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# The use of indigenous plant species and calcium phosphate for the stabilization of highly metal-polluted sites in southern Poland

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## Abstract

Highly metal-polluted (Pb, Cd, Zn) soil from a non-ferrous mine and smelter site in southern Poland, further referred to as "Waryński" soil, was used to test indigenous plant species for stabilization effectiveness of heavy metals in soils. Results of pilot investigations with commercially available cultivars of plant species showed that these cultivars could not grow on this highly polluted soil even with the application of soil amendments to stabilize the heavy metals. Based on these results, mesocosm and field experiments with an indigenous, metal-tolerant ecotype of *Deschampsia cespitosa* from the Waryński site were carried out. The mesocosm experiment showed that applications of calcium phosphate (3.8% w/w) as a heavy metal-stabilizing amendment decreased Cd and Zn concentrations 2 and 3-fold respectively in leachates, whereas lead content was not significantly changed. This decrease in the concentration of heavy metals in leachates was correlated with a lower accumulation of Pb, Cd and Zn in the roots and shoots of *D. cespitosa*, ecotype Waryński. In the field experiment, lower accumulations of Cd in roots and shoots and Zn in shoots in the amendment added plot were observed during the second year of investigations. In the first growing season, *D. cespitosa* plant cover in the amendment enriched mesocosms ranged from 95 to 100%, compared to 10% in mesocosms without calcium phosphate. In the second year of the experiment, in non-amendment enriched mesocosms *D. cespitosa* was substituted with *Cardaminopsis arenosa* (95% cover). *C. arenosa* is an undesirable species for phytostabilization, as it accumulates high amounts of zinc and cadmium in its shoots, even thought it provided better growth cover in not amended soils. However, in amended mesocosms, soil surface cover by *D. cespitosa* was still very high (90%). Similar results were obtained in field experiments. Addition of calcium phosphate to the soil also resulted in excellent *D. cespitosa* root system development when compared to soils without amendment. In amended mesocosms, high plant cover and root system development significantly decreased the volume of leachates and improved water retention. These results indicate that the use of *D. cespitosa*, ecotype Waryński in combination with calcium phosphate as a heavy metals immobilizing agent is sufficient to restore a dense vegetative cover to highly heavy metal-polluted soil.

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## Introduction

The countries of the former Eastern European Block continue to suffer from environmental contamination resulting from former economic practices, where environmental issues were not given a priority. Considerable environmental progress has already been made over the past decade, especially concerning air quality. In Poland, average concentrations of sulfur dioxide and nitrogen dioxide in air have decreased over the years 1989–2002 from 75 to 36 $\mu$g/m$^3$ and 45 to 22 $\mu$g/m$^3$, respectively. Concentrations of suspended matter have also decreased in the same period from 143 to 42 $\mu$g/m$^3$ (Hławiczka and Fudała, 2003). Nevertheless, persistent pollutants in soils, particularly heavy metals deposited occasionally in a highly irresponsible manner as waste, still pose a serious threat to living organisms (Knox et al., 2001). The bioavailable fraction of heavy metal contaminants is an issue of particular concern from an ecological, toxicological and health standpoint, as these may easily penetrate most environmental compartments, including the food chain.

Heavy metal contaminated areas mainly consist of hotspots located close to former or operational non-ferrous metal works. Dwellings for ore mine and smelter workers, which were built over a century ago and continue to have a limited occupancy today, surround these waste sites. Occasionally, these former industrial areas occupy highly valuable land, which must be left as brownfields awaiting restoration following site decommissioning. This is a worldwide problem, as the estimated number of sites requiring remediation in Europe alone amount to 1,500,000, of which 300,000 pose a potential threat to their surrounding environment (Janikowski and Korcz, 2001).

A variety of technical soil remediation methods exist. Most of them, however, are expensive, technically complicated and cause additional adverse side effects on the environment (Dushenkov et al., 1997; Vangronsveld and Cunningham, 1998; Vangronsveld et al., 1995). The cost issue is a driving factor in all remediation activities and even in well-developed countries economical considerations have caused postponements in land restoration.

Phytoremediation, a biological method, is an environmentally friendly and relatively inexpensive technology, which uses plants and soil amendments to extract, degrade or immobilize contaminants in soil (Knox et al., 2001; McGrath et al., 2002). Phytostabilization is a method of phytoremediation that converts soil contaminants into inert, immobile forms using metal tolerating plants and soil amendments (Berti et al., 1998; Salt et al., 1995). The mechanism may include absorption, adsorption, accumulation, precipitation or physical stabilization of contaminants in the root zone. Plants with well-developed root systems prevent contaminant migration via wind, hydrological processes and runoff (Berti and Cunningham, 2000; Knox et al., 2000; Vangronsveld and Cunningham, 1998). Phytostabilization may be applicable to large areas of contaminated soil, sludge and sediments that are not amenable to alternative forms of treatment for the remediation of heavily polluted sites (Li and Chaney, 1998; Vangronsveld, 1998). The best plants for phytostabilization are indigenous species of grasses, which are metal-tolerant as well as tolerant of growing conditions for a given site and thus able to develop a dense and strong root system (Geebelen et al., 2003; Smith and Bradshaw, 1979; Vangronsveld and Cunningham, 1998). Phytostabilization of heavily polluted sites may be achieved using a combination of chemical and biological methods (Brown et al., 2003; Mench et al., 2003) so the term "chemo-phytostabilization" has been recently coined (Knox et al., 2000). In this case, the upper layers of soils are first treated with chemicals (lime, commercial fertilizers as needed and various amendments) to adjust the soil pH, fertilize and transform the metal compounds into non-soluble forms. The next step is to develop a robust plant cover to reinforce the soil surface, maintain the desired soil-chemical composition and minimize soil transport processes (e.g., erosion and wind transport) (Vangronsveld and Cunningham, 1998; Vangronsveld et al., 1995).

