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Continuous and Induced Phytoextraction — Plant-Based Methods to Remove Heavy Metals from Contaminated Soil

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Additional information is available at the end of the chapter

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1. Introduction

As a result of the continuous civilization progress and the increasing human population we have been observing an accelerating process of environmental pollution, frequently leading to its complete degradation. The primary cause of environmental pollution is associated with the rapid development of motorization and industry (particularly power industry and mining), progressing urbanization, improved standards of living worldwide, intensive farming (application of high amounts of mineral fertilizers and herbicides), along with numerous other anthropogenic factors. These sources contribute to increased concentrations of many chemical elements and compounds in the atmosphere, soil, water and plants (including crops with edible parts for human consumption).

The cycle of chemical elements and compounds in nature is influenced not only by human activity, but also nature itself, in which progressing geological processes occur such as volcanic eruptions, shifts of tectonic plates or natural disasters.

In recent years the ecological awareness of the general public has increased, with decontamination of the polluted environment being perceived as an essential requirement. The aim of reclamation of polluted areas is to restore ecosystems polluted by human activity to the condition resembling their former natural state.

There are many methods applied in environment decontamination worldwide, including const-intensive, conventional physico-chemical methods. Scientists continue to search for novel, more effective and economically viable methods of pollutant inactivation. In recent

years an increasing body of research has focused on engineering bioremediation, such as e.g. an *in situ* technology of phytoremediation, utilizing the capacity of plants to accumulate high amounts of pollutants from the environment. Research conducted for many years now has identified a relatively numerous group of plants potentially applicable in this new environment decontamination technology.

At present a major ecological problem is connected with the penetration to the environment of heavy metals, which at higher concentrations are strongly toxic to humans and animals (resulting in e.g. increased disease incidence), and have a negative effect on soil properties as well as quality and physiological activity of plants.

Literature sources present various definitions of an element to be considered a heavy metal. One of these hypotheses says that they are elements with specific gravity greater than 4.5, 5, 6 and 7 g cm⁻³. It is a physical term, which is understood and used differently in various contexts. There are also many definitions based on atomic number. Some of them are considered to be nutrients for living organisms (essential nutrients e.g. Fe, Cu, Zn, Mn, Ni) and others are redundant or toxic (e.g. Cd, Pb, Hg, Al, As). Their common characteristic is connected with the fact that at excessive concentrations in the environment they have an adverse effect on plant growth and development, and when incorporated in the food chain may also pose a hazard for animals and humans.

2. Soil factors influencing availability of heavy metals to plants

Physico-chemical properties of soil influencing contents of phytotoxic forms of heavy metals include the type of soil, its grain size distribution, reaction, organic matter content, sorption properties and redox potential [43, 67, 68, 73].

Mother rock is a natural source of heavy metals in soils. The amounts of elements coming from mother rock constitute the geochemical background posing no threat to soil fertility [67]. Other sources of heavy metals include geochemical processes and anthropogenic factors. In soil heavy metals are subjected to processes affecting their concentrations and chemical forms [4]. In individual soil horizons their content depends on anthropogenic and climatic factors [67, 114].

Soil reaction is a major factor influencing the form and availability of heavy metals to plants [67]. Soil acidity may lead to their increased concentrations in soil [3, 13, 30, 48, 93, 112] and their excessive uptake by plants [42, 93]. According to Tyler and Olsson [118], concentrations of Cu and Pb increase also at pH 7.5 – 7.8 as a result of formation of stable complexes with ligands, which solubility is connected with solubility of organic substance. Liming results in a reduced content of available forms of heavy metals in soil [39]. Soil reaction is a factor determining the force with which heavy metals are bound by organic substance and mineral compounds [2, 60].

Organic substance found in soil contributes to the limitation of the amounts of heavy metals available to plants [38, 47], since it binds very strongly Cr³⁺, Fe³⁺, Pb²⁺, Ni²⁺ and Co²⁺ ions and

to a lesser extent also Mn^{2+} and Zn^{2+} [68]. Organic matter binds heavy metals into water insoluble forms or forms sparingly soluble in water [79], thus reducing the share of plant-available forms of heavy metals [19], and in this way it limits their toxicity to plants [48, 87].

