

# Remediation of Soil-Free Grounds Contaminated by Zinc, Lead and Cadmium with the Use of Metallophytes

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## Abstract

Our work addresses the issue of remediation of grounds heavily contaminated with zinc, lead and cadmium using local plant species *Armeria maritima* and *Silene vulgaris*. The results of the field experiment showed that the cultivation of tested plant species caused a significant decrease of zinc and lead content in post-flotation waste material and both tested plant species could be applied to phytostabilisation of areas contaminated by heavy metals. Moreover, the use for remediation of the Olkusz settling ponds can be favourable for the restoration of native ecosystems on degraded areas.

**Keywords:** *Armeria maritima*, *Silene vulgaris*, heavy metals, phytoremediation

## Introduction

Mining and refining ores of non-ferrous metals cause a strong spot contamination of the environment. Management of heaps and ponds of post-flotation materials creates a serious problem regarding their contamination with heavy metals, especially in cases of the contamination reaching the level which prevent growth of a protective layer of vegetation by succession. In such situation these areas are prone to erosion of the ground and dispersion of the contaminants to adjacent aquifers and areas [1, 2]. Revegetation of degraded areas helps to stabilize the substrate and is a first step in decontamination through phytoremediation, leading to the reduction of heavy metal concentrations. For remediation of such sites there is a great need to identify native plant species capable of accumulating elevated amounts of heavy metals in their tissues, so-called metallophytes which

grow on naturally metal-enriched soils or contaminated with metals and accumulate them in quantities toxic for other plant species [3-5]. *Armeria maritima* (Mill.) Willd. and *Silene vulgaris* (Moench) Garcke are species frequently occurring in Europe [6]. These plants, characterized by population-specific tolerance to the metallic elements, have long been known as distinct subspecies, that is respectively *A. maritima* subsp. *halleri* and *S. vulgaris* subsp. *angustifolia*. They are the portion of vegetation resulting from the process of succession on the natural metal-bearing areas near Olkusz in Poland, and also on calamine waste heaps in this region. In those plants the mechanisms of resistance against the toxic influence of elevated heavy metal levels occurring in the substrate have been developed. Those mechanisms are the avoidance of metals, working at the organismal level or the metal-specific tolerance at the cellular level, such as enhanced chelation in the cytoplasm by metal-binding compounds, like phytochelatins or aminoacids which prevents metal from being transported across the plasma membrane [7-10].

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The idea of using metallophyte hypertolerance to heavy metals and their capacity to accumulate metals has been recently developed as an effective technique of phytoremediation of grounds contaminated with heavy metals [1, 3, 11-13]. Another interest is in the use of plants accumulating considerable amounts of heavy metals and growing in polluted areas as biomonitors of the metals, especially in cases of significant correlation between metal content in foliage and soil [14-16].

Specific aims of the paper were to:

- I. test if and to what degree *Armeria maritima* and *Silene vulgaris* possess the potential for remediation of substrates polluted with zinc, lead and cadmium,
- II. check which of the tested plant species is more useful for that aim and
- III. verify if tested metallophytes can be used as phytoextractors of heavy metals.

### Experimental Procedures

Studies were carried out on waste materials coming from an active settling pond disposed after zinc and lead ore flotations carried by "Bolesław" Mining and Metallurgy Plant in Bukowno near Olkusz. The experiment was established in 3 variants on 3 experimental plots: on the smoothed shelf surface of the post-flotation settling pond and 2 variants on the experimental plot of the Horticulture Faculty of Agriculture University in Kraków. Each experimental plot had a 10 m<sup>2</sup> surface.

As starting plant material, seed samples taken from the Olkusz populations of *Armeria maritima* (Mill.) Willd. subsp. *halleri* (Wallr.) Á. Löve & D. Löve and *Silene vulgaris* (Moench) Garcke subsp. *angustifolia* (Mill.) Hayek were used. Seeds were surface sterilized and sown on agar medium. Seedlings obtained in vitro were donors to get explants to initiate tissue culture in order to micropropagate plant material according to the protocol elaborated by Hanus-Fajerska [17]. Shoot cultures were tested for the content of heavy metals in biomass of respective samples. Contents of zinc, lead and cadmium were determined, which respectively amounted to 278.6 mg, 3.44 mg and 2.17 mg/kg (*Armeria maritima* subsp. *halleri*) and 606.4 mg, 174.6 mg and 3.5 mg/kg (*Silene vulgaris* subsp. *angustifolia*). The obtained shoots were rooted in vitro and afterwards acclimatized to ex vitro conditions in the greenhouse. Plant material (90 plants) obtained in this way was divided into 3 parts. Two parts (60 plants) were planted into plastic rings with diameter of 15 cm filled with the post-flotation waste material. 30 plants prepared in such a way were placed in substrate on the experimental plot situated on the shelf of the post-flotation settling pond. The second part (30 pieces) was planted in the soil on the experimental plot of the Agricultural University. The third part of plant material was planted into analogous rings filled with garden plant soil and placed also in the soil on the experimental plot of the Agricultural University. Both tested plant species were planted into the grounds in the middle of June and grew there for 4.5 months until the end of October 2007.

