Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2015.1017330

57 (2016) 1469–1477
January

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Assessment of the availability of heavy metals to plants based on the translocation index and the bioaccumulation factor

Piotr Pachura, Agnieszka Ociepa-Kubicka*, Beata Skowron-Grabowska

Faculty of Management, Czestochowa University of Technology, 42-200 Czestochowa, Poland, Tel. +48608469741; email: agnieszkaociepa22@wp.pl (A. Ociepa-Kubicka)

Received 10 July 2014; Accepted 19 January 2015

ABSTRACT

The bioconcentration factors and the translocation index have been determined for the assessment of the level of phytoextraction in plants. The bioaccumulation factor, determined as the quotient of the content of a given metal of the plant to its content of soil, defines the ability of the plant to accumulate heavy metals. The translocation index, T_i, is a measure of the phytoextraction capacity of plants. This index provides a useful means of the quantitative description of relative differences in the biological availability of metals to plants. This paper presents the results of studies on the effect of fertilizing soil on the ability to translocate cadmium, zinc, lead, and nickel from the soil to the roots, and then to the above-ground parts of Virginia fanpetals. The source of the results was a pot experiment conducted under semi-natural conditions from April 2007 to November 2011. The studies described in this paper concern the last year of the experiment (the plant crop in November 2011). Six fertilization combinations plus soil control were used in the experiment, each in three repetitions. Sewage sludge in three doses: 10, 20, and 40 t/ha, and two composts (one made from Dano urban greenery, and one produced from sewage sludge and forest waste) in a dose of 20 t/ha were used for plant cultivation. Great differences in bioaccumulation indices were observed between the above-ground parts and the roots of the plants and between individual metals, while there were small differences between individual fertilization types. The level of translocation of metals from the underground parts to the above-ground ones depended primarily on the kind of metal and fertilization applied. By analyzing the values of the translocation index, T_i for individual metals, the following relationship was obtained. It should be noted that definitely higher T_i index values were obtained for cadmium and zinc than for lead. The zinc translocation amounted to approx. 40–50%, while the translocation of cadmium reached a level of even approx. 70–80%. The lead translocation was very low, amounting to about 10% at the maximum. The low mobility of lead in the root—above-ground part system reduced the phytoextraction process.

Keywords: Heavy metals; Translocation; BFC

*Corresponding author.

Presented at the 12th Scientific Conference on Microcontaminants in Human Environment 25–27 September 2014, Czestochowa, Poland

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1. Introduction

The bioavailability of heavy metals depends on soil conditions, the plant species, the kind of metal, and environmental factors [1,2]. All of the above-mentioned factors jointly determine what quantity of heavy metals will be absorbed by the plants, and what will be immobilized in the soil environment. Fertilization may significantly modify the soil environment by influencing the pH, the organic matter content, sorption capacity, the contents of Fe and Mn oxides, and grain size distribution, which has an effect on the accumulation of metals in plant biomass. Metals can be immobilized in the soil, due to exchangeable, biological, chemical, physical, and mechanical sorption [3–6]. Organic matter forms simple or complex chelate compounds with heavy metals. These prevent trace elements from moving by immobilizing them in the soil.

Contradictory views on the relationship between the overall contents of metals of soil and their availability to plants can be found in the literature. Some studies indicate a close correlation between the above factors, while others do not confirm the relationship between the overall trace element contents of soil and the quantity of trace elements taken in by the plants [7,8].

The purpose of the studies presented in this paper was to determine the effect of diversified fertilization on the contents of zinc, cadmium, and lead of Virginia fanpetals biomass. When introduced into the soil with sewage sludge and composts organic matter while maintaining a neutral, it can effectively reduce the phytoavailability of metals. An important issue is the durability of the effect of such soil fertilization on the reduction of the migration of metals. There are no exhaustive studies on this subject. For a more complete assessment of the phytoextraction and phytostabilization process conditions varying under the influence of fertilizing substances applied, the bioaccumulation factor (BCF) and translocation factor (T_i) were determined.

2. Material and methods

2.1. Materials

A grain size analysis showed that the soil used for the experiment belonged to light soils. It was characterized by a slight contamination with zinc and cadmium (acc. to the IUNG (Institute of Soil Science and Plant Cultivation) scale) and an increased content of lead (Table 1)[9]. The contents of copper, nickel, and chromium were at the level of natural contents.

