella* (where Rb was not detected in samples from the tailings) and *S. officinalis*.

Mean Fe concentration (Fig. 3) in plants growing in the wild ranged from 100 to 850 $mg kg^{-1}$ and was positively correlated only with Ti (p<0.05). Mean Fe concentration in plants from the tailings exceeded 7,500 $mg kg^{-1}$(Fig. 3) and became significantly correlated with Mn, Pb, Y and Zn (Table 3). Among wild populations, *P. cinerea* and *Verbascum thapsus* were the most efficient accumulators of Fe (Fig. 3). *V. thapsus* was also very efficient Fe-accumulator (7.4%) on the waste, sharing this property with *C. pannonicum* (1.8%), *H. pilosella* (1.5%), *S. vulgaris* (1.2%) and *P. media* (1.1%).

High concentration of Zn (Fig. 3) was noted only in plants growing on the waste with 2- to 37-fold difference when compared to grassland specimens. In the case of the grassland *Inula ensifolia* was very efficient in Zn accumulation, while *V. thapsus* was the most effective at both sites. A very high accumulation of Zn on industrial wastes could be attributed to *C. pannonicum*, *P. media*, *V. spicata*—all easily exceed-

Table 1 Total element content (in mgkg^{-1}) in soil (N=6) of xerothermic grasslands and substratum of Zn-Pb waste; different letters indicate statistically significant differences, b.l.- below detection level. The results are presented as mean ± standard deviation

	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	As	Rb	Sr	Y	Pb
Kalina Lisiniec	9320±244 b	222000±282 b	351±99.5 b	70.1±9.5 ac	127±26.2 ac	8020±878 ac	90.0±41.4 b	b.l.	61.6±6.4 a	b.l.	17.0±5.0 ad	1040±130 b	b.l.	20.1±4.6 a
Glanow	7590±341 b	76600±334 ab	196±25.0 ab	93.0±9.9 ab	921±36.2 bc	16000±590 bc	41.4±4.9 bc	15.0±1.5 ac	204.3±5.0 ab	17.4±2.9 ab	51.2±3.2 bd	49.9±2.1 acd	21.2±2.5 bc	98.7±6.9 ab
Bukowska Wola	8400±768 b	7710±933 ac	364±123 b	124.5±6.6 bc	276±39.9 ac	21300±261 b	19.7±3.5 ac	16.1±2.3 ac	118±15.6 ac	b.l.	61.7±6.6 b	93.0±9.5 bc	19.3±2.3 ab	42.9±7.2 ac
Slawice	6880±540 bd	130000±11400 bc	89.8±11.9 a	73.7±21.3 ac	129±24.9 ac	9240±819 ac	57.2±9.0 bd	b.l.	81.8±12.5 a	11.0±1.6 a	29.0±2.7 bcd	532±36.6 b	5.9±2.3 ac	17.0±4.1 a
Skolczanka	2460±188 ac	78300±5950 ab	131±14.1 acd	69.3±13.3 ac	636±36.8 ab	13300±620 abc	40.6±4.0 bc	3.3±3.8 a	187±2.6 ac	47.6±2.7 ab	12.1±1.3 ac	27.8±1.8 a	15.8±1.7 ab	65.4±2.5 ac
Krzemionki	4040±65.9 ab	14500±4220 ac	252±13.0 bd	86.1±10.0 ab	376±18.5 ac	12800±131 abc	13.6±2.2 ac	34.4±2.6 bc	208±1.3 ab	5.6±1.5 a	28.3±1.2 bcd	150±31 bd	10.8±1.4 ac	87.9±2.0 ab
Chrzanow	2600±121 acd	36700±303 ac	153±4.9 ab	53.4±2.4 a	642±7.7 ab	9680±79.3 ac	20.8±2.6 acd	50.4±74.8 ab	478±2.2 bc	13.0±1.8 a	25.5±1.2 ab	33.8±0.8 ac	19.0±1.5 ab	158±3.4 bc
Kostrze	1050±38.2 a	3940±95.7 a	173±4.6 ab	43.4±4.8 a	105±5.0 a	7000±111 a	6.8±0.9 a	11.6±1.9 ac	139±2.1 ac	8.0±3.0 a	6.3±0.5 a	32.5±0.8 ac	5.4±0.9 a	46.8±9.0 ac
Zn-Pb waste	4490±562 bc	199000±23300 b	147±65.7 acd	211±87.3 b	1890±157 b	28500±18600 b	34.0±13.2 b	4990±6260 b	10700±896 b	202±153 b	b.l.	93.1±44.3 bc	44.4±18.9 b	1480±585 b