Results of pilot investigations conducted at an abandoned industrial site situated in the vicinity of a former metal works, drew our attention to the phytoremediation potential of *Deschampsia cespitosa (*tufted hair grass), a perennial grass spontaneously growing across the site. This plant species is further referred as *D. cespitosa*, ecotype *Waryński. D. cespitosa* is a typical native European grass with expansive hyper competitive qualities and limited growth requirements (Falkowski, 1982; Rutkowski, 1998). The plants growing in the vicinity of the former metal works appeared relatively strong and healthy, in spite of poor soil conditions and inadequate watering. Its distribution across the site, however, was highly irregular. These findings motivated further research with this species with respect to its potential use for metal stabilization. In parallel, pilot observations were performed with other local species (*Silene inflata*, *Melandrium album*, *Cardaminopsis arenosa*), which spontaneously invaded the area, to determine if they form a co-operative relationship with *D. cespitosa*, ecotype *Waryński* during the process of soil stabilization. Toward this end, mesocosm and field experiments were performed, attempting to optimize the conditions for plant growth in an adverse environment and to assess the suitability of using local weeds to stabilize highly metal-polluted soil.

There are a number of well-recognized plants species resistant to high concentrations of metals in soil (Rostański, 1997; Smith and Bradshaw, 1979; Turner, 1994). The most suitable for stabilization are those which retain pollutants in their underground parts, thus precluding unwanted substance mobilization by grazing animals and penetrating the food chain (Berti and Cunningham, 2000; Vangronsveld and Cunningham, 1998).

Various modifications to the phytostabilization process have been implemented in recent years with good effects, preventing heavy metal migration and water or wind erosion of the land (Berti et al., 1998; Li and Chaney, 1998; Vangronsveld, 1998). A variety of plant species, mainly commercially available agricultural grasses, were established successfully if planted after soil conditioning with the application of fertilizers and different amendments e.g. lime and compost (Johnson et al., 1977; Li and Chaney, 1998; Vangronsveld et al., 1995). The issue becomes problematic, however, when dealing with highly contaminated land where the soil barely supports plant growth. In this case, using local, metal-tolerant populations of grasses, along with fertilizers and amendments was found to be successful (Smith and Bradshaw, 1979). Good fertilizing and watering should be routine in all land reclamation activities, as the plants are forced to grow under highly unfavorable environmental conditions. The key factor however, is the species of plant used, which must be highly tolerant to the pollutants contained in the contaminated soil. In extreme conditions the soil will not support growth and the plants will soon die.

Desirable features of species selected for land phytostabilization are as follows (Berti and Cunningham, 2000; Berti et al., 1998; Vangronsveld and Cunningham, 1998):

- tolerance to high concentrations of the pollutant in question,
- ability to develop a large root system and create a dense root mat,
- accumulation of pollutants in non-edible underground parts to prevent penetration of toxicants to the food chain,
- low maintenance requirements (watering, pest and weed control),
- relatively high transpiration rates,
- relatively long life or ability for self-propagation,
- adaptation to the local climate.

Our research consisted of two separate investigations: mesocosm and field experiments. The outdoor mesocosm experiment was carried out to test the ability of indigenous plant species (*Deschampsia cespitosa*) to restore a vegetative cover on a highly metal-polluted soil from southern Poland. To support plant growth, calcium phosphate was used as a soil amendment for immobilization of lead, cadmium and zinc. In parallel, changes in chemistry of mesocosm leachates were monitored. Metal accumulation in roots and shoots, plant cover and root system development were also examined. Based on the results of mesocosm experiment a full field experiment was conducted at an abandoned site located in the vicinity of a former non-ferrous metal works. The same plant species was tested in field experiment. The effect of calcium phosphate on the accumulation of target metals, the development of root system, plant cover and characteristics of plant communities were studied.

## Materials and methods

### *Site description*

The experimental site is a former mine and smelter area, situated between the towns of Bytom and Piekary Śląskie, in the Upper Silesia Industrial

Region of southern Poland. The enterprise operated for approximately 70 years, and the primary minerals of concern were zinc, lead and cadmium ore, dolomite, silt and gravel. The metal ores were thermally processed on-site, applying the Welz and Doerschel process (Stuczyński et al., 2000).

Mining activities have caused deformations of the land, subsidence and a considerable lowering of the groundwater table. In 1989, production was terminated with all facilities decommissioned and activities began towards the revitalisation of the huge property (460 ha). Currently, many of the old tailing piles and surrounding wastelands are overgrown with grasses and short trees. Nevertheless, a large area of land remains unvegetated (Stuczyński et al., 2000).

The area of concern, where the experimental plots were located and soil for laboratory experiments was taken, is situated in the immediate vicinity of the former smelter. The land was used for agricultural purposes until early eighties, when farming ceased due to poor crop yield (Stuczyński et al., 2000).

### *Mesocosm experiment*

Contaminated soil for mesocosm experiments, further referred to as *Waryński* soil, was obtained from an abandoned agricultural area located close to the Waryński polymetalic smelter, where later field experiments were also performed. The material was excavated approximately from the 0–30 cm layer of soil. From approximately 3 tons of soil, the solid debris such as pieces of wood, concrete, steel scraps etc., were removed and the soil sifted to pass a 4 mm sieve. Finally, the soil was homogenized using a concrete mixer and stored on the pile in the Institute for Ecology of Industrial Areas (IETU's) facilities for the further use. The mesocosms, consisting of PVC vessels 30 cm in diameter, 50 cm height, working volume of 35 L, and equipped with outlet valves at the bottom to collect leachates, were situated outdoors at the IETU's experimental grounds. To prevent migration of soil particulates with the leachates, the bottom of the mesocosms were covered with a 1-cm layer of sand. A fiberglass anti-root mat mitigated root penetration into the sand and outlet valve. For each mesocosm, a separate charge of 26 kg of air-dried soil mixed with fertilizers and amendment was prepared.

Since metalliferous wastes are very often deficient in nitrogen, phosphorus and sometimes other macronutrients (Smith and Bradshaw, 1979), fertilization with macroelements was done according to Boisson et al. (1999) using commercially available fertilizers, *Azofoska* (Inco VERITAS S.A., Poland, $NO_3$ – 5.5%; $NH_4$ – 8.1%; $P_2O_5$ – 6.4%; $K_2O$ – 19.1%; MgO – 4.5%; and microelements) and ammonium nitrate. Fertilizers were applied in all mesocosms in the following ratios: *Azofoska* 10.4 g per mesocosm and ammonium nitrate 5.35 g per mesocosm.