Table 1
The soil characteristic used for the experiment

pH	6.0
C_{or}, %	1.38
N, %	0.10
P, mg/kg d.m.	19.4
K, mg/kg d.m.	24.0
Pb, mg/kg d.m.	39.0
Cd, mg/kg d.m.	1.18
Zn, mg/kg d.m.	122.2
Cu, mg/kg d.m.	8.3
Ni, mg/kg d.m.	9.20
Cr, mg/kg d.m.	13.0

2.1.1. Description of the sewage sludge and composts used in the experiment

Stabilized, dehydrated sewage sludge, with a slightly acid reaction, a high organic matter content, and a small amount of heavy metals, was used for fertilizing. [10] The sewage sludge was taken from the small sewage treatment plant. The characteristics of the substrates used for soil fertilization is given in Table 2.

The sewage sludge and composts used for fertilization were characterized by good fertilizing properties owing to their nitrogen, phosphorus, and potassium contents. Their total heavy metal contents were relatively low, which allowed them to be used for agricultural purposes and for energy crops**.** The examined composts and sewage sludge met the requirements of the regulation **[**10], therefore were eligible for being used for soil reclamation.

2.2. Experimental procedure

The investigation was based on the analysis of soil and plant samples obtained from a pot experiment which started in 2007, and the results presented in this work come from the year 2011 data. For the experiment, about 300 kg of soil from an area located in the southern polish was taken from a 0–25 cm deep layer. The soil material was taken following the applicable procedure, so that it would reflect the condition of the examined area as faithfully as possible [11]. The pot experiment was prepared in accordance with the scheme shown in Table 3.

Six fertilization types plus unfertilized soil were used in the experiment. Each of the fertilization combinations was executed in three repetitions. In total, 21 pots were setup, each with 12 kg soil. Two *V. fanpetals* seedlings were planted in each pot. The sewage

Table 2
Characteristics of substrates

Indicator	Sewage sludge	Compost from sewage and waste from forestry	Compost Dano
C, % d.m	25.06	18.80	19.00
N, % d.m	4.24	2.50	2.28
P, % d.m	2.50	1.20	0.98
K, % d.m	0.50	1.05	1.1
Ca, mg/kg	28,000.00	8,500.00	1,540.00
Mg, mg/kg	4,600.00	1,000.00	580.00
Pb, mg/kg	40.00	25.00	52.00
Cd, mg/kg	2.40	1.20	1.40
Zn, mg/kg	1,000.00	270.00	242.00
Cu, mg/kg	160.00	48.00	40.00
Ni, mg/kg	18.00	19.00	14.00
Cr, mg/kg	22.00	16.00	15.00
Salmonella	lack	lack	lack
Ascaris sp., *Trichuris* sp., and *Toxocara* sp., szt./kg s.m.	3	lack	lack
pH w H_2O	6.5	6.80	7.80

Table 3
The fertilization combinations investigated in the pot experiment

Fertilization combination	Type and dose of fertilizer
O	Sample—soil without fertilization
I os	Soil amended with sewage sludge in a dose of 10 t d.m./ha
II os	Soil amended with sewage sludge in a dose of 20 t d.m./ha /ha
III os	Soil amended with sewage sludge in a dose of 40 t d.m./ha /ha
D	Soil amended with Dano-type compost from urban greenery, in a dose of 20 t d.m./ha
K	Soil amended with compost from sewage sludge and forest waste in a dose of 20 t d.m./ha
NPK	Soil amended with NPK (carbamide, potash salt, and superphosphate), in a dose of N–120 kg/ha, P_2O_5–80 kg/ha, K_2O–100 kg/ha

sludge used for the experiment originated from the municipal sewage treatment plant. In addition to the sewage sludge, compost produced from the small sewage and forest waste was also used for fertilization. Moreover, fertilization with Dano compost produced from waste urban greenery was used (Table 1).