Table 2 Availability of elements (in mgkg^{-1}) in soil (N=3) of xerothermic grasslands and substratum of Zn-Pb waste measured after extraction in water; different letters indicate statistically significant differences, b.l.- below detection level. The results are presented as mean ± standard deviation

	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Sr	As	Y	Pb
Kalina Lisiniec	137±14.1 b	682±43.5 b	b.l.	1.0±0.5 ab	b.l.	5.0±1.1 ab	0.8±0.8	0.6±0.3 ab	b.l.	2.9±0.3 b	b.l.	b.l.	b.l.
Glanow	101±1.7 bc	337±2.6 ab	b.l.	0.7±0.1 ab	3.9±0.2 b	7.0±0.8 b	0.5±0.1	0.3±0.2 a	0.2±0.2 a	0.2±0.0 a	b.l.	b.l.	b.l.
Bukowska Wola	47.6±4.5 ab	190±3.1 ac	b.l.	0.7±0.1 ab	0.7±0.2 ab	19.9±2.6 b	0.3±0.1	0.4±0.0 ab	0.3±0.0 ab	1.0±0.1 ab	b.l.	b.l.	b.l.
Slawice	108±7.0 bc	358±4.0 ab	b.l.	1.1±0.3 b	b.l.	6.5±0.8 ab	0.4±0.1	0.6±0.2 ab	0.3±0.1 ab	1.8±0.0 ab	b.l.	b.l.	b.l.
Skolczanka	56.8±3.4 ab	410±7.6 bc	b.l.	0.9±0.0 ab	2.1±0.3 bc	4.5±0.3 ab	0.1±0.2	b.l.	b.l.	0.1±0.1 a	b.l.	b.l.	b.l.
Krzemionki	41.1±2.2 ab	293±2.6 ab	b.l.	1.1±0.5 ab	0.2±0.3 a	7.4±0.7 b	0.2±0.2	0.7±0.1 ab	0.3±0.0 a	1.5±0.5 ab	b.l.	b.l.	b.l.
Chrzanow	10.8±2.6 ac	419±148 ab	b.l.	0.9±0.1 ab	0.4±03 ac	6.2±0.6 ab	b.l.	2.1±0.1 b	1.1±0.1 ab	0.5±0.0 ab	b.l.	b.l.	b.l.
Kostrze	9.2±1.6 ac	187±3.8 ac	b.l.	1.1±0.2 b	1.2±0.2 ab	5.4±2.2 ab	b.l.	0.1±0.2 a	0.4±0.2 ab	0.7±0.0 ab	b.l.	b.l.	b.l.
Zn-Pb waste	6.2±3.0 a	92.0±3.3 a	0.3±0.1	0.3±0.2 a	0.4±0.2 ac	3.4±0.6 a	b.l.	0.5±0.2 ab	11.7±3.4 b	0.3±0.2 a	0.3±0.1	0.1±0.0	2.7±0.8