In a preliminary study with *Waryński* soil, two phosphate compounds (calcium phosphate and ammonium polyphosphate), each under two different concentrations, were tested for potential as metal-stabilizing amendments. Calcium phosphate ($Ca(H_2PO_4)_2$) at concentration of 3.8% (w/w) was the most effective for immobilizing metals and was thus the concentration of choice for the mesocosm experiment as a metal-stabilizing agent in combination with fertilizers.

Three mesocosms with soil and fertilizers (control) and three mesocosms with soil, fertilizers and amendment were prepared. Three weeks after fertilizer or fertilizer and amendment application, 2 g of *D. cespitosa* ecotype *Waryński* seeds per mesocosm were planted into all of the mesocosms.

As the soil from mesocosm experiment was collected directly from the site, the presence of different plant species can also be expected. This may result in their voluntary growth in course of experiment.

The experiment was carried out over 2 years. The leachates were collected monthly (from June to September) and analyzed for pH, electrical conductivity (EC) and heavy metal concentration (Pb, Cd, Zn) during first year of experiment. The leachates were filtered through 0.45 $\mu$m membrane filter (Nalge Nunc International, USA) prior to metal analysis.

Water retention was evaluated as the difference between input and output of water from the mesocosms.

### *Field experiment*

#### *Preparation and design of plots*

The experimental plot, situated near the Waryński smelter, was divided into two subplots of 4 m × 4 m each. The soil was tilled to a depth of 20 cm and all visible plant fragments were removed. Commercially available fertilizers *Azofoska* at the rate of 1280 g/subplot (800 kg/ha) and ammonium nitrate at the rate of 658 g/subplot (411 kg/ha) were applied to each subplot including control, and mixed with the 0–20 cm soil layer. The rate of fertilizer applied per subplot was proportionally calculated from the rate of fertilizers used in the mesocosm experiments.

Based on the results of mesocosm experiment, lime at the rate of 12 t/ha was applied to correct the pH on the subplot where calcium phosphate at the rate of 61 kg/subplot (38 t/ha) was added.

Two weeks after amendment application, 125 g (77 kg/ha) of *D. cespitosa*, ecotype Waryński seeds were planted in each subplot.

### *Assessment of root system development by pinboard method*

A well-developed root system is one of the chief plant properties used for stabilization (Berti et al., 1998).

The pinboard method (Oliveira et al., 2000) allows for a practical assessment of root system architecture. This method makes it possible to isolate the entire plant, including the root system, in an undisturbed form from the soil.

A 50 × 70-cm board equipped with 14-cm spikes fastened at 5-cm intervals was pushed into the vertical wall of a 1-m deep soil trench. After the spikes had fully penetrated into the soil, the soil sample was cut away from the ground on both sides, the back and the bottom of the board. Using this procedure, the entire plant, with an almost undamaged root system, was removed together with the soil in between the spikes. The soil was subsequently washed out with water to expose the roots for examination.

### *Sampling procedures*

Mesocosm experiment: for analysis of the soil used during the mesocosm experiment, 30 subsamples, were collected from a pile of Waryński soil and well mixed together to prepare three composite samples.

Field experiment: in the beginning of field experiment three composite samples per subplot from each depth (0–20 cm and 20–40 cm) were analyzed.

In the end of the mesocosm experiment, soil was removed from mesocosm while the shoots and roots of *D. cespitosa* and *C. arenosa* were carefully removed and collected. Plant material, for each plant species collected from one mesocosm, formed one individual sample.

During the field experiments in 2002 and 2003, three shoot and root samples of *D. cespitosa* were collected from each subplot at the end of the growing season.

### *Analysis*

Physical and chemical soil properties were analyzed using standard methods as follows: pH (KCl) and EC in water extract were measured in soil to solution ratio of 1:2.5 (combination glass/calomel electrode and pH meter); organic matter (loss-on ignition, Houba et al., 1995); texture (hydrometric method); total metal concentration (*aqua regia* extraction).

Cation exchange capacity (CEC) was analyzed according to Houba et al. (1995). Concentration of $CaCl_2$ extractable metals was measured as follows: 5 g of air-dried soil ground $< 0.25$ mm was extracted with 50 mL 0.01 *M* $CaCl_2$ for 5 h and the concentration of metals was analyzed in the extract.

Leachates collected from mesocosms were filtrated through a 0.45 $\mu$m membrane filter (Nalge Nunc International, USA) and first analyzed for pH and EC with combination electrodes and a pH meter, then analyzed for metal content.

Plant material collected from mesocosms and experimental plots was washed with tap water in an ultrasonic washer to remove soil particles and then dried at 70 °C for three days. Approximately 1 g of dried ground material was wet-ashed using concentrated nitric acid in a microwave system (MDS 2000, CEM, USA).

Concentrations of metals were analyzed by flame atomic absorption spectrophotometer (Varian Spectra AA300) or by inductive coupled plasma spectroscopy (ICP-AES) (Varian, USA).

Data reported in this paper was processed using the computer software Statistica for Windows (Statistica'99). A probability of 0.05 or less was considered to be statistically significant.

### *Braun-Blanquet method*

Plant cover and contribution of various plant species on plot surface were determined based on Braun–Blanquet approach (Cilliers and Bredenkamp, 2000, Gombert et al., 2004).

The Braun-Blanquet scale (Gombert et al., 2004) was used to estimate the cover of each species in a following scale: 0.5 ( < 1% cover); 1 (1–5% cover); 2 (6–25% cover); 3 (26–50% cover); 4 (51–75% cover); 5 (76–100% cover); R (only several plants were found).

## Results

### *Soil properties*

Physical and chemical properties of the soil used in the mesocosm experiments are presented in Table 1. The soil was classified as loamy and highly polluted with Zn, Cd and Pb. The pH was almost neutral, followed by high concentration of organic matter (OM) and low EC. Cadmium exists in highly bioavailable form (7.80% of total metal concentration in 0.01 *M* $CaCl_2$), whereas bioavailability of Pb was relatively low (Table 1).