2.3. Analytical methods

(1) pH in H_2O—the measurement was made by the potentiometric method in accordance with with PN-ISO-10390:1997.
(2) The organic carbon content was determined by the modified Tiurin colorimetric method in accordance with PN-ISO 14235:2003.
(3) The overall Kjeldahl nitrogen quantity was determined in accordance with PN-ISO 11261:2002 using a BUCHI 426 mineralizer and a BUCHI 323 distilling apparatus.
(4) The overall phosphorus (in the phosphate from) was determined by the molybdate method in accordance with PN-EN 1189:2000.
(5) The potassium was determined by the Egner–Riehm method (PN-R-04023:1996), by extracting it with a calcium lactate solution and determining the color of the phosphomolybdate complex on an HACH spectrophotometer for a wavelength of L = 660 nm.
(6) The heavy metal contents of the soil and plant biomass were assayed on a Thermo ICP-AES plasma spectrophotometer according to PN-ISO 11047: 2001, after the material having been previously mineralized in concentrated nitric acid using a Plasmotronik UniClever microwave mineralizer.

Table 4
The content of cadmium in soil

Fertilization combination	Cadmium in soil geochemical after reaching equilibrium			Cadmium in the soil after the fourth year of the experiment		
		Bioavailable forms			Bioavailable forms	
	Total content (mg/kg)	(mg/kg)	The content of total (%)	The content of total (mg/kg)	(mg/kg)	The content of total (%)
O	1.18	0.06	5.08	1.12	0.08	7.14
I os	1.19*	0.06	5.04	1.11	0.06	5.41
II os	1.20*	0.07*	8.40	1.12	0.08	7.14
III os	1.23*	0.05*	6.15	1.15*	0.08	6.96
D	1.19*	0.07*	8.33	1.14*	0.09*	7.70
K	1.19*	0.06	7.14	1.10*	0.07*	6.36
NPK	1.19^{n}	0.06	7.14	1.16*	0.08	6.90

Significance at a confidence level:
$^{*}p = 0.05$.

Table 5
The content of lead in soil

Fertilization combination	Lead in soil geochemical after reaching equilibrium			Lead in the soil after the fourth year of the experiment		
		Bioavailable forms			Bioavailable forms	
	The content of total (mg/kg)	(mg/kg)	The content of total (%)	The content of total (mg/kg)	(mg/kg)	The content of total (%)
O	39.00	0.24	0.69	37.40	0.27	0.72
I os	39.15*	0.20*	0.62	37.50	0.22*	0.59
II os	39.26*	0.21	0.53	36.90*	0.22*	0.60
III os	40.01*	0.19*	0.47	38.80*	0.19*	0.49
D	39.60*	0.19*	0.48	38.20*	0.20*	0.52
K	38.50*	0.20*	0.51	37.10*	0.19*	0.51
NPK	39.15*	0.14*	0.43	37.60	0.19*	0.50

Significance at a confidence level:
$^{*}p = 0.05$.

(7) Bioavailable forms of 0.01 M $CaCl_2$.
(8) The presence of the ova of the intestinal parasites *Ascaris* sp., *Trichuris* sp., and *Toxocara* sp. was determined in accordance with Standard PN-Z-19000-4:2001. Salmonella were determined in accordance with Standard PN-ISO 6579:1998.

2.4. BCF and translocation index

The BCF is determined as the quotient of the content of a given metal of the plant to its total content of soil. The calculation of BCF

$$\mathrm{BCF} = \frac{C_B}{C_G}$$

where C_B—metal concentration in the tissues of the plant overground/underground organs (g kg^{-1}), C_G—initial concentration of metal in the soil (mg kg^{-1}).

Translocation index T_i:

$$T_i = (C_B/C_K) \times 100$$

where T_i—translocation index of metals (%); C_B—metal concentration in the tissues of organs above ground plant (mg kg^{-1}); and C_K—metal concentration in the tissues of the plant roots (mg kg^{-1}).

2.5. Statistical methods

The obtained results were subjected to statistical analysis by the variance analysis and single-factor

Table 6
The content of zinc in soil

Fertilization combination	Zinc in soil geochemical after reaching equilibrium			Zinc in the soil after the fourth year of the experiment		
		Bioavailable forms			Bioavailable forms	
	The content of total (mg/kg)	(mg/kg)	The content of total (%)	The content of total (mg/kg)	(mg/kg)	The content of total (%)
O	122.20	4.70	3.85	118.50	5.10	4.30
I os	124.30*	4.31*	3.47	121.80*	4.35*	3.57
II os	126.40*	3.92*	3.10	122.45*	3.90*	3.18
III os	130.00*	3.15*	2.42	126.00*	4.00*	3.17
D	122.60	3.18*	2.59	120.00*	4.45*	3.71
K	123.40*	3.01*	2.44	120.30*	5.00	4.15
NPK	122.80*	2.80*	2.28	119.70	5.70*	4.76

Significance at a confidence level:
$^*p = 0.05$.

regression methods. A detailed analysis of the significance of differences between the results of individual fertilization combinations as against the control was made using the student test at a significance level of $p = 0.05$.