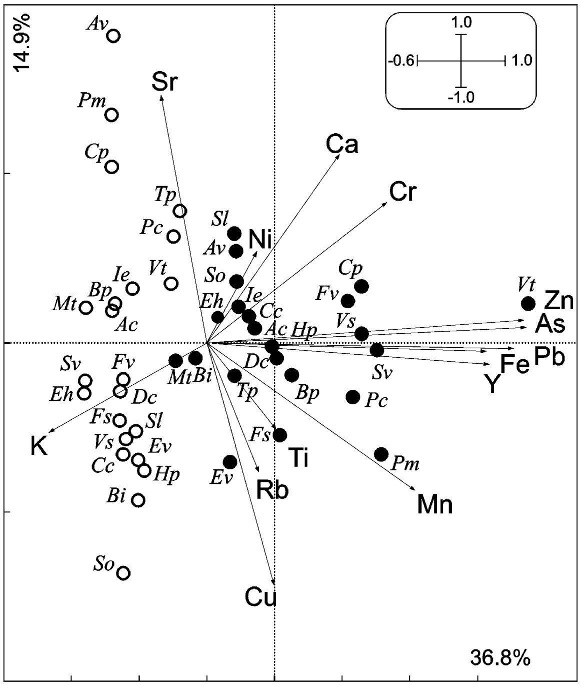


Fig. 2 Principal Component Analysis (PCA) ordination diagrams grouping sampling of plant species with respect to concentration of metals. Numbers above the axis show percent of explained variance; black circle—Zn-Pb waste, open circle—sites of xerothermic grasslands; *At Anthyllis vulneraria, Ac Astragalus cicer, Bi Bromus inermis, Cc Carex caryophyllea, Cp Cirsium pannonicum, Dc Dianthus carthusianorum, Ev Echium vulgare, Eh Elymus hispidus, Fs Festuca sulcata, Fv Fragaria viridis, Hp Hieracium pilosella, Ie Inula ensifolia, Mt Melica transsilvanica, Pm Plantago media, Pc Potentilla cinnerea, Sl Seseli libanotis, Sv Silene vulgaris, So Stachys officinalis, Tp Thymus pulegioides, Vt Verbascum thapsus, Vs Veronica spicata*

ing 0.12% Zn dry weight concentration characteristic for *S. vulgaris*. A few more cases of plant species with Zn concentration similar to *S. vulgaris* were noticed (Fig. 3).

Mn concentration in plants from xerothermic grasslands ranged from 10 to 450 $mgkg^{-1}$ with most plants not exceeding 100 $mgkg^{-1}$(Fig. 3) and its concentration was positively correlated with Pb only (Table 3). For leaves of plants grown on industrial waste, this range was much broader with the maximum reaching 868 $mgkg^{-1}$ and leaf Mn concentrations became correlated positively with leaf Ca, As, Cr, Fe, Pb, Y and Zn concentrations (Table 3). Less than half of plant species grown on industrial waste contained more Mn when compared to the wild individuals (Fig. 3). The highest Mn concentrations were found in *C. pannonicum*, *P. media*, *P. cinnerea*, *F. viridis* and *S. vulgaris*. On the contrary, the lowest Mn concentrations on wastes were shown in *D. carthusianorum*, *S. libanotis* and *S. officinalis* (Fig. 3).

Fig. 3 Concentrations of As, Ca, Cr, Cu, Fe, K, Mn, Ni, Pb, Rb, Sr, As, Y and Zn ($mgkg^{-1}DW$) in dry leaves of species collected from xerothermic grasslands (white bars) and from Zn-Pb tailings (black bars). Values are mean ± standard deviation (n=5 for all species); asterisk above two adjacent bars mean statistically significant difference at $p<0.005$; plant species abbreviations as in Fig. 2