Before starting the experiment both substrates, i.e. post-flotation waste material coming from the settling pond and garden plant soil, were analyzed. Soil reaction was determined potentiometrically in soil–water 1:2.5 suspension. The levels of total nitrogen and carbon were analyzed in solid homogenized soil samples with the use of TOC–TN 1200 Thermo Euroglas apparatus. The level of organic carbon was calculated as a difference between total and inorganic carbon. In substrate samples collected before and after the tested plant cultivation, the total content of Zn, Pb and Cd were determined. Shoot cultures and plants taken after the experiment were rinsed with distilled water, roots and shoots separated and oven dried (105°C) for 12 hours and weighed. Plant material (2 g) was ashed in the muffle furnace at 460°C for 12 hours. The ash was finally dissolved in HNO<sub>3</sub> (1:2). The 2 g substrate samples were digested in the mixture of nitric and perchloric concentrated acids. The analyzes of the elements were performed in solutions with the use of an atomic emission spectrophotometer with inductively coupled argon plasma ICP-AES JY 238 ULTRACE using ICP multi-element standard solution IV (Merck). The accuracy of the analytical methods was verified with the reference to the certified reference material GSS-8 (GBW 07408 - State Bureau of Metrology, Beijing, China).

Studied heavy metal concentrations were analyzed on three replicated samples per plant part, species and variant of the experiment. Growth data were obtained from 30 samples of plants, above ground parts and roots separately per species per variant of the experiment. The results were subjected to one way STATISTICA 6.1, ANOVA analysis. *A posteriori* Fisher's test was used to determine the significance of differences between studied objects at significance level 0.05. In tables and figures values marked with the same letters are not significantly different at  $p < 0.05$ .

### Results

#### Content of Heavy Metals in Applied Substrates

Contents of zinc, lead and cadmium determined in post-flotation waste material and in garden plant soil before and after the experiment are presented in Tables 1 and 2. Contents of these metals in post-flotation waste material were extremely high, ranging from 15.894 to 14.005 mg Zn/kg, 6100–5044 mg Pb/kg d.m. and 86.1–78.1 mg Cd/kg d.m. before and after plant cultivation. The determined amounts exceeded several times limiting levels, defined by the Ministry of the Environment in the directive for industrial lands issued on 9 September 2002 [18] regarding soil and ground quality standards.

The cultivation of *Armeria maritima* and *Silene vulgaris* during one growing season caused a statistically significant decrease of zinc and lead content in post-flotation waste material used as substrate, both in the case of plant cultivated on the shelf of settling pond and plants grown in post-flotation material on the university experimental plot

Table 1. Selected properties of applied substrates before the experiment.

Substrates	org. C	tot. N	pH H <sub>2</sub> O	Zn	Pb	Cd
	[g/kg]			[mg/kg]		
waste material	0.68 <sup>1)</sup>	0.035	7.4	15894 <sup>f1)</sup>	6100 <sup>f</sup>	86.1 <sup>f</sup>
garden plant soil	228.3	14.03	5.8	296.1 <sup>a</sup>	117.3 <sup>a</sup>	1.9 <sup>a</sup>

<sup>1)</sup> the same letters indicate lack of statistically significant differences between objects, at the level =0.05

Table 2. Mean contents of heavy metals in substrates (mg/kg d.m.) after the cultivation of *Armeria maritima* and *Silene vulgaris*.

Substrates		<i>Armeria maritima</i>			<i>Silene vulgaris</i>		
		Zn	Pb	Cd	Zn	Pb	Cd
seedling pond	waste material	14005 <sup>e1)</sup>	5078.2 <sup>e</sup>	78.14 <sup>f</sup>	14213 <sup>e</sup>	5043.8 <sup>e</sup>	79.96 <sup>f</sup>
university plot	waste material	15076 <sup>ef</sup>	5439.7 <sup>e</sup>	84.48 <sup>f</sup>	15243 <sup>ef</sup>	5249.3 <sup>e</sup>	85.98 <sup>f</sup>
	garden plant soil	283.9 <sup>a</sup>	112.3 <sup>a</sup>	1.43 <sup>a</sup>	278.6 <sup>a</sup>	94.16 <sup>a</sup>	1.75 <sup>a</sup>

<sup>1)</sup> the same letters indicate lack of statistically significant differences between objects, at the level =0.05

(Table 2). Cadmium contents determined in substrates, in which both tested plant species were cultivated after the experiment, were lower than before but the differences were statistically insignificant.