Heavy metals differ in the force, with which they are bound by the sorption complex and they are connected mainly with the silt fraction [67]. In soil they undergo exchange and biological sorption. They may also be precipitated in the form of insoluble compounds [83]. Availability of heavy metals in soil is influenced by the cation sorption capacity. Introduction of compounds enhancing sorption capacity to soil causes a reduction of amounts of available metal forms in soil [106].

Availability of heavy metals is also dependent on the redox reactions taking place in soil [83]. Oxide forms of heavy metals become readily available to plants at a low redox potential [33].

The application of mineral fertilizers changes amounts of phytoavailable forms of heavy metals in soil. The effect of used fertilizers on physico-chemical and biological properties of soil causes a reduction or an increase of amounts of phytotoxic forms in the soil environment [105]. When applying mineral fertilizers we introduce heavy metals to soil, which contents in fertilizers are connected both with the raw material from which they were produced and the technological process of fertilizer production. The greatest contamination with heavy metals, particularly cadmium, is found in phosphorus fertilizers [52] and calcium fertilizers, mainly being by-products of various branches of industry [27, 69]. Heavy metal contents in phosphorus fertilizers depend on the fertilizer type [52] and solubility [57]. The application of phosphorus fertilizers leads to the transition of soluble phosphate forms into sparingly soluble zinc, copper, cadmium and lead phosphates [30], leading to the reduction of heavy metal contents in plants.

3. Admissible heavy metal contents in soil binding in Poland

In Poland respective boundary heavy metal contents are specified in the Ordinance of the Minister of the Environment of 9 September 2002 on soil quality standards and land quality standards. These standards were established taking into consideration the current and forecasted functions for the following categories of land types (Table 1):

1. Category A:
 - a. Landed property incorporated into an area legally protected on the power of the regulations of the Water Act,
 - b. Areas protected on the power of regulations on nature protection if maintenance of the current soil pollution levels does not pose a threat for human health or the environment – for these areas the concentrations meet the standards resulting from the actual status, subject to points 2 and 3;
2. Category B – land classified as agriculturally utilized area except for land covered by ponds and ditches, forested areas as well as areas covered by trees and shrubs, barren

land, as well as developed and urbanized areas except for industrial areas, surface mining land in use and municipal areas;

3. Category C – industrial areas, surface mining land in use, transportation areas.

Attachment to the Ordinance of the Minister of the Environment of 9 September 2002 (item 1359)

Pollutant	Category A	Category B					Category C			
		Depth [m below ground level]								
		0-0.3	0.3-15.0		>15		0-2	2-15		
		Water permeability of soil [m/s]								
		Up to		below	Up to		below	Up to		below
		1•10 ⁻⁷		1•10 ⁻⁷		1•10 ⁻⁷				
Arsenic	20	20	20	25	25	55	60	25	100	
Barium	200	200	250	320	300	650	1000	300	3000	
Chromium	50	150	150	190	150	380	500	150	800	
Tin	20	20	30	50	40	300	350	40	300	
Zinc	100	300	350	300	300	720	1000	300	3000	
Cadmium	1	4	5	6	4	10	15	6	20	
Cobalt	20	20	30	60	50	120	200	50	300	
Copper	30	150	100	100	100	200	600	200	1000	
Molibdenum	10	10	10	40	30	210	250	30	200	
Nickel	35	100	50	100	70	210	300	70	500	
Lead	50	100	100	200	100	200	600	200	1000	
Mercury	0,5	2	3	5	4	10	30	4	50	

Table 1. Admissible concentrations in soil (mg kg⁻¹ dry matter)

Based on the multiannual analyses the Institute of Soil Science and Plant Cultivation in Puławy, Poland (IUNG) specified boundary heavy metal contents in soils to be utilized agriculturally (Table 2). As it was reported by Ociepa et al. [94], the mean content of heavy metals in agriculturally utilized soils in Poland is lower than in countries of Western Europe or the USA, which results mainly from a lesser share of farms with intensive agricultural production systems and a lesser intensity of industrial processes. The same authors reported that approx. 90% agriculturally utilized area have natural levels of toxic metals. Several percent of this area have elevated contents (I⁰) of mainly cadmium and zinc, while approx. 3% are polluted (II-V⁰) with metals. The greatest percentages of polluted soils are found in the Śląskie, Małopolskie and Wrocławskie provinces of Poland.