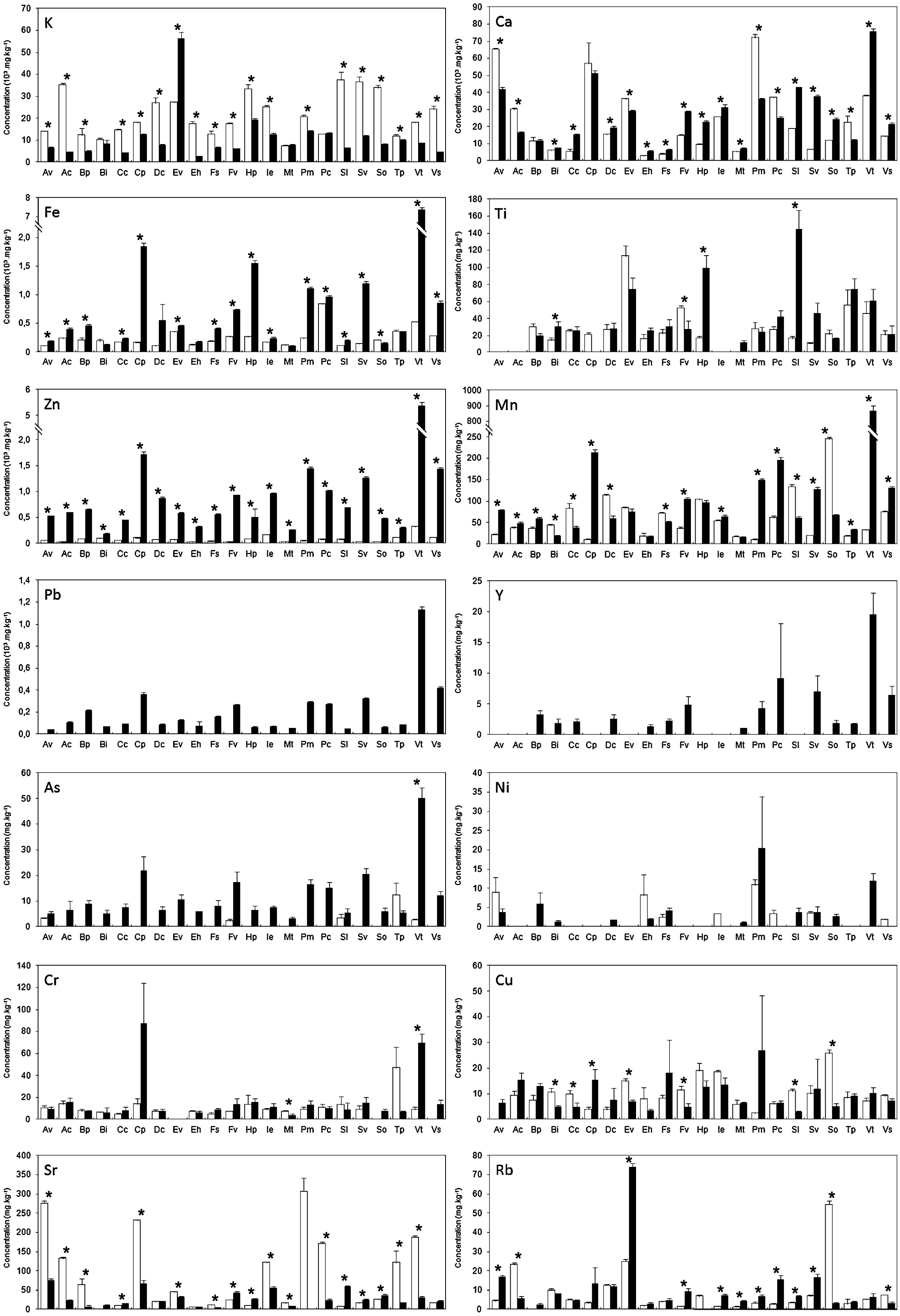
Pb was never detected in wild plants, while it was abundant in leaves of plants grown on the tailings. The highest concentrations of this element were found in *V. thapsus*, *V. spicata*, *S. vulgaris*, *P. media*, *C. pannonicum*, *F. viridis* and *B. pinnatum* (Fig. 3).

Y was another element detected only in leaves of plants grown on industrial waste; however, unlike Pb, Y was also present in grassland soil. The highest Y concentrations were noted in *V. thapsus* and *P. cinnerea* (Fig. 3).

Although Ti was not found in the water extract from soils, it was present in the tissues of almost all plants examined (Fig. 3). Leaves of most species did not exhibit significant differences in the Ti concentrations between sites of origin. The highest leaf Ti concentration was found in *E. vulgare* growing in the wild, while the most efficient in Ti concentration on the waste areas was *S. libanotis*. The latter plant showed 8 fold increase in Ti concentration following the change from unpolluted to polluted habitats.

Unlike Ti, high Ni concentration (Fig. 3) was not a usual feature for plants from either environment and even when present in the tissues, its concentration appeared to be site-independent. The highest mean level of Ni on both wild and contaminated sites was shown in *P. media* with two-fold difference between places of origin.

Cr and Cu were regularly detected in leaves from both waste and grassland plants (Fig. 3). The highest level of Cr among plants from the tailings was shown for *C. pannonicum* together with *V. thapsus*. In over 50% cases Cu concentrations in leaves from both sites showed no statistically significant differences. The case of *C. pannonicum* was exceptional—individuals from the waste had significantly higher Cu concentration than individuals from the wild; six other exceptional species exhibited the reverse situation.


