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*Original Research*

# Efficient Pb Translocation by 'Purple Petticoats' cv. of *Heuchera* L. from Contaminated Soil in a Coal Basin

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**Abstract**

The post-mining area of Dąbrowskie Coal Basin is highly contaminated with toxic metals such as Cd, Pb, and Zn. In our previous research *Heuchera* cv. 'Purple Petticoats' has shown the ability to Pb uptake and effective transport into leaves. The purpose of this experiment was to check whether the time of cultivation and use of nutrients would change the reaction of plants to Pb uptake in soil with low-level pollution (75 $mg \cdot kg^{-1}$). Pb content was analyzed in soil and plant tissues. Young plants (11 months old) absorb more Pb but stop it in their roots. Contrarily, plants grown for three consecutive seasons in one place were characterized by BAF~0.5 and TF~0.5. Pb amount in soil on plots where fertilizer was used was reduced by 9%, and without fertilizing by 20%.

**Keywords:** *Heuchera*, lead, translocation factor, contaminated soil, phytoremediation

## Introduction

Current efforts to raise the standard of living are associated with the idea of a clean environment. In the soils of industrial areas contents of heavy metals such as lead, cadmium, and zinc are elevated [1]. However, population growth generates an escalation of the colonization of urban peripherals, which were brownfields before. This causes an environmental threat for human health. This problem also occurs in Dąbrowskie Coal Basin. In 80% of the results Pb determinations in the upper soil layer (0-30 cm) on the monitored area exceed the value 50 $mg \cdot kg^{-1}$ d.m., defined by The Ministry of the Environment as the permissible concentration of lead in unpolluted soils [2, 3]. Lead is toxic for plants, animals, and people. It is especially dangerous for small children. Breaching from the environment into their quickly-growing organisms, can cause damage of nervous systems and organs. Any Pb exposure is dangerous for children [4-6]. Reducing the risk of exposure to environmental Pb poisoning is subject to limitation of availability from all sources [7]. One of the main sources of this element for small children is polluted soil surface [8]. Therefore the level of toxic metals in soil, Pb included, should be reduced in the urban areas, above all in places like playgrounds, squares, and gardens.

Remediation of the contaminated land, using the best available techniques, is a responsibility of the managers of cities of Dąbrowskie Coal Basin [9]. The interest is focused on strategy for sustainable soil use without risk for human health. Phytoexstraction – one of the techniques of phytoremediation – is an environmentally friendly and relatively inexpensive method of soil treatment [10]. Use of

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Table 1. Chemical soil properties of experimental plot in Dąbrowskie Coal Basin.

| Chemical soil properties | pH | $N(NO_3)$ | P | K | Ca |
|---|---|---|---|---|---|
| | | mg·kg$^{-1}$ d.wt. | | | |
| | 6.9±0.21 | 3±2.1 | 82±1.8 | 74±1.1 | 974±3.9 |

ornamental plants additionally enrich the urban space [11]. So far many plants have been identified as useful for remediation of heavy metals-contaminated soils. However, very few among them are decorative perennials [12].

*Heuchera L.* is an evergreen perennial plant from North America. 'Purple Petticoats' cv. is a compact, hardy variety with decorative dark purple foliage (Fig. 1). The field experiment described previously [13] indicated that 'Purple Petticoats' cv. uptake Pb from soil and efficiently collects it in aboveground parts. This research was also carried out in outdoor conditions. The purpose of this experiment is to determine whether Pb uptake and its distribution in plant tissues can be affected by the age of 'Purple Petticoats' plants and soil fertilization.

## Material and Methods

Our research subjects were surface layers (0-0.2 m) of soil as well as roots and aerial parts of *Heuchera* cv. 'Purple Petticoats'. The biological material was obtained from plants that were grown August 2009-September 2012 on plots localized on post-mining, peripheral area in Będzin (Dąbrowskie Coal Basin). The estimated area concerned in this research is approximately 50 km$^2$ (Fig. 2). Soils of the Dąbrowskie Basin area belong to very light or medium light type, characterized by soil reaction in range: moderately acid (pH 5.64) to slightly alkaline (pH 7.85) [3]. Experimental plots under study have not been used for agriculture for the last 10 years. Basic analysis of soil fertility were made on an average soil sample before the start of this experiment. Nutrients essential for plants (N, P, K, Ca) were determined by the methodology specified by Breś et al. [14]. Results are summarized in Table 1.