Metal	Soil class*	Soil pollution rates					
		0	I	II	III	IV	V
lead (Pb)	a	30	70	100	500	2500	>2500
	b	50	100	250	1000	5000	>5000
	c	70	200	500	2000	7000	>7000
zinc (Zn)	a	50	100	300	700	3000	>3000
	b	70	200	500	1500	5000	>5000
	c	100	300	1000	3000	8000	>8000
copper (Cu)	a	15	30	50	150	750	>750
	b	25	50	80	100	500	>500
	c	40	70	100	150	750	>750
nickel (Ni)	a	10	30	50	100	400	>400
	b	25	50	75	150	600	>600
	c	50	75	100	300	1000	>1000
cadmium (Cd)	a	0,3	1,0	2	3	5	>5
	b	0,5	1,5	3	5	10	>10
	c	1,0	3,0	5	10	20	>20

Pollution rate: 0 – natural content, I – elevated content, II – weak pollution, III – medium pollution, IV – strong pollution, V – very strong pollution

*soil classes:

a. Very light soils with low silt and clay contents (< 10%), irrespective of pH (in KCl)

- light soils (10 - 20% silt and clay), highly acid (pH < 4.5) and acid (pH 4.5 – 5.5)

b. Light soils (10 - 20% silt and clay), neutral reaction (pH > 6.5)

- medium soils (20 - 35% silt and clay), highly acid (pH < 4.5) and acid (pH 4.5 – 5.5)

c. heavy soils (>35% silt and clay), highly acid (pH < 4.5)

- mineral organic soils (organic substance at 6 - 10%)

d. Medium heavy soils (20-30% silt and clay) and heavy soils (> 35%).

Table 2. Boundary values in mg kg⁻¹ heavy metals in the surface layer (0 – 20 cm) of soils with different degrees of contamination (IUNG 1992)

4. Contents of heavy metal soluble forms and reaction of surface horizon (0 - 20 cm) of soils in green belts adjacent to selected transportation routes in the city of Poznań (western Poland)

Anthropopressure affects physical, biological and chemical properties of soil. Soils in urban areas, located along transportation routes, are exposed to heavy metal pollution, originating from substances produced during combustion of fuels, abrasion of road surfaces and tires,

granular materials falling onto the ground during transport, etc. [24, 36]. Platinum metals, which have been employed in production of car catalysts, reach environment and cause contamination of soil, plants and water [84]. Moreover, chemical substances used in winter to eliminate black ice (e.g. sodium or calcium chlorides) as well as deposition of dusts and water migration of elements contribute to soil degradation and deterioration of plant growth conditions. In urban areas strong alkalization of soil is frequently observed, which significantly reduces contents of soluble forms of metallic components [12, 27, 74].

According to the Ordinance of the Minister of the Environment of 9 September 2002 on soil quality standards and land quality standards currently binding in Poland (the Journal of Law Dziennik Ustaw no. 165, item 1359), soil category B comprises soils in urbanized areas, for which the admissible heavy metal level (mg kg^{-1} dry matter) in the upper 0 - 30 cm layer is Ni 100, Cd 4, Pb 100 and Cr 150, respectively.

Legal regulations pertain to total heavy metal contents in soils; however, many authors claim that it is not always a direct indicator of their bioavailability [44]. In the opinion of Gorlach and Gambús [53], the most appropriate measure is to assess soil contents of soluble forms of trace elements, as they may be absorbed by plants.

The aim of the studies conducted by the authors was to determine what amounts of soluble forms of heavy metals are available for plants in the 0 - 20 cm layer of soil in green areas located in the vicinity of selected transportation routes in the city of Poznań, Poland. Collected soil samples were tested for soil reaction and contents of selected heavy metals (cadmium, lead, chromium and nickel, classified as metallic micronutrients, essential elements at the same time having a negative effect on plants when found in greater amounts).