K
Ca
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Av Ac Bp Bi Cc Cp Dc Ev Eh Fs Fv Hp Ie Mt Pm Pc Sl Sv So Tp Vt Vs

Table 3 Pearson correlations between elements (r values) in leaves of species collected from xerothermic grasslands and from Zn-Pb tailings; * $0.01<p<0.05$, ** $0.0005<p<0.01$, *** $p<0.0005$

Zn-Pb tailings													
	Ca	Cr	Cu	Fe	K	Mn	Ni	Pb	Rb	Sr	Ti	Y	Zn
As	0.76***	0.72***	0.27	0.93***	0.07	0.94***	0.48*	0.97***	0.21	0.11	0.11	0.86***	0.87***
Ca		0.71***	0.20	0.74***	0.19	0.78***	0.42*	0.69***	0.46*	0.63**	0.19	0.52**	0.79***
Cr			0.28	0.72***	−0.04	0.71***	0.19	0.69***	0.26	0.39*	−0.10	0.41*	0.73***
Cu				0.22	0.12	0.18	0.61**	0.24	0.09	−0.15	−0.16	0.06	0.21
Fe					0.05	0.98***	0.45*	0.94***	0.11	0.09	0.19	0.83***	0.92***
K						0.04	−0.02	−0.01	0.19	0.13	0.31	−0.11	0.01
Mn							0.47*	0.95***	0.14	0.14	0.15	0.86***	0.94***
Ni								0.48*	−0.02	−0.21	0.06	0.44*	0.47*
Pb									0.08	0.02	0.08	0.91***	0.90***
Rb										0.53**	−0.03	0.09	0.14
Sr											0.05	−0.17	0.17
Ti												0.11	0.04
Y													0.80***
Xerothermic grasslands													
	Ca	Cr	Cu	Fe	K	Mn	Ni	Pb	Rb	Sr	Ti	Zn	
As	0.14	0.86***	−0.08	0.14	−0.12	−0.17	−0.08	−0.11	−0.14	0.20	0.27	0.04	
Ca		0.16	−0.36	0.23	0.03	−0.31	0.49*	−0.18	−0.02	0.94***	0.13	0.19	
Cr			−0.16	0.14	−0.10	−0.23	−0.07	−0.04	−0.27	0.28	0.04	0.04	
Cu				0.00	0.55**	0.35	−0.36	−0.17	0.63**	−0.42*	0.17	−0.04	
Fe					−0.16	−0.13	−0.06	−0.13	−0.01	0.28	0.39*	0.19	
K						0.23	−0.09	−0.21	0.52**	−0.10	0.01	−0.14	
Mn							−0.34	0.64***	0.41*	−0.37	−0.10	−0.13	
Ni								−0.12	−0.22	0.51**	−0.22	0.05	
Pb									−0.14	−0.15	−0.16	−0.09	
Rb										−0.13	0.19	−0.09	
Sr											−0.02	0.11	
Ti												0.02	

TXRF revealed also the presence of Sr and Rb (Fig. 3) in plant tissues originating from both environments. However, while Sr was detected in both waste and grassland substrata, this could not be achieved for Rb using the water extraction protocol (Table 2). Despite this phenomenon, Rb was usually demonstrated to be present in plant tissues, but also it was shown to be positively correlated with K (as mentioned above) and negatively with Cu (Table 3). The highest concentration of Rb in wild plant specimens was found in *S. officinalis,* while on industrial wastes the highest level was detected in *E. vulgare*. Sr was present in both types of environment but its higher total and extractable level was found in plants growing on grassland soil. Only six plant species (*C. caryophyllea*, *F. vesca*, *H. pilosella*, *S. libanotis*, *S. vulgaris* and *S. officinalis*) contained more Sr when grown on industrial wastes. The others showed either no differences between sites of origin or had higher concentration when originating from grasslands. The highest Sr accumulations were found in *P. media*, *A. vulneraria* and *C. pannonicum*.

When comparing green and brown leaves of plants that were often seen in *E. vulgare*, *V. thapsus* from the wastes low K concentration was observed when the viability of plant tissues was lost. K decrease was associated in the brown leaves with the increased concentration of heavy metals. This was usually detected either in rosette leaves or in brown leaves which were remnants from the previous year (Table 4).