Table 2. Total content of metal in soil before an experiment.

| Content | Cd | Pb | Zn |
|---|---|---|---|
| | mg·kg$^{-1}$ d.wt. | | |
| | 2.02±0.05 | 75.3±0.96 | 276.8±2.45 |
| Admissible concentration Soil standard for A group | 1 | 50 | 100 |

Cadmium, lead, and zinc are the basic pollution of heavy metal in the soil in this area. Their contents were determined at the beginning and at the end of the experiment. The concentrations of total form of the investigated metals together with the soil standards (admissible values) fixed by the Minister of the Environment's Decree for land protected on the basis of environmental protection regulations (grounds belonging to A group) [2] are shown in Table 2.

The experimental area was divided into three quarters sized at 6.5 m$^2$ each. A group of 10 six-week-old 'Purple Petticoats' seedlings was planted in each subplot. To improve the properties of the soil, and thus increase the productivity of plants, fertilizers were applied: Azofoska – artificial mineral fertilizer in powder form and Eko-użyźniacz – organic liquid based on vermicompost. Azofoska was used on one plot and Eko-użyźniacz on an adjacent plot. Azofoska is composed of such elements as: Mg, S, B, Cu, Fe, Mn, and Mo. Due to *Heuchera* plants' low demand for

Fig. 1. 'Purple Petticoats' cultivar of *Heuchera* L.

Table 3. Total dose of macronutrients with fertilizers.

| Element | Azofoska* | Eko-użyźniacz* |
|---|---|---|
| | $mg \cdot plant^{-1}$ | |
| Nitrogen (N) | 652.8 | 27.2 |
| Phosphorus (P) | 307.2 | 11.2 |
| Potassium (K) | 917.8 | 94.4 |

*Azofoska and Eko-użyźniacz are self-standing names describing subsequently nitrogen-phosphate artificial and chemicals free natural fertilizers.

nutrients, the amounts of N, P, and K provided with Azofoska was only about 30% demand of arable crops. The eco-friendly Eko-użyźniacz contains a high amount of microorganisms and enzymes associated with metabolism of earthworms. It improves soil properties and provides plants with nutrients. The amount of macronutrients from the fertilizers per one plant in the three-year period (2009-12) is given in Table 3. The dose of nutrients was obtained by a sum based on the chemical composition given by the manufacturer and mass of substances used to fertilize. Plants in the control group were not treated with any nutrients. Aerial parts and roots were manually separated after harvesting. Pieces of plant were washed separately for each group under running water and later in distilled water. Parts of plants were individually dried first on filter paper and then in an oven at 105ºC until they reached constant weight. Dried material was weighed to calculate the mass of leaves and root per plant. Calibrated micro-analytical balance SE Genius Sartorius with accuracy 0.000001 g and AS 220/C/2 Radwag with accuracy 0.0001 mg were used. Powdering of plant samples in a porcelain grinder was the next step. Soil samples were dried in room conditions and sieved to 1 mm diameter particles. Obtained material was stored until analysis in sealed polyethylene containers without light access.

## Chemical Analysis

Samples of leaves, roots, and soils, approximately 1 g each, were wet mineralized in concentrated nitric acid especially clean, company Baker. 5 $cm^3$ of acid and 1 $cm^3$ hydrogen peroxide was used on each sample. Special attention was given to cleanliness, homogeneity, and representativeness of samples. Propylene sieves, laboratory bags, and spatulas were used. Samples were analyzed in three repetitions. The contents of metallic elements in the soil at the start of the experiment were marked with atomic absorption spectrometry methods (AAS) using a PU 9100 spectrophotometer (Philips, UK) (Zn) and Perkin-Elmer 1400 ZL apparatus (Perkin-Elmer, USA) for Cd and Pb determination. Metallic element content was marked with flame atomic absorption spectrometry in the flame air-acetylene using a Karl Zeiss Jena-3 spectrometer at the end of the experimental period. The accuracy and precision were verified in each measuring series by analyzing certified reference material INCT-1 Mixed Polish Herbs.

Field research took 36 months. During this time the acidity of the soil fluctuated due to weather condition changes or seasons of the year. Soil pH was monitored twice in each growing season: at the beginning of April and at the end of September. Measurement of pH in soil was performed in ratio 1:2 (suspension in deionized $H_2O$) using a CP-125 pH-meter (Elmetron, Poland). The range of values was between 6.14-7.0. It can be presumed that shares of easily available fraction of metals were also changing. Interpretation of the results of determination of heavy metal concentration in soil at the beginning of the experiment performed according to the instructions of the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy shows slightly elevated level (I°) of Pb (75.3 $mg \cdot kg^{-1}$), and the amounts of Cd (2.02 $mg \cdot kg^{-1}$) and Zn (275.8 $mg \cdot kg^{-1}$) exemplified slight pollution (II°) [15].