#### Content of Heavy Metals in the Studied Plant Material

##### *Armeria Maritima* Subsp. *Halleri*

The highest contents of zinc (6472 mg/kg d.m.), lead (2107 mg/kg d.m.) and cadmium (61.0 mg/kg d.m.) were determined in plants cultivated on the shelf of settling pond (Fig. 1). These amounts are several times higher than critical values of the metals found in guidelines of plants assessment for their industrial use capacity [19]. In roots of the tested plant species planted into rings containing post-flotation waste material zinc and cadmium contents were about sixfold and the lead content about tenfold higher than in roots of plants cultivated in rings with garden plant soil (Fig. 1). Zinc, lead and cadmium contents determined in roots are generally statistically significantly higher than in shoots. Only in plants cultivated on the shelf of settling pond were similar contents of elements in both analyzed parts determined (Fig. 1). In this case the accumulation of trace elements in shoots is probably caused by the inflow of the metals together with atmospherical dust rich in these elements.

##### *Silene vulgaris* Subsp. *angustifolia*

The highest contents of zinc, lead and cadmium amounting respectively to 3681 mg/kg d.m., 1515 mg/kg d.m. and 38.0 mg/kg d.m. were determined in roots of the plant in question cultivated on the plot situated on the shelf of the settling pond (Fig. 1), and again in case of this

species the determined contents exceeded several times critical values of these metals found in guidelines of plant assessment for their industrial use capacity [19]. Contents of trace metals determined in roots of plants cultivated in post-flotation waste material were several dozen times higher than those determined in roots of plants grown in garden plant soil.

Fig. 1 shows that *Armeria maritima* cultivated on post-flotation waste materials, both on the university experimental plot and on the shelf of settling pond, accumulates in roots statistically higher amounts of metals than *Silene vulgaris*.

#### General Characteristics of Substrates and the Heavy Metals Uptake by Plants

Contents of organic carbon, total nitrogen and pH values determined in both substrates used in the experiment are presented in Table 1. Other characteristic features of post-flotation material are also low water capacity, high susceptibility to wind erosion and nutrient deficiency, which together make it an inappropriate substrate for plant growth [20]. The garden plant soil was enriched in basic macro- and microelements and contained about 80% of medium decomposed peat, thus it was not only rich in nutrients but also due to a large share of peat was characterized by high water capacity.

Dry mass of roots and shoots expressed in one plant mass of both species cultivated in garden plant soil were similar and amounted respectively to 2.52 g and 2.14 g (*Armeria maritima*) and 2.52 g and 2.31 g (*Silene vulgaris*). *Armeria maritima* grown in rings containing post-flotation waste material, both on the university experimental plot and on the shelf of settling pond had a considerably larger mass of roots than those grown in garden plant soil. *Silene vulgaris* was characterized by a smaller mass of plants, both roots and shoots, grown in post flotation waste

material in both variants of the experiment than cultivated in garden plant soil, yet contrary to *Armeria maritima* its mass of roots was smaller than the mass of aboveground parts (Table 3).

Basing on the plant mass and the metal concentrations in their vegetative organs and assuming that at the ground surface of 1 m<sup>2</sup> 25 plants can be cultivated, the uptake of metals by roots and shoots of both experimental plant species was calculated. Table 3 presents amounts which indicate that *Armeria maritima* has higher capacity to uptake zinc and lead especially by roots, when cultivated in post-flotation material (respectively 547.4 mg/kg and 152.4 mg/kg) than *Silene vulgaris* (155.5 mg Zn/kg and 64.0 mg Pb/kg). Only in the case of cadmium is the amount of the metal uptake expressed in one plant per one m<sup>2</sup> is similar for both plant species. Table 3 indicates also that both plant species accumulate trace elements mainly in roots.