3. Results and discussion

3.1. *The effect of fertilization on the cadmium, lead, and zinc contents of* V. fanpetals *biomass*

The unfertilized soil (control) had an overall cadmium content of 1.18 mg/kg soil, a lead content of 39.0 mg/kg, and a zinc content of 122.2 mg/kg. As a result of fertilization, the overall Cd, Pb, and Zn contents increased compared to the control, which was due to the introduction of these metals with the fertilizers. The highest metal contents were found upon introducing sewage sludges in a dose of 40 t/ha to the soil; they were as follows: Cd = 1.23 mg/kg d.m., Pb = 40.00 mg/kg d.m., and Zn = 130.0 mg/kg d.m soil. It should be noted that the applied fertilizing materials changed the total Zn, Cd, and Pb contents of soil to a small extent, which did not change the class of contamination with the metals in question. However, they could have had the effect of changing the form of the metals in the soil, thus influencing their bioavailability (Tables 4–6).

Therefore, the main aim of the investigation was to determine the effect of the applied fertilization on the absorption of cadmium, lead, and zinc by *V. fanpetals*. The investigation results given in Table 7 apply to the fourth year of the experiment (the 2011 crop). In the biomass of plants grown on the control soil, the Zn, Cd, and Pb contents of the above-ground parts were higher compared with the plants grown on the improved soils (except for cadmium, where its highest content was found in plants fertilized with compost). Studies by other authors indicate much broader ranges of metal accumulations in *V. fanpetals*, amounting to 0.57–24.6 mg/kg d.m. for Cd, 0.2–22.7 mg/kg d.m. for Pb, 11.7–489.0 mg/kg d.m for Zn, 1.2–190.0 mg/kg d.m. for Ni, and up to 10 mg/kg d.m. for Cu [12,13]. According to the studies by Borkowska and Styk [14], the range of metal contents found in *V. fanpetals* is up to 25 mg/kg d.m. for Cd, up to 23 mg/kg d.m. for Ni, up to 270 mg/kg d.m. for Zn, and up to 245 mg/kg d.m for Cr. These differences are primarily due to the very diverse contamination of the soils with metals, and the different organic matter content, and pH of the examined soils, which has a direct impact on the uptake of metals. Some authors suggest that the availability of heavy metals to plants may increase with the simultaneous accumulation of some of them in the soil. In soils, where excessive contents of zinc, cadmium, and lead occurred at the same time, such a phenomenon was observed by Bradl [15] and Karaca [16]. This is most probably linked with the desorption action of the metals [17].

The analysis of the results indicates a significant influence of fertilization on the content of Zn and Cd, while a generally statistically insignificant effect of fertilization on the Pb content of the above-ground parts of plants. It should be emphasized that most preferred to reduce the Cd content in the biomass of *V. fanpetals* fertilization sediments (II os), Zn—fertilization sediments (III os), and for Pb—fertilization with composts (K) (Table 7). The following metal content reductions

Table 7
The metal contents of *V. fanpetals* biomass (mg/kg d.m.)

	Cd		Pb		Zn	
Fertilization combination	Partial-ground	Root	Partial-ground	Root	Partial-ground	Root
O	2.13	2.56	2.62	24.2	62.8	100.0
I os	2.00*	2.80*	2.60	24.3	62.0	106.2*
II os	1.92*	3.00*	2.70*	24.8	62.5	115.0*
III os	2.13*	2.95*	2.60	30.0*	55.5*	120.5*
D	2.10*	2.85*	2.56	32.0*	60.0^{ns}	119.0*
K	2.19*	3.05*	2.40*	33.5*	56.0*	115.0*
NPK	2.05*	2.50	2.58	31.5*	58.0*	105.0

Significance at a confidence level:
$^{*}p = 0.05$.