Properties of soil used for field experiment are presented in Table 2. In general, concentrations of the investigated metals, total and $CaCl_2$ extractable fractions, as well as pH and EC were in the same range as in the soil used in the mesocosm experiments (Table 1). It was particularly observed when soil from the mesocosm experiments is compared to soil from field experiment taken from the depth 0–20 cm. Soil pollution on experimental site is a typical example of man made contamination, where various human activities took place. Therefore the concentration of metals vary from place to place. Such a distribution of metals is typical for the areas situated close to the former non-ferrous works. That may explain the differences in concentration of metals in particular subplots and soil gathered for mesocosm experiment (Tables 1 and 2).

### *Characteristics of leachate from mesocosms*

Following the addition of calcium phosphate, a decrease in leachate pH from 7.5 to about 6 and a significant increase in EC were found compared to controls (Figure 1). The increase of EC was particularly high in the first sampling event in June. It is supposed that high EC of leachates from amended mesocosms during the whole growing season was an effect of gradual dissolution of applied calcium phosphate.

*Table 1.* Characteristics of *Waryński* soil used during mesocosm experiments

| Property | Value |
|---|---|
| pH (1 : 2.5 soil/KCl ratio) | 6.71 ± 0.03 |
| EC ($\mu$S cm$^{-1}$) | 248 ± 26 |
| Organic matter content (%) | 8.52 ± 0.12 |
| CEC (cmol(+)/kg) | 6.67 ± 0.24 |
| Sand (1–0.05 mm), % | 37.3 |
| Silt (0.05–0.002 mm), % | 56.3 |
| Clay ( < 0.002 mm), % | 6.8 |
| *Total heavy metal concentration (extraction with aqua regia)* | |
| Pb (mg kg$^{-1}$) | 9712 ± 562 |
| Cd (mg kg$^{-1}$) | 537 ± 23 |
| Zn (mg kg$^{-1}$) | 11,498 ± 417 |
| *$CaCl_2$ extractable metal fraction*[a] | |
| Pb (mg kg$^{-1}$) | 5.23 ± 0.13 (0.06)[b] |
| Cd (mg kg$^{-1}$) | 41.78 ± 0.69 (7.80)[b] |
| Zn (mg kg$^{-1}$) | 363.00 ± 7.50 (3.30)[b] |

Values represent mean of three replicates samples ± SE.

[a] Extraction with 0.01 *M* $CaCl_2$.

[b] In parentheses % of total metal concentration is presented.

*Table 2.* Soil characteristics at the beginning of field experimentation. Soil samples were taken at two depths: 0–20 cm and 20–40 cm

| Property | Depth (cm) | |
|---|---|---|
| | 0–20 | 20–40 |
| pH (1 : 2.5 soil/KCl ratio) | 6.57 ± 0.07 | 6.18 ± 0.08 |
| EC ($\mu$S cm$^{-1}$) | 154 ± 11 | 125 ± 10 |
| *Total heavy metal concentration (extraction with aqua regia)* | | |
| Pb (mg kg$^{-1}$) | 8265 ± 1143 | 2890 ± 822 |
| Cd (mg kg$^{-1}$) | 392 ± 45 | 155 ± 23 |
| Zn (mg kg$^{-1}$) | 9673 ± 925 | 4854 ± 760 |
| *$CaCl_2$ extractable metal fraction*[a] | | |
| Pb (mg kg$^{-1}$) | 4.36 ± 0.21 | 1.99 ± 0.60 |
| Cd (mg kg$^{-1}$) | 75 ± 3.7 | 39 ± 3.3 |
| Zn (mg kg$^{-1}$) | 474 ± 16 | 366 ± 15 |

Values represent mean of three replicate samples ± SE.
[a] extraction with 0.01 *M* $CaCl_2$.

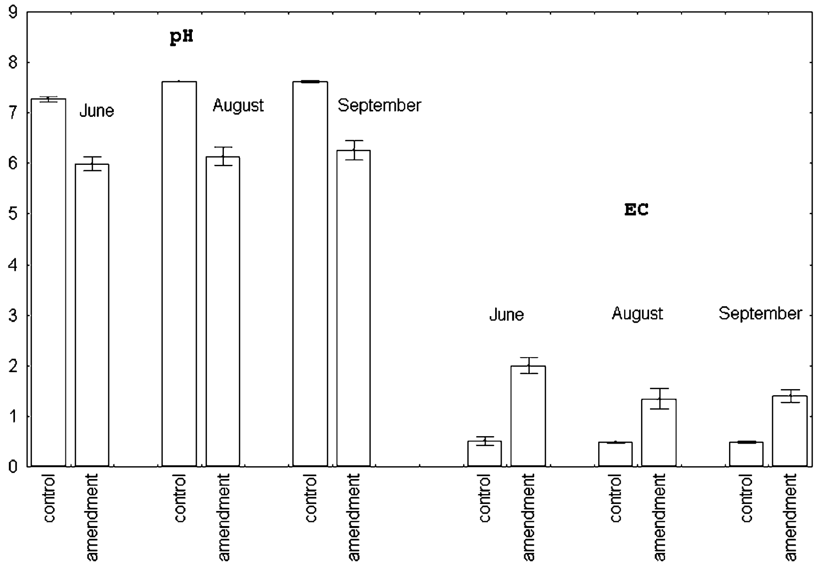

*Figure 1.* pH and EC (mS cm$^{-1}$) of leachates from mesocosm. Means ± standard error ($n = 3$) are shown.

One month after calcium phosphate application (June), cadmium concentration in leachates was significantly reduced (Figure 2a), the decrease of zinc concentration was small and not statistically significant (Figure 2b), whereas lead concentration in leachates was at unchanged level (Figure 2c).

At the end of growing season (September) 2.5-fold decrease of cadmium concentration in leachates from mesocosms with amendment compared to control mesocosms was found (Figure 2a). Similar effect of amendment on concentration of zinc (3-fold decrease) in leacheates was observed (Figure 2b). By contrast to zinc and cadmium, increase in lead concentration in leachates at the end of growing season was noticed.