On average, a relatively low concentration of potentially toxic metals was characteristic for grasses such as *M. transsilvanica*, *B. innermis* and *E. hispidus*. The highest concentrations of such elements within tissues of this particular plant family was found in *B. pinnatum*. At the same time, grasses exhibited much lower concentration of Ca when compared to other species from both environments.

Discussion

As previously shown, selected plants originating from xerothermic grasslands (Turnau et al. 2008) are able to grow on industrial wastes and are tolerant to drought and high temperatures. Not only are harsh atmospheric conditions common for both environments, but there is an increased Ca concentration in the substratum. Calcicoles—plants growing on calcareous soils—have various adaptations which allow them to grow under such conditions (Marschner 1995). One such adaptationI is high calcium concentration in tissues. Members of Brassicaceae are known to be calciotrophic. As discussed by Kinzel and Lechner (1992), this feature is advantageous for osmoregulation on dry limestone sites. The Ca concentration in plants varies between 0.1% and 5% of dry weight (White and Broadley 2009). As shown in the present study, *Silene vulgaris*, a member of this group, contained 0.7% Ca when growing on unpolluted soil but was particularly effective in Ca accumulation on the wastes (almost 6 times more). Only 6 other plant species contained less Ca in shoots on unpolluted soils, but they increased the Ca concentration 1–3 times following the change of habitats.

Various mechanisms have been described as helping plants against Ca toxicity within tissues. One of them depends on synthesis of calcium-binding proteins in the cytoplasm (White and Broadley 2003) and further precipitation of calcium oxalate or carbonate within cell vacuoles. Oxalate producing plants accumulate such compounds in the range of 3–80% dry weight and its possible functions include Ca equilibrium, plant defense, tissue support, light reflection and most importantly detoxification (as reviewed by Franceschi and Nakata 2005; Nakata 2003; Nakata and McConn 2000). The latter function has been described for toxic or potentially toxic elements like Pb, Sr, Cd and Cu (Franceschi and Schueren 1986; Mazen and El Maghraby 1998; Yang et al. 2000; Choi et al. 2001;). The correlation between Ca and heavy metal concentration shown in the present paper suggests a possible role of oxalate in the detoxification of these elements, although other mechanisms cannot be excluded (Broadley et al. 2007). Interestingly, despite the lower availability of Ca and Fe in the waste substrata, many plants exhibited increased concentration of both elements. On the other hand, grasses, contained less Ca when grown on polluted sites. This result is in accordance with Broadley et al. (2003) and may be correlated with low levels of pectin in monocot cell walls. It may also indicate better performance of grasses in degraded habitats confirming the finding that they

Table 4 Element concentration comparison (in mgkg^{-1}) between green and brown leaves of *Echium vulgare and Verbascum thapsus* growing on Zn-Pb waste; different letters indicate statistically significant differences, b.l.- below detection level. The results presented as mean ± standard deviation

species/ element		K	Ca	Mn	Cr	Cu	As	Fe	Ni	Pb	Rb	Sr	Ti	Y	Zn
Echium vulgare	G	56200±3130 a	29200±527 a	76.3±5.9 a	b.l.	7.1±0.4 a	10.6±1.8 a	461±12.4 a	b.l.	127±3.1 a	74.3±1.7 a	32.9±1.2 a	75.1±12.9 a	b.l.	594±6.6 a
	B	3690±129 b	57300±1620 b	280±9.6 b	25.8±4.7	23.9±3.0 b	46.2±5.1 b	1990±95.7 b	7.4±1.7	1640±53.6 b	5.3±1.3 b	58.7±4.3 b	164.7±10.8 b	25.3±2.7	3210±88.3 b
Verbascum thapsus	G	8770±243 a	75800±1650 a	868±31.4 a	69.6±8.3 a	10.3±2.4 a	50.3±4.1 a	7370±135 a	11.9±2.0	1140±22.0 a	6.5±1.7	30.8±3.2 a	60.8±14.0 a	19.5±3.6 a	5370±137 a
	B	2750±248 b	116000±5300 b	1370±69.6 b	76.7±14.4 a	17.0±5.2 b	84.9±7.2 b	11500±127 b	b.l.	2440±121 b	b.l.	48.3±2.9 b	61.8±11.1 a	40.0±2.8 b	8780±424 b

are suitable for restoration of polluted areas (Ryszka and Turnau 2007).