were found for respective fertilization types, as against the control: a 10% cadmium content reduction for fertilization with IIos; a 22% zinc content reduction for fertilization with III o; and a 9% lead content reduction for fertilization with K. The lower heavy metal accumulation in the plants grown on the fertilized soil was due to an increase in soil pH and its enrichment in organic matter. In soils fertilized organically, the complexing properties are enhanced, due to which stable organometallic bonds form that reduce the availability of heavy metals to plants. In order to reduce heavy metal uptake by plants, numerous authors recommend organic fertilizers that slowly undergo mineralization processes, within the neutral range of pH [18,19]. Based on the 25 year long fertilization of soils with manure, Hao and Chang [20] found a significant increase in the degree of soil saturation with basic elements, which resulted in a reduction of heavy metal contents of the plants. The obtained fourth year's results show that *V. fanpetals* has the capability to take in metals, such as Cd, Zn, and Pb, and extract them from the soil. The biomass of these plants contains larger heavy metal quantities than the respective quantities for purple willow, privet, or Jerusalem artichoke reported in the literature. They also take in larger quantities of Cd and Zn than cereals do [21]. The most commonly reported average contents of selected metals, depending on the species, are as follows: straw of cereals: Zn, 37.0 mg/kg d.m; Cd, approx. 0.05–0.19 mg/kg d.m; and Pb, 0.20–5.0 mg/kg d.m. In the cereal grains, these contents are considerably lower, amounting to, respectively: Zn, 20.0 mg/kg d.m; Cd, 0.05 mg/kg d.m; and Pb, approx. 0.10 mg/kg d.m. The concentration of heavy metals in the above-ground plant parts was relatively high compared with straws or cereals, but much lower than in hyperaccumulators.

Higher contents of all metals of the roots of plants, as compared to their above-ground parts, were found. For Pb, these differences were exceptionally large. The Pb content of the roots was several or even a dozen or sometimes greater than the above-ground parts. The smallest differences in the metal content between the roots and the above-ground parts were found for Cd, which gives the grounds for presuming that for cadmium, in contrast to Pb, the root system does not provide an effective barrier. The level of metal accumulation in the roots and above-ground parts was dependent primarily on the kind of metal and, to a lesser extent, on soil fertilization.

3.2. Bioaccumulation of metals

Bioaccumulation defines the ability of the plant to accumulate heavy metals, while allowing for their initial substrate content. The higher the values that it takes on, the higher the concentration of the element is found in the plant biomass as against its initial substrate content. As a basis for assessing the bioaccumulation of metals, the four-degree scale described in [22] was adopted. According to this scale, a BCF of < 0.01 means no accumulation; 0.01–0.1—low bioaccumulation; 0.1–1.0—medium bioaccumulation; and above 1.0—high bioaccumulation.

It should be noted that the accumulation factors for all the examined metals were considerably higher for roots than for above-ground parts (Figs. 1 and 2). The *V. fanpetals* roots accumulated Cd at the high level with small differences between individual soil improvement modes, while Zn and Pb at the medium level. The above-ground parts accumulated Cd at the high level, Zn at the medium level, and Pb at the low level. The BCF factors calculated for the above-ground parts, similarly as their counterparts for the roots, indicate a small effect of fertilization on the accumulation of metals in plants. Among the examined metals, the highest accumulation was exhibited by cadmium,

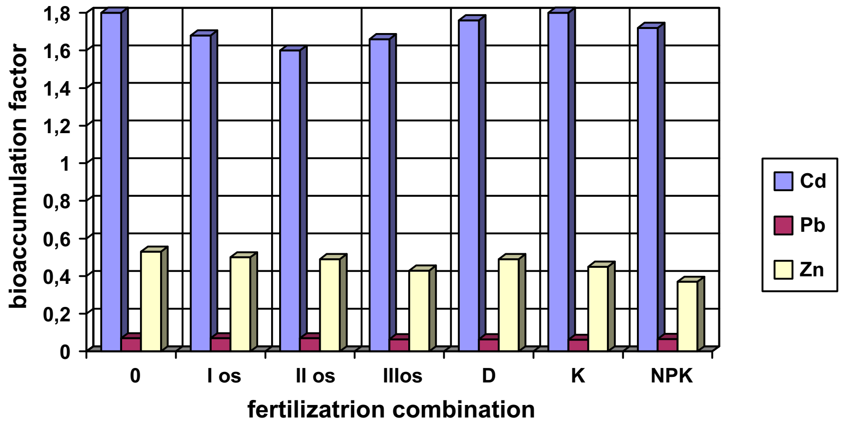

Fig. 1. The BCF for the ground Virginia.