Figure 3 shows the relationship between soil plant cover and water retention. Over the course of the entire experiment, an appropriate plant cover significantly improved water retention. This effect did not change during the entire growing season.

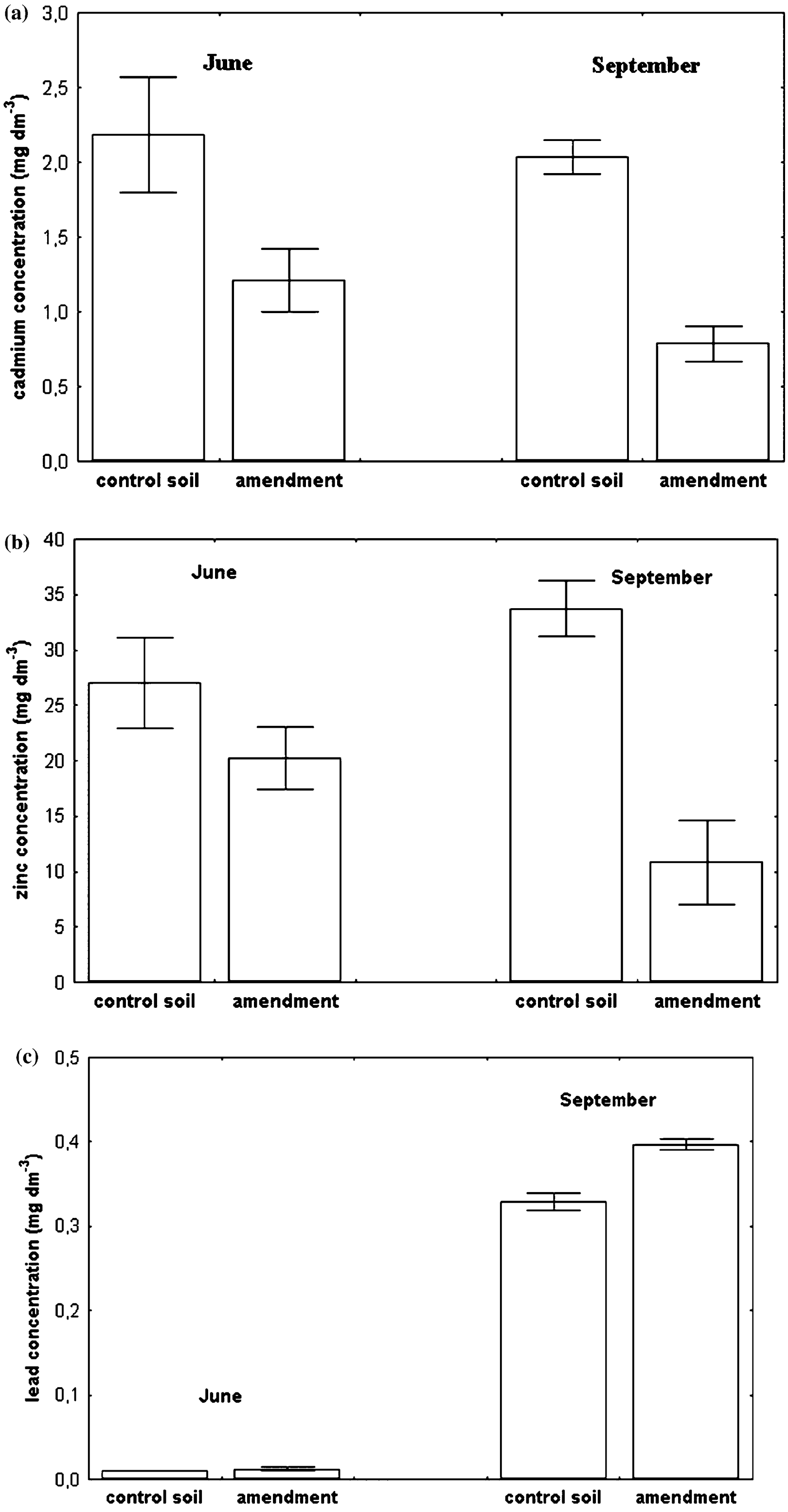

*Figure 2.* Effect of amendment addition to the soil on (a) cadmium, (b) zinc and (c) lead concentrations in leachates from mesocosm. Means ± standard error ($n = 3$) are shown.

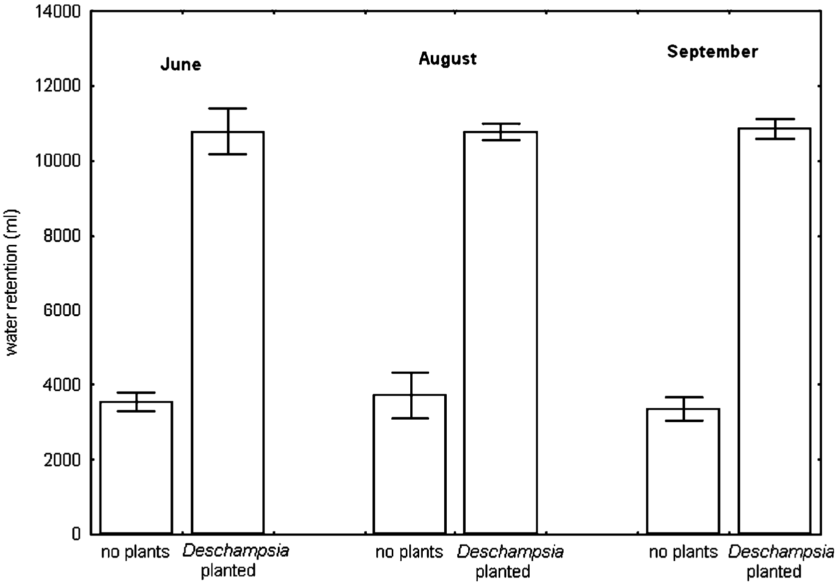

*Figure 3.* Water retention in mesocosm in relation to plant cover. Means ± standard error ($n = 3$) are shown.