## Statistical Analysis

This experiment was completely randomized. The type of fertilization was a single factor. Each variant of experiment was carried out in 10 replicates. Mean and standard deviation in the individual samples were calculated and considered by Student t-test. The examined differences

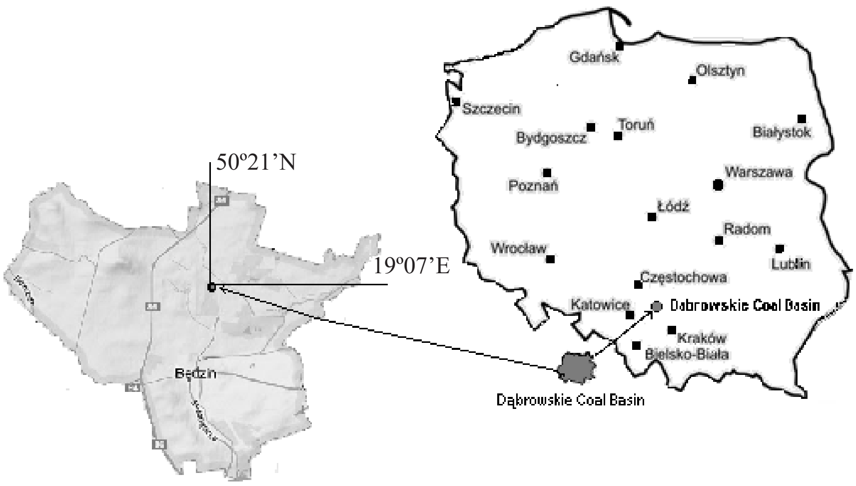


Fig. 2. Location of the study area on a map of Poland and a map of Będzin. Our own work is based on portals: Sosnowiec virtual and Pine virtuals.

Table 4. Content of Pb in soil and in organs *Heuchera* cv. 'Purple Petticoats' under a different methods of fertilization.

| Method of cultivation | Plant tissue | Yield | Pb content |
|---|---|---|---|
| | | mg·plant$^{-1}$ | mg·kg$^{-1}$ d.wt. |
| Azofoska** | leaves | 69.7±2.07 | 14.7±0.79a* |
| | roots | 13.2±0.90 | 27.9±1.12a |
| | soil | - | 64.7±1.62b |
| Eko-użyźniacz** | leaves | 71.9±2.33 | 13.0± 0.65a |
| | roots | 29.2±1.79 | 27.7±1.10a |
| | soil | - | 67.5±1.01b |
| Control | leaves | 40.2±1.15 | 13.7±0.70a |
| | roots | 14.3±1.69 | 29.8±1.15a |
| | soil | - | 58.6±1.50b |

*Means followed by the same letters are not significantly different at $\alpha$-0.05.
**Azofoska and Eko-użyźniacz are self-standing names describing subsequently nitrogen-phosphate artificial and chemicals free natural fertilizers.

were not statistically significant when probability was < 0.05. Data reported in this paper was analyzed with the use of the program Statistica 9.

## Results

Data in Table 4 show the Pb concentration in plant tissues of *Heuchera* cv. 'Purple Petticoats' and in soil after the plant harvest.

The total mass of Pb in plants at each plot, calculated by multiplication of the average amount of Pb accumulated by leaves and roots of one plant, mean that the value of dry mass of plant tissues and number of plants was 1392 mg (with Azofoska), 1744 mg (with Eko-użyźniacz) and respectively 975 mg on the control plot without fertilizing. Bioaccumulation factor (BAF) is the ratio of metal content in the plant to its concentration in the soil. This value informs about the plants' ability to uptake metal from the soil. An interpretation of degree accumulation by BAF is: ≤ 0.01 – non, ≤ 0.1 – poor, ≤ 1.0 – medium, > 1.0 – intensive ("hiperaccumulator") [20].

The mobility of the metal in plants determines the translocation factor (TF). It is the ratio of metal content in the aboveground parts (leaves) to its quantity in the roots. Both factors were calculated to assess uptake and movement of Pb in the test plants (Table 5).

## Discussion

Scientific reports show that some angiospermae plants have the ability to highly concentrate metals [21]. The absorption of toxic elements by the root system of plants is regulated by multiple relationships between the soil and the plants. Toxic effects of metals are due to the type of metal ions, their concentration, and type of plant (species or variety) and its growth stage. Several parameters of the soil that affect the absorption of metals by plants were specified:

- concentration of metallic elements
- enrichment of nutrients
- pH value
- content of calcium compounds.