### Discussion

Phytoremediation is still considered a new technology, yet in the last few years a lot of research has been carried out in an attempt to understand mechanisms of heavy metal phytoextraction from soils and their translocation from

roots to shoots with use of different cultivated plants, mainly vegetables [21-25] and storage of metals performed by hyperaccumulators [1, 9, 11, 13]. By definition, a hyperaccumulator must accumulate in its tissues at least 10,000 mg/kg d.m. of Zn, 1000 mg/kg d.m. of Pb and 100 mg/kg d.m. of Cd [26]. According to this opinion both studied plant species meet the requirements of hyperaccumulators regarding only concentration of lead in roots of plants growing on the experimental plot situated on the settling pond shelf. A factor limiting the uptake of metals from the post-flotation material by experimental plants is the alkaline reaction of this substrate, which contributes to the presence of large amounts of heavy metals in forms unavailable for plants. With reference to the concentration of metals determined in plant material obtained through in vitro culture and prepared for acclimatization, the content of trace elements in roots and shoots of *Armeria maritima* grown on the shelf of settling pond increased 23 times for Zn, more than 500 times for Pb and 28 times for Cd. In the case of *Silene vulgaris* these values were much lower and oscillate around 10. In the opinion of McGrath and Zhao [26] the common feature of all hyperaccumulators is a value of bio-concentration coefficient, which means the ratio of the concentration of the toxic substance in the organism tissues to this substance concentration in the life environment

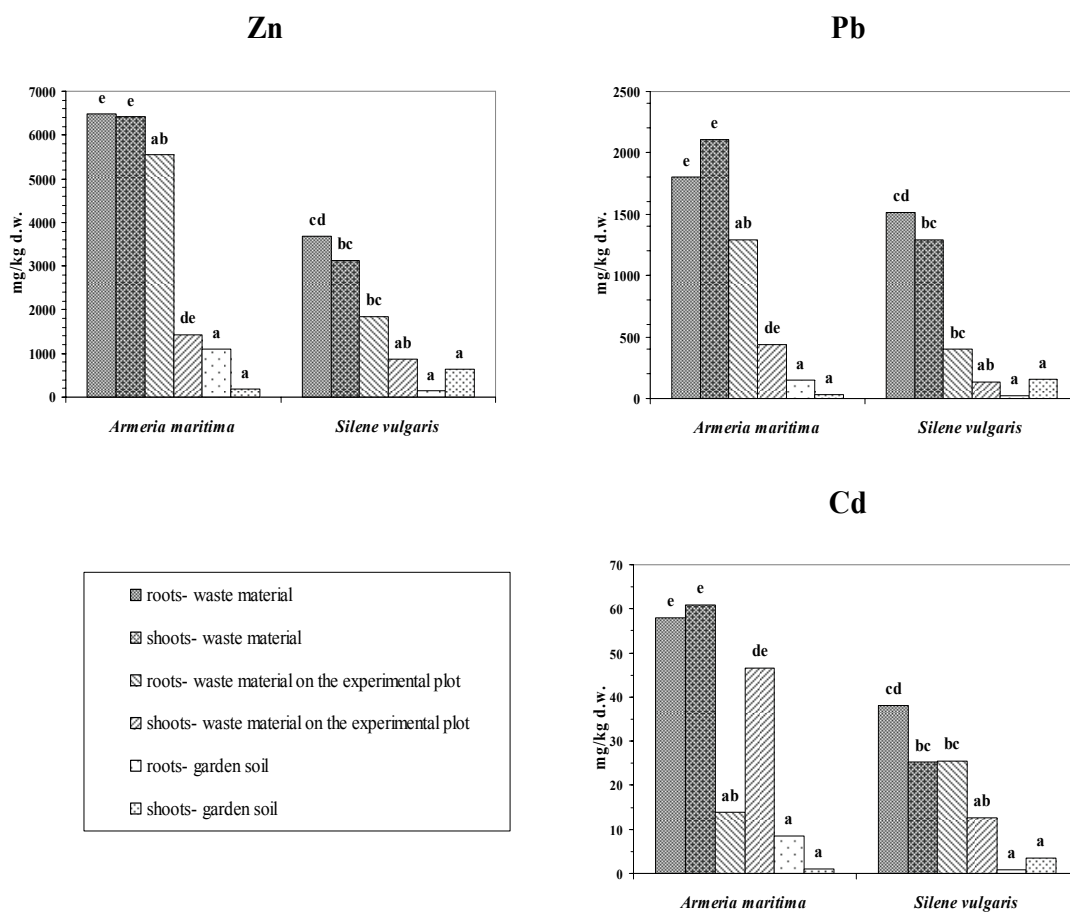


Fig. 1. Mean concentrations of zinc, lead and cadmium in roots and shoots of *Armeria maritima* and *Silene vulgaris* cultivated in waste material on the shelf of seedling pond, in waste material on a university plot and in the garden plant soil on a university plot (the same letters indicate lack of statistically significant differences between objects, at the level = 0.05).