### *Concentration of metals (Pb, Cd and Zn) in* Deschampsia cespitosa*, ecotype Waryński from mesocosm and field experiments*

Accumulation of Pb and Cd in the shoots and roots of *D. cespitosa* was effectively lowered by the addition of calcium phosphate in the mesocosm experiments. Concentrations of Pb in shoots and roots were diminished by 80% and the concentration of Cd by approximately 75%, compared to plants from mesocosms without amendment (Table 3). After addition of calcium phosphate, accumulation of zinc in shoots was also lowered by 78%, whereas concentrations of zinc in the roots were diminished by 48% compared to control (Table 3). In Table 3, metal concentrations in shoots and roots of *C. arenosa* are also presented. That species accidentally appeared in mesocosms together with seed-contaminated soil. *C. arenosa* accumulates significant amounts of metals in shoots, even after addition of calcium phosphate to the soil, which may exert an adverse effect on the food chain (Table 3).

The effect of calcium phosphate on accumulation of metals by *D. cespitosa* was also investigated

*Table 3.* Metal accumulation (mg kg$^{-1}$) in roots and shoots of *Deschampsia cespitosa* and *Cardaminopsis arenosa* in mesocosm experiment

| Plant species | Treatment | Pb (mg kg$^{-1}$) | | Cd (mg kg$^{-1}$) | | Zn (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | | Shoot | Root | Shoot | Root | Shoot | root |
| *Deschampsia cespitosa* | control (no amendment) | 520 ± 128 | 4915 ± 2704 | 67.4 ± 19.8 | 871 ± 183 | 1363 ± 277 | 6392 ± 2403 |
| | calcium phosphate | 98.5 ± 35 | 868 ± 359 | 16.2 ± 4.9 | 220 ± 45 | 296 ± 74 | 3318 ± 1174 |
| *Cardaminopsis arenosa* | control (no amendment) | 579 ± 12 | 2123 ± 341 | 1025 ± 110 | 565 ± 33 | 15,354 ± 985.8 | 5226 ± 363.4 |
| | calcium phosphate | 101.2 ± 14.3 | 603 ± 303 | 107 ± 12.8 | 83.2 ± 15.9 | 1479 ± 282 | 1730 ± 572 |

Values represent mean of three replicate samples ± SE.

*Table 4.* Metal accumulation (mg kg$^{-1}$) in roots and shoots of *Deschampsia cespitosa* in field experiment

| | Treatment | Pb (mg kg$^{-1}$) | | Cd (mg kg$^{-1}$) | | Zn (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | | Shoot | Root | Shoot | Root | Shoot | Root |
| First year | Control (no amendment) | 759 ± 215 | 10,419 ± 1031 | 44.4 ± 11.4 | 972 ± 28.2 | 1282 ± 207 | 8171 ± 1149 |
| | Calcium phosphate | 648 ± 11 | 387 ± 28 | 63.0 ± 1.3 | 513 ± 29 | 1108 ± 35 | 6332 ± 164 |
| Second year | Control (no amendment) | 157 ± 14 | 526 ± 112 | 47.0 ± 1.7 | 1258 ± 73 | 1154 ± 44 | 2785 ± 78 |
| | Calcium phosphate | 140 ± 10 | 585 ± 122 | 19.2 ± 1.8 | 437 ± 24 | 754 ± 32 | 2992 ± 178 |

Values represent mean of three replicate samples ± SE.

under field conditions. Due to the lowering of leachates pH in the mesocosm experiments by the addition of calcium phosphate (Figure 1), lime was added in the field experiment to stabilize soil pH. During the first growing season, a positive effect of calcium phosphate in reducing the accumulation of the metals in *D. cespitosa* plants was observed for lead and cadmium, but was restricted only to the roots (Table 4). A 27-fold lower concentration of lead and 2-fold lower cadmium concentration in roots *of D. cespitosa* was observed from the subplot with amendment when compared to the control subplot with no amendment. However, the positive effect of calcium phosphate for decreasing zinc concentrations in roots was not noticed (Table 4).

In the following year, the influence of amendment on lowering concentrations of cadmium in shoots and roots was observed. However, reduced concentrations of lead in shoots and roots were not found (Table 4). Also in the second year, uptake of zinc by *D. cespitosa* shoots was lowered by calcium phosphate, but no such effect was observed in the roots (Table 4).

*Table 5.* Plant cover characteristics in mesocosm experiment

| Soil treatment and plant species sown | Replicates (mesocosms) | First year | | | Second year | | |
|---|---|---|---|---|---|---|---|
| | | Plant cover (%) | Dominate species | Accompanying species | Plant cover (%) | Dominate species | Accompanying species |
| Control (no amendment) *Deschampsia cespitosa* | 1 | 10 | 99% *Deschampsia cespitosa* | Few *Cardaminopsis arenosa* | 95 | *100% Cardaminopsis arenosa* | None |
| | 2 | <1 | *100% Deschampsia cespitosa* | None | 40 | 99% *Cardaminopsis arenosa* | Few *Deschampsia cespitosa* |
| | 3 | 10 | 99% *Deschampsia cespitosa* | Few *Cardaminopsis arenosa* | 5 | *100% Deschampsia cespitosa* | None |
| Calcium phosphate *Deschampsia cespitosa* | 1 | 100 | *100% Deschampsia cespitosa* | None | 100 | 85% *Deschampsia cespitosa* | 15% *Cardaminopsis arenosa,*<br>few *Silene inflata* |
| | 2 | 100 | *100% Deschampsia cespitosa* | None | 100 | 70% *Deschampsia cespitosa* | 30% *Cardaminopsis arenosa* |
| | 3 | 95 | *100% Deschampsia cespitosa* | None | 100 | 90% *Deschampsia cespitosa* | 10% *Cardaminopsis arenosa* |

### *Characteristics of plant cover in the mesocosm experiment and plant communities in field experiment*

Plant cover in each mesocosm was evaluated during the first and second year of the experiment. The addition of calcium phosphate resulted in intensive growth of *D. cespitosa* and good plant cover which ranged from 95 to 100% during the first growing season (Table 5). In mesocosms without the amendment, plant growth was poor and plant cover did not exceed 10%.