Cd, Pb, and Zn are found associated in soils of the Dąbrowski Coal Basin. Factors in the soil that could favor the uptake of metals by plants in this experiment were: elevated trace element concentrations and a small amount of nutrients. This study focuses on the phytoexstraction Pb by *Heuchera* cv. 'Purple Petticoats.' The upper limit of "normal" concentration of Pb in the tissues of plants is 10 mg·kg$^{-1}$ [16]. The amount of Pb in organs of 'Purple Petticoats' exceeds this value, regardless of soil cultivation type. Parameters of soil quality that restrict the availability of metal ions for the plant from the soil solution were: neutral pH and high calcium content [17]. Although according to the statement by Herms and Brummer in the publication from 1991 [18], in the case of lead ions soil pH does not affect the bonding strength of the organic matter. Also, studies done by Yoon [19] have not shown the impact of soil pH on metals absorption in wild plants. The inhibition of Pb uptake by Ca is well described [20]. Wu and coauthors report on the antagonism of Pb assimilation when there was a high Zn concentration in the soil [21]. It is difficult to distinctly determine the occurrence of this effect in our study.

Due to the toxical effects of Pb on plants (i.a. disturbance of antioxidant enzyme synthesis, the formation of chlorophyll, and the photosynthetic process) translocation

Table 5. Comparison of BAF and TF for plants of *Heuchera* cv. 'Purple Petticoats' for different time and methods of fertilization.

| Index \ Method of growing | Azofoska** | Eko-użyźniacz** | Control (without fertilizers) | |
|---|---|---|---|---|
| Age [in monts] | 36 | 36 | 11* | 36 |
| Bioaccumulation factor | 0.57 | 0.54 | 0.11 | 0.58 |
| Translocation factor | 0.53 | 0.47 | 1.0 | 0.46 |

*Sąkol et al. 2012 [25]
**Azofoska and Eko-użyźniacz are self-standing names describing subsequently nitrogen-phosphate artificial and chemicals free natural fertilizers.

to shoots is low [20]. The vast majority of plants that absorb $Pb^{+2}$ from the soil stops them primarily in the root system. The phenomenon of effective lead movement to aboveground tissues occurs relatively rarely [17]. Based on studies of plants growing in the wild in areas contaminated by Pb in Florida, Yoon claims that in 95% of samples of the plants the concentration of Pb was higher in roots than in aerial parts [19]. Only a few plant species are characterized by a Pb translocation factor higher than 1. They belong i.a. to the Thsalpi and Brassica genus [22]. Pb is an element poorly mobile in soil and lowly bioaccumulated in plants, unlike Cd and Zn, which concentrates in plant tissues, especially in roots, where it is much higher than the concentration in the soil. This regularity was also observed in 3-year-old heucheras. In our research the median Cd content in soil was 1.5 $mg \cdot kg^{-1}$ to 7.15 $mg \cdot kg^{-1}$ in roots, respectively Zn content 171 $mg \cdot kg^{-1}$ unto 319 $mg \cdot kg^{-1}$, while median Pb content in soil was over two times higher than in roots (63.6 $mg \cdot kg^{-1}$ and 28.5 $mg \cdot kg^{-1}$).

Low-level Pb pollution of soil limits the growth of the roots and aboveground parts of the plants, though it does not cause necrosis or discoloration of leaves [23]. In these studies it was confirmed (visually and based on the dry mass of plant tissue) that the presence of Pb in the soil together with paucity of nutritional elements disturbed the development of all organs of plants on the control plot.

Plant tolerance to the destructive impact of toxic metals is based on a number of strategies of adaptation and the detoxification mechanisms, thanks to which ions of these metals can actively uptake and transport to the aboveground part. Metal form in plants has a decisive role. Toxic forms can be bonded at cell walls, away from sensitive sites, in a vacuolar compartment; they may create metal complexes with peptides (glutathione-phytochelatins, cysteine-metallothioneins), organic acids, and some amino acids [27].

Bioaccumulation factor (BAF) value is the main criterion for classification in terms of plant tolerance and ability to absorb metals. Together with the translocation factor (TF), it may be grounds to determine a plant's usefulness for phytoexstraction.

Young or annual plants have higher rates of translocation than plants growing on the same plot for a couple of seasons [24]. Though the 11-month-old plants *Heuchera* cv. 'Purple Petticoats' demonstrated greater ability for efficient Pb ion transfer from the roots to the leaves (TF = 1.0), the metal uptake was very limited (BAF = 0.11). Three-year cultivation on the polluted plot has changed their reaction to toxic elements. Plants were actively uptaking Pb from soil, and BAF increased more than 5 fold. However, long-lasting (36 months) toxic metal stress probably launched defensive processes in plants. A plant's response measured in this experiment was reduction of Pb displacement. TF value decreased almost two times. The soil enrichment in nutrients from fertilizers did not change the model of Pb uptake and transport in three-year-old plants in this test.