Table 3. Zinc, lead and cadmium uptake by roots and shoots of *Armeria maritima* and *Silene vulgaris* growing in different substrates.

Plant species	Site	Type of substrate	Plant parts	Dry mass* [g]	Uptake [mg/m <sup>2</sup> ]		
					Zn	Pb	Cd
<i>Armeria maritima</i>	settling pond	waste material	roots	3.38	547.4	152.4	4.91
			shoots	3.25	521.9	171.3	4.96
	university plot	waste material	roots	3.57	586.6	134.7	4.81
			shoots	2.18	77.41	23.63	0.75
		garden plant soil	roots	2.52	69.60	9.34	0.54
			shoots	2.14	10.52	1.50	0.06
<i>Silene vulgaris</i>	settling pond	waste material	roots	1.69	155.5	64.01	1.61
			shoots	2.44	191.2	79.00	1.54
	university plot	waste material	roots	1.61	70.74	14.93	0.94
			shoots	2.43	52.32	8.33	0.77
		garden plant soil	roots	2.52	8.86	1.33	0.06
			shoots	2.31	37.51	8.89	0.21

\* mean content of dry mass expressed in one plant, n=30

is higher than 1. In both studied plant species a value of this coefficient considerably exceeds 1 only in the case of their cultivation in the garden plant soil.

Phytoextractors should be able to accumulate metals mainly in aboveground organs, have a high biomass increase and be easily harvestable [4, 11, 13]. The plant species used in the presented experiment do not meet these requirements. Instead, they could be applied to phytostabilisation of waste heaps contaminated with heavy metals. According to Ernst [4], phytostabilization is an even more recommended process for soils with a high load or deep penetration of heavy metals for which phytoextraction is not a realistic option. Both tested plant species *Armeria maritima* and *Silene vulgaris* are characterized by considerable accumulation of metals in roots and thus root ability to limit contaminant mobility, preservation of vitality on substrates characterized by extremely disadvantageous physical conditions, the facility for propagation and development during numerous growing seasons. The obtained results indicate a slightly higher potential for soil-free grounds stabilization that could be obtained using *A. maritima* subsp. *halleri* than *S. vulgaris* subsp. *angustifolia*. It is the consequence of a better biomass increment, a higher accumulation of zinc, lead and cadmium in vegetative organs and as a result a better uptake of these metallic elements.

One of the general principles of phytoremediation is to match the proper plant species with the site, taking into consideration both ground conditions and the contamination to be cleaned [11, 12]. Applying two locally occurring metallophytes species is at the same time assisting in the natural process of revegetation. In the consequence, making use of natural vegetation of adjacent areas creates the possibility of initiating ecological restoration on areas degraded as a result of industrial activity. Utilization of local metallophyte

species or subspecies in the process of reclamation of polluted fields is also advantageous from the point of view of avoiding the introduction of non-native species to destroyed ecosystems with simultaneous preservation of precious genetic pool of galmanic flora [27, 28]. Planting local species on such areas permits us to enhance a natural succession, since according to Bradshaw [29] spontaneous succession of vegetation can eventually be restored, even heavily degraded mineland, but it often takes 40-100 years if unaided and the biggest problem constitutes immigration of species. This opinion is confirmed by Abratowska [10] and Szarek-Lukaszewska with collaborators [20] in their papers regarding heavy metal tolerance strategies developed by *Armeria maritima* that enable it to grow on soils strongly contaminated with heavy metals and owing to that it can be successfully used for reclamation of such areas. Similar utilization of calamine spoil forms of *Silene vulgaris* notices Wierzbička [7]. In her opinion these species populations growing on waste-heaps in the area of Olkusz are especially valuable as they have genetically established features that allow them to grow under the harsh and pioneering conditions prevailing on waste heaps rich in zinc and lead. It also should be taken into consideration that one of the key problems is to restore degraded lands to their original landscape values. Both plant species are pretty flowering, look good in dense field and when planning management of such areas an aesthetic aspect should not be neglected.

In summary it can be stated that the cultivation of *Armeria maritima* nad *Silene vulgaris* caused a statistically significant decrease of zinc and lead content in post-flotation waste material and both tested plant species could be applied to phytostabilisation of areas contaminated with heavy metals. Moreover, as they are local subspecies their

use for remediation of the Olkusz settling ponds can be favourable for the restoration of native ecosystems on degraded areas.

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