Ca closely related to Sr. As shown by White (2001), both elements travel apoplastically across the roots to the Casparian band and then symplastically to the endodermis, where there is no competition or interaction between Ca^{2+} and Sr^{2+} for transport to shoots despite that they compete for uptake into root cells. In the present study, the uptake of these elements by plants from xerothermic grasslands is strongly correlated. The uptake of these two elements is, however, affected in plants from the tailings where lower Sr concentrations were found.

K is another macronutrient detected in plant and soil samples. It is generally accepted that the average concentration of this element necessary for growth equals approximately 1% of dry mass for shoots; however, the amount optimal for growth lies between 2% to 5% (Karley and White 2009). K uptake is associated with metabolic activity, plays an important role in water relations, and may affect photosynthesis at various levels. Deficiency of this element results in reduced growth and impaired translocation from mature to developing tissues (Karley and White 2009). According to the present study, K concentration in plants growing on grassland soils ranged from sufficient to optimal levels, with most species being closer to optimal. When grown on industrial waste, where K availability was strongly limited, most of the plants exhibited up to 6 times lower concentration of this macronutrient. This may explain why periodically some plants growing in this site showed loss of turgor and wilting. Thus, the use of K fertilizers could improve phytoremediation processes. However, any fertilizers should be used with special care, as the existence of plants on waste areas depend upon their symbiosis with arbuscular mycorrhizal fungi as indicated in pilot tests carried out under laboratory conditions (unpublished data). Extensive use of fertilizers may thwart restoration attempts involving the use of xerothermic grassland plants, for such agents can negatively affect symbiotic soil microbiota (Oehl et al. 2004). Arbuscular mycorrhizal fungi are not only essential for xerothermic plants development but also serve as a key factor in sustaining high diversity within plant communities on destroyed lands.

Rb, considered as ultra-trace element, was detected in the plant tissue. Rb behaves as an analog of K and thus was often used as radiotracer in several studies of K uptake (Epstein 1972). The concentration of Rb in leaves was even higher in plants on the tailings which might be influenced by the presence of other metals.

The comparision of vital, yellow and brown leaves of selected plant species grown on industrial waste has shown that the loss of leaf viability is accompanied by a decline in K concentration. As discussed by Marschner (1995), this might indicate K deficiency in the soil. This is supported by our observations of lower availability of K in the waste substratum. On the other hand, brown and yellow leaves had higher concentrations of heavy metals. This phenomenon was already described for *Biscutella laevigata* growing on Zn-Pb wastes in Olkusz, Poland (Pielichowska and Wierzbicka 2004) and other plant species at various locations (Baker 1981; Ernst et al. 1992; Hall 2002). Such behaviour is considered to act as a plant detoxification mechanism in that metals are deposited within fading leaves and then shed which enable the elimination of toxic compounds taken up by the roots.

Mn is the next element with possible limiting role in terms of plant nutrition on alkaline soils. Mn amounts of less than 20 ppm were shown to be detrimental for most of the plant species, 20–500 sufficient and quantities exceeding 500—toxic (Mengel and Kirkby 2001). Nevertheless, some plants were found to tolerate as much as double the mentioned toxic dose (Kabata-Pendias and Pendias 2001). In our study, this was apparently the case for *V. spicata* growing on the waste. Most of the other plant species from both sites contained "sufficient" quantities of this element. However, one should keep in mind that Mn concentration itself is not as important as the Fe/Mn ratio. According to the published data, values below 1.5 are accompanied by Mn toxicity symptoms, while values above 2.5 result in Fe toxicity and Mn deficiency symptoms (Kabata-Pendias and Pendias 2001). The Fe/Mn ratio is very high in most xerothermic plants. On the other hand, plants grown on the waste have either low or very high Fe/Mn rate (Fig). Neverthless, it is important to realize that Mn can form antagonistic interactions with several other elements such as Pb, Cd, P, Mg, Si, K etc. Further studies of this subject should lead to optimisation of restoration practices.