Adding fertilizers to the soil has resulted in biomass increase. Compared to unfertilized plants, even small doses of N, P, and K increased the average dry weight of plants respectively from 53% (Azofoska) to 86% (Eko-użyźniacz). Plant size is specified by number of leaves. The average number of leaves counted at the end of the experiment was 3.7 times greater than the number of leaves of plants after 11 months under review as described in a previous issue [25]. In effect the amount of Pb removed from the soil by aerial parts of one-season-grown plant is smaller than in an evergreen plant. Obtained results match the results of other authors. In older parts of plants the concentration of metal is many times higher than in younger [26].

According to the very sharp criterion of the phytoremediation concept for a specific environment treatment technology which is phytoexstraction, only species having BAF>1 and TF>1 can potentially be used. Phytoexstraction is defined as using plants to absorb toxic contaminants from the soil matrix, translocate and store into their root and shoot tissues which can be harvested as a yield, and then utilized [27]. Compared to other technical methods of purifying contaminated sites, the phytoexstraction is low cost, environmentally friendly, and an *in situ* applicable technique. That is why it is more and more often proposed for reclaiming metal-polluted soil, especially in areas with a medium degree of pollution. Selection of suitable plants is essential for this technique. According to Chakroun [28], plants that tolerate pollutants well and are able to collect and accumulate them in the tissues can be used in this method even if they don't meet the BAF criterion for "hyperaccumulator." Tomaszewska and Mazur [29] in their study of content of microelements in the leaves of *Schisandra chinensis*, conducted in similar variants as this experiment, received results in range of 14.50-22.17 $mg \cdot kg^{-1}$ for the accumulation of Pb. They have noted that *Schisandra chinensis* can be used for phytoremediation. The range of Pb concentrations in leaves of *Heuchera* is slightly less: 13.0-14.7 $mg \cdot kg^{-1}$.

As given in Tables 2 and 4 after the completion of the experiment there was less Pb in the soil of each plot than before the start. The best results in remediation in our experiment were obtained for control plot. Pb content in soil after the experiment was reduced by approximately 20%. The nutrient application to plants is closely related to the decreased toxic metal uptake [30]. This is a likely reason for the decrease of Pb content in plots dosed with Azofoska and Eko-użyźniacz only by 9%. In this context *Heuchera* cv. 'Purple Pettiocoats' may be used *in situ* as repeated crops to reduce the amount of Pb in the soil until the pollution decreases to the lowest acceptable level.

The contaminated biomass must be subjected to disposal, which can be obtained by ashing at 900-1000ºC in energy production or in hydrometallurgical processes [31]. The last solution is the newest technology – clean metal recovery from bio-ores made from waste-based products. The other method is thermal decomposition in pyrolysis process [32]. In the framework of ensuring chemical safety the Ministry of the Environment of Poland designates best available technologies, including lead-contaminated waste. A lead disposal technology of hazardous waste that consists of manufacturing of vitrified slag was implemented in 2011 [33].

The 'Purple Petticoats' cultivar of *Heuchera* is an attractive, decorative evergreen perennial. Its habitat and nutrition requirements are minimal [25]. The plants feature fast growth. The diameter of the rosette of leaves of two-year-old plants is about 0.35 m. 'Purple Petticoats' may be used for the arrangement of urban green spaces as a permanent "green carpet" to reduce exposure to lead. According to the general recommendations of landscape architects, six items can be planted on 1 $m^2$.

## Conclusions

*Heuchera* cv. 'Purple Petticoats' is a decorative perennial resistant to adverse environmental conditions and tolerant to toxic metals in polluted soil. The test plants *Heuchera* cv. 'Purple Petticoats' showed a greater concentration of lead in tissues than given in the literature as normal. The ability to collect lead in the tissues of the plants based on the value of the bioaccumulation factor was classified as medium. The ability of Pb translocation from roots to aboveground parts is decreasing with time, but increasing biomass increases the amount of Pb in the plant tissues. Due to the medium level of lead translocation (TF~0.5) of a several-year-old 'Purple Petticoats' plant, it can be used for natural lead phytoexstraction on slightly polluted sites. Removal and disposal of whole plants gives a greater clean-up effect of soil. Plants of *Heuchera* cv. 'Purple Petticoats' growing three years in a post-mining area in Dąbrowskie Coal Basin reduced approximately 9% of Pb pollution in soil.

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