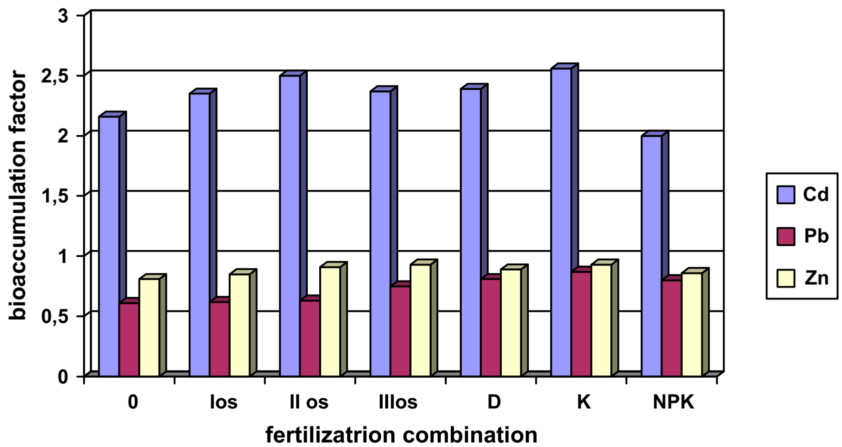

Fig. 2. The BCF for the roots of Virginia.

both in the above-ground parts and in the roots. In the above-ground parts, the lead accumulation was several or even a dozen or sometimes lower than in the roots, depending on the fertilization mode.

The translocation index, T_i, is a measure of the phytoextraction capacity of plants. It indicates that there is a possibility of the translocation of metals from the roots to the above-ground parts of plants. The degree of metal translocation from the underground parts to the above-ground parts depended primarily on the kind of metal and the substrate used (Table 8). By analyzing the values of T_i for individual metals, the relationship Cd > Zn > Pb was obtained. The lead translocation was very low and was contained in the range from 7.16 to 10.86%, depending on the fertilization mode. This means that the mobility of lead in the root—above-ground part system was very low, which reduced the phytoextraction process. Studies by other authors confirm that the quantity of the lead retained in the roots may amount to approx. 90%

Table 8
Metal translocation factor values for *V. fanpetals* after year IV of the experiment

Fertilization combination	Cd	Pb	Zn
O	83.0	10.8	62.8
I os	71.0	10.7	58.4
II os	64.0	10.8	54.3
III os	72.2	8.7	46.3
D	73.7	8.0	50.0
K	65.6	7.2	48.7
NPK	82.4	8.2	55.0

of the metal taken up [4,23]. The translocation of cadmium from the roots to the above-ground parts under the conditions of the conducted experiment was lower than the average of approx. 20% reported for this metal in the literature [24]. Definitely higher was zinc translocation, amounting to approx. 40–50%; while translocation of cadmium reached levels as high as approx. 70–80%. The investigation results show clearly that the quantities of metals moving from the roots to the above-ground parts of *V. fanpetals* were determined primarily by the kind of metal and, to a much lesser extent, by the type of fertilization. The sewage sludges and composts introduced to the soil only reduced translocation of metals to the above-ground parts to a small extent.

4. Conclusion

From the investigation carried out, the following conclusions have been drawn:

(1) The quantity of heavy metals taken in by *V. fanpetals* depended primarily on the kind of metal, and to a lesser extent on the fertilization applied. Among the examined heavy metals, the highest mobility was exhibited by cadmium, while the lowest one by lead.

(2) Fertilization with sewage sludge in the highest dose of 40 t/ha, in spite of causing the highest concentration of all Cd, Pb, and Zn in the soil, did not result in the highest metal uptake from the soil. The reduction of the heavy metal mobility with this fertilization resulted from the considerable enrichment of the soil in the organic matter. The content metal in biomass plant fertilization sediments in doses of 10, 20, and 40 Mg/ha was at the similar level.

(3) A higher content of all of the investigated metals of plant roots was found, compared to the above-ground part. Lead bioaccumulation in the roots was by several or even a dozen or sometimes higher than in the above-ground parts.

(4) Cadmium and zinc showed much higher translocation from the roots to the above-ground parts than lead. The maximum value of cadmium translocation was about 80% and about 60% zinc. Lead translocation was very low, amounting to a maximum of about 10%. The low mobility of lead in the root—above-ground part system reduced the phytoextraction process.

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