The soil used to fill the mesocosms was passed through a 4 mm sieve, so seeds from other plant species such as *Cardaminopsis arenosa* and *Silene inflata* still were found in the soil, which resulted in their presence in mesocosms (Table 5).

In the second growing season, as a result of the growth of additional plant species (*C. arenosa* and *S. inflata*), soil surface cover by *D. cespitosa* in mesocosms with amendment was lower, compared to the first year and ranged from 70 to 90% (Table 5). Plant cover of *D. cespitosa* in mesocosms without amendment, compared to first year of experiment, was very poor and the grass was displaced in two mesocosms by *C. arenosa* which covered up to 95% of the soil surface in one mesocosm (Table 5).

During the course of the 2-year field experiment, plant cover and the contribution of various plant species in soil surface coverings were observed (Table 6). Similarly, as in the case of the mesocosms, the subplot amended with calcium phosphate showed better growth and soil cover with *D. cespitosa*, when compared to the control subplot.

The cover of the non-amended subplot reached only 40% in the second year of the experiment (Table 6). In the first and second year of the experiment in the non-amended subplot the dominant species was *D. cespitosa*, which formed separated tufts where uncovered soil among the tufts was observed. Additionally plants of *C. arenosa* and *Silene inflata* were found throughout the 2 years of the experiment, but their contribution in soil cover ranged only to 5% (Table 6).

Plant cover in the amended subplot ranged from 90% in the first year to 100% in the second year (Table 6). In both years the dominant species was *D. cespitosa*, which formed a dense vegetative stratum instead of the separated tufts observed in the control subplot. In the first year, *D. cespitosa* growth was accompanied by *C. arenosa*, *S. inflata* and *Melandrium album*. These accompanying species covered in the first year less than 3% of the subplot area. In the second year only several plants of C. *arenosa* were found. *C. arenosa* was replaced by *M. album* (Table 6), which is more beneficial species for the process of phytostabilization, because it accumulates zinc and lead mainly in roots (data not shown).

### *Development of* D. cespitosa *root system in mesocosm and field experiments*

Analysis of the *D. cespitosa* root system was performed after completion of the mesocosm experiment (Table 7). The density and length of

*Table 6*. Characteristics of plants cover and contribution of various plant species in soil surface covering in the field experiment

| Treatment/plant | First year | | | Second year | | |
|---|---|---|---|---|---|---|
| | Plant cover (%) | Contribution of various plant species in soil surface covering* | | Plant cover (%) | Contribution of various plant species in soil surface covering* | |
| Control (no amendment)<br>*Deschampsia cespitosa* | 25 | *Deschampsia cespitosa* | 3 | 40 | *Deschampsia cespitosa* | 4 |
| | | *Cardaminopsis arenosa* | 1 | | *Silene inflata* | 1 |
| | | *Silene inflata* | 0.5 | | *Cardaminopsis arenosa* | 1 |
| Calcium phosphate<br>*Deschampsia cespitosa* | 90 | *Deschampsia cespitosa* | 5 | 100 | *Deschampsia cespitosa* | 5 |
| | | *Melandrium album* | 0.5 | | *Melandrium album* | 1 |
| | | *Silene inflata* | 0.5 | | *Silene inflata* | 0.5 |
| | | *Cardaminopsis arenosa* | 0.5 | | *Cardaminopsis arenosa* | R |

*The Braun–Blanquet scale was used to estimate the cover of each species a following scale: 0.5 (<1% cover); 1 (1–5% cover); 2 (6–25% cover); 3 (26–50% cover); 4 (51–75% cover); 5 (76–100% cover); R (only several plants were found).

the root systems was significantly higher in amended than in non-amended mesocosms. In the mesocosms with calcium phosphate added, the *D. cespitosa* root system was well developed, with dense and thin roots dispersed in the whole volume of mesocosm, whereas in the control mesocosms root system was medium developed with single, thin roots.

The positive effect of calcium phosphate on root system development was fully confirmed in field experiment (Table 7). The application of the amendment resulted in developing a strong root systems, effectively penetrating soil to a depth of 80 cm, whereas plants from the control subplot had poorly developed root systems, particularly at the 40–80 cm depth (Table 7).

## Discussion

The present study showed that *D. cespitosa* ecotype Waryński is able to grow on soil highly contaminated with Zn, Pb and Cd and, in combination with calcium phosphate, to create complete coverage of the soil. This species grows spontaneously on many metal contaminated sites (Baker et al., 1986; Cox and Hutchinson, 1980; Rostański, 1997; Smith and Bradshaw, 1979), however scientist paid little attention to this species compared to other species as *Festuca rubra*, *Festuca ovina*, *Agrostis capilaris* and *Agrostis stolonifera* (Li and Chaney, 1998; Smith and Bradshaw, 1979; Tordoff et al., 2000; Vangronsveld et al., 1995). Although there are many papers dealing with heavy metals accumulation and heavy metal tolerances in *D. cespitosa* (Baker et al., 1986; Cox and Hutchinson, 1980; Godbold et al., 1983, 1984; Von Frenckell-Insam and Hutchinson, 1993 a, b), there is a lack of data on application of this plant species for revegetation and reclamation of metal contaminated soils.

Investigations by Smith and Bradshaw (1979) on various commercial cultivars and indigenous populations or different plant species (*Festuca rubra*, *Festuca ovina*, *Agrostis stolonifera*, *Agrostis capilaris*) were made with the intent of using them to revegetate metal polluted land. Good results in this experiment were achieved only in case of well-fertilized and watered populations of local origin. Yield of local origin population, after fertilization, was 10-fold greater in comparison with commercially available cultivars. Commercial cultivars are suitable for less contaminated soils. A successful trial using such grasses and leguminous plants was performed in the UK on soil slightly polluted with Pb and Zn and amended with sewage sludge (Tordoff et al., 2000).