All of the plants used in the experiment at the Trzebionka waste belong to a group called "pseudometalophytes." These usually feature the metal exclusion strategy, which consists of avoiding metal uptake and restricting their transport to the shoots (De Vos et al. 1991). It is especially important in phytostabilisation practices that the plant species used actually exhibit low concentrations of toxic elements in shoots so as to exclude the possibility of entry into the food chain. According to the data presented here, the concentration of Pb, Zn and As in shoots of all plant species examined exceeded the amounts usually considered as suitable for animal food (Meers et al. 2010). Although such concentrations are common on industrial areas, we should still try to select species containing as little metals as possible. Among the species studied, the most useful were shown to be *Melica transsilvanica*, *Bromus innermis*, *Elymus hispidus* and *Anthyllis vulneraria*. The first three species belong to the grass family, once again confirming that grasses are one of the best choices among plants to be used in phytoremediation of industrial wastes (Ryszka and Turnau 2007). Those plants together with *A. vulneraria* when grown on industrial waste did not exhibit differences in photosynthesis parameters when compared to the specimens originating from xerothermic grasslands (Turnau et al. 2008). Contrary to *M. transsilvanica*, *B. innermis*, *E. hispidus* and *A. vulneraria*, a very effective accumulator of potentially toxic metals such as Pb and Zn was *Verbascum thapsus*. The possibility of using this plant species in phytoextraction attempts needs further examination, however.

Most plants originating from dry calcareous grasslands showed appropriate performance on the waste unless they were introduced as seedlings. This implies higher costs of introduction, but still lower than the costs of covering the waste surface with soil transported from other areas and constant site-watering. Plants from xerothermic grasslands are tolerant enough to heavy metals to survive in vegetative form and even to produce seeds. Already within this relatively short, 3-year experiment we were able to observe multiplication of one species (*V. thapsus*) with the use of self-produced seeds. Moreover, these seeds when tested in the laboratory were shown to be highly vital. For comparison, plants adapted to growth on wastes such as *Silene vulgaris*, produce seeds with a lower rate of germination than individuals grown on unpolluted site (Wierzbicka and Panufnik 1998). We expect that in the near future other plant species introduced on the waste will also establish sexual reproduction.

As reported previously by Turnau et al. (2008), except for such plants as *S. vulgaris* or *Carex* spp, it was not possible to grow nonmycorrhizal plants on industrial wastes. Thus, the role of mycorrhiza in metal uptake can be only estimated under laboratory conditions. Most of the plants used in the present study were usually strongly mycorrhizal. *S. vulgaris*, exceptional in this way was used for comparison in the present study since it is known to hyperaccumulate heavy metals (Broadley et al. 2007). Unfortunately its use in phytoextraction, similarly to nonmycorrhizal Brassicaceae plants, is limited due to the low biomass production (Meers et al. 2005; Liang et al. 2009). On the basis of data shown in the present study, there are several other plant species that can compete with *Silene* in metal uptake for phytoextraction purposes by simply having much higher yield. *Carex caryophyllea*, another species showing no AMF, showed the presence of unidentified fungi in roots (unpublished data) and was unexpectedly successful on the tailings. Further studies in this direction seem very promising. Also, *Anthylis vulneraria* should be taken into consideration for further research because this plant forms AM and rhizobial symbiosis that would be of importance in nutrient poor substrata. In some areas of the Zn-Pb tailings where, *Anthylis vulneraria* appears spontaneously, it can be devoid of one or both symbiosis, and the introduction of efficient inocula may improve its growth. Another gap in the present study is the lack of data concerning Cd concentration both in plant tissues and substratum. This is simply because detection of this particular element is impossible with the use of TXRF, because energy of cadmium L-line is in the range of 3 keV and lines characterisctic for K and Ca are placed in the same range.

Conclusions

This study shows differences in element composition between plants originating from xerothermic grasslands and the same species grown on industrial waste containing heavy metals. Significantly, the data does not originate from a laboratory trial, which may not explain the actual situation in the field. Among 23 species chosen in the present investigation, three members of grass family and one legume exhibited the lowest levels of heavy metals in shoots. On the other hand, several other species were shown to be good candidates for further studies of heavy metal accumulation properties. It seems very probable that there are many effective accumulators in the wild still waiting to be discovered. These plants may also turn out to be much more effective in phytoextraction technologies than the hyperaccumulating plants which are currently used.

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