The mesocosm experiment showed that calcium phosphate decreases the concentration of bivalent heavy metals in leachates, however it binds these heavy metals only to certain extent. Concentrations of unbound bioavailable metals

*Table 7.* Development of root system of *Deschampsia cespitosa* in mesocosm and field experiments

| Treatment | Root length (cm) | Root system development | | |
|---|---|---|---|---|
| | | Mesocosm experiment | | |
| | | Depth 0–20 cm | Depth 20–40 cm | Depth 40–50 cm |
| Control (no amendment) | 40 | Medium developed | Poorly developed, only single roots occurring | No root system[a] |
| Calcium phosphate | Up to 60 | Strong, well-penetrating root system, creating a dense robust mat | As at 0–20 cm depth | Well-developed, well shaped roots[a] |
| | | Field experiment | | |
| | | Depth 0–20 cm | Depth 20–40 cm | Depth 40–80 cm |
| Control (no amendment) | Up to 80 | Medium developed | Poorly developed, | Only single roots occurred |
| Calcium phosphate | Up to 80 | Strong, well-penetrating root system, creating a dense robust mat | As at 0–20 cm depth | Well-developed, well shaped roots |

[a] mesocosm length was limited to 50 cm and therefore root system development was measured at a depth of 40–50 cm.

remained high enough to inhibit the growth of commercially available cultivars of different plant species (data not shown). Therefore, the efforts were focused on local species.

Phytostabilization of metal-contaminated soils requires plants tolerant to local environment of a given site. These plants should accumulate low concentrations of contaminants in aboveground plant tissue, in order to limit possible metal transfer to herbivores and to other compartments of the food chain (Vangronsveld and Cunningham, 1998).

In the current study good stabilization features of *D. cespitosa* ecotype Waryński were demonstrated. This plant species is resistant to lead, zinc and cadmium, as it was shown, on *Waryński* soil highly contaminated with metals. Mesocosm experiment showed that in non-amended soil *D. cespitosa* ecotype Waryński accumulates lead, zinc and cadmium predominantly in roots, in contrast to second indigenous plant species *C. arenosa* (Table 3). The amendment application significantly decreased uptake of lead, cadmium and zinc by *D. cespitosa* roots and their transport to shoots. Cao et al. (2003a) have recently reported that addition of calcium phosphate to contaminated soil reduced shoot tissue lead and zinc concentration but enhanced content of both metals in the roots of *Stenotaphrum secundatum* which is a predominant grass growing on the soil contaminated with lead, zinc and copper (Cao et al., 2003a). Contrary to *S. secundatum*, application of calcium phosphate decreased the concentration of metals in shoots and roots of *D. cespitosa* further supports its good phytostabilization features (Table 3). *D. cespitosa* ecotype Waryński grows well on different types of polluted material. Recently, the plant has been successfully used to stabilize metals in polluted dredge sediment deposits (Girondelot et al., 2003), which texture is different from texture of *Waryński* soil. The rhizosphere of this perennial grass is also known to be an important factor in humus creation (Miklosz, 1966), which in turn can further improve metal immobilization.

The possibility of ingestion of contaminated aboveground parts of plant by invertebrates or mammals and entering into the food chain is to be considered during the phytostabilization process. It has been documented, however, that some animals instinctively avoid such contaminated food (Migula and Binkowska, 1993; Pollard and Baker, 1997). On the other hand the high content of silica in leaf tissue may render *Deschampsia cespitosa* unattractive to grazing animals (Kozłowski et al., 1998).

The research aimed at application of indigenous plant species to stabilize to metal contaminated soils was conducted by Li and Chaney (1998) in the vicinity of the Palmerton zinc smelter. The species (*Festuca rubra* L., "Merlin"), indigenous to heavy metals contaminated soil in Great Britain, was planted on a soil polluted comparably to the *Waryński* soil. They found that three months after planting *Festuca rubra* cv. Merlin plants covered only 30% of the surface in both untreated and treated with the biosolids compost plots (Li and Chaney, 1998). In contrary *D. cespitosa* ecotype Waryński plants colonized *Waryński* soil very quickly, which prove the supremacy of this species of plant.

In phytostabilization process, water retention is very important and should be stable during growing season. In mesocosm experiment It was shown that retention of water did not change from June to September (spring to autumn) (Figure 3) so plant cover reduced effectively volume of leachates.

Application of calcium phosphate had positive effect on *D. cespitosa* growth and development. The amendment addition improved plant growth and caused better soil cover by plants. In the second year of the field experiment *D. cespitosa* covered only 51–75% of the control subplot, and formed tufts with bare soil surface in between. Such uncompleted vegetation cover pose a risk from dust resuspension (Tordoff et al., 2000). However, in the subplot with the amendment *D. cespitosa* formed a dense vegetative stratum instead of the separated tufts observed at the control subplot. Calcium phosphate also had positive effect on root system development. It has to be concluded that addition of amendment resulted in mesocosm and field experiments in creation of strong, well penetrating root system and dense robust mat.

In the current study relatively high concentrations of phosphate (38 ton/ha; 3.8% w/w), as calcium phosphate, were used compared to the other authors, who tried to stabilize Cd or Pb in contaminated soil (Bolan et al., 2003: Geebelen et al., 2003). Philips (1998), however, used higher concentrations of calcium phosphate (10 and 20%) to immobilize heavy metals compared to amount of phosphate applied in the present study. To immobilize metals in heavily contaminated soils application of high amount of amendments is often necessary. Vangronsveld et al. (1995) applied Beringite and compost from municipal waste at the rate of 120 and 100 tons/ha respectively. Li and Chaney (1988) used limstone at 8 tons/ha and compost at 224 tons/ha.

At the Waryński site, application of calcium phosphate resulted in high biomass production by *D. cespitosa*. Harvesting in this case is not recommended, as the nodules which are important for further plant development, can be easily damaged (Kozłowski et al., 1998). On the other hand, a costly contaminated crop disposal process can be avoided that way (Sas-Nowosielska et al., 2004).

Phosphate is not recommended for arsenic polluted soils, as competition between arsenate and phosphate can provoke increased arsenic levels in plants, causing risks of food chain propagation and accumulation (Alam et al., 2001, Cao et al., 2003b, Tokunaga and Hakuta, 2002, Theodoratos et al., 2002).

An optimization study between phosphorus added to the soil and satisfactory plant growth remains to be done, as the cost of amendment is a matter of serious concern. The expected output of this further study will result in precise information on the lowest dose of phosphorus necessary for satisfactory plant growth.

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