Material and methods

Soil samples from green areas were collected in October 2012 in the vicinity of transportation routes in the city of Poznań, Poland. Forty five streets were selected for analyses and soil samples were collected using an Egner sampling stick from a 0 - 20 cm layer at a distance of 0.5 - 2.0 m from the roadway. Along each analyzed street 4 bulk samples were collected, comprising 15 individual samples ($4 \times 15 = 60$ individual samples). Heavy metals (Cd, Pb, Cr and Ni) were extracted from soil using Lindsay's solution containing in 1 dm^3 : 5 g EDTA (ethylenediaminetetraacetic acid), 9 cm^3 25% NH_4OH solution, 4 g citric acid and 2 g $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$. Next they were assayed by flame atomic absorption spectroscopy (FAAS) with an AAS 3 Zeiss apparatus. Active acidity expressed in pH (H_2O) was determined by potentiometry (soil : water = 1:2), [50].

Results were analyzed determining their minimum, maximum and mean values (in the case of pH its logarithmic value was considered), standard deviation, coefficients of variation and empirical distribution for individual chemical parameters. Mean results of chemical analyses are presented in Table 3, while stem-and-leaf displays are given in Figs. 1 - 2.

In these analyses soil pH fell within the range of 4.32 – 8.26, while its coefficient of variation was as low as 9% (Table 3). For most plants optimal soil reaction is $\text{pH}_{\text{H}_2\text{O}}$ 6.0 – 6.5. Only 2.2% soils had a highly acid reaction (at pH 4.3 – 5.0), whereas a vast majority had an alkaline reaction $\text{pH} > 7.4$ (46.7% soil samples) and a neutral reaction (37.8%), comprising jointly 84.5% samples

(Figure 1). Elevated soil pH is connected with an excessive content of alkaline ions, i.e. calcium, magnesium, sodium and potassium. Alkalization of urban soils may result e.g. from the use of salts such as sodium chloride to remove black ice from roadways, as well as strongly alkalizing dusts (containing e.g. CaO, MgO, Na₂O, K₂O), being by-products of carbon combustion to heat houses [27, 74]. In turn, Kleiber [74] to a much greater degree suggests strong alkalization of soil (pH in H₂O up to 10.95) as a factor having a potentially negative effect on plant growth and development.

In the opinion of Klimowicz and Melke [75], in urban areas traffic-related pollution is more dangerous than industrial pollution, since it is spread in relatively large amounts, at low heights, in the respiration zone of humans, animals and plants. Relief features, distance from a roadway and intensity of vehicle traffic have a decisive effect on the contents of heavy metals in soils adjacent to transportation routes in urban green areas. In the soil found at the car market in Słomczyn near Warsaw (one of the biggest car markets in Poland) contents of zinc, lead and copper are as follows (in mg kg⁻¹ d.m.): the car sale point Zn at 612.3, Pb at 397.8 and Ni at 94.5, while at the spare part sale point and warehouse they are: Zn at 679.1, Pb at 420.4 and Ni at 114.0, respectively [115].

Soil pollution with heavy metals is typically assessed on the basis of total contents of elements [44]. Those authors claimed that this assessment should be supplemented with an analysis of heavy metal contents directly available to living organisms.

This study consisted in the determination of contents of soluble heavy metal forms (Table 3). Cadmium content in soils fell within the range of 0.16–0.42 mg Cd dm⁻³ and it was of relatively limited variability (CV=17.50%). As many as 55.1 % samples contained this heavy metal at <0.21–0.26 mg Cd dm⁻³ (Figure 2). Bach [9] in soils of green areas adjacent to transportation routes in the city of Krakow, Poland found contents of soluble cadmium forms to range from 0.21 to 1.54 mg kg⁻¹ d.m. soil. In most tested soils Bach [9] recorded cadmium content from 0.4 to 0.8 mg kg⁻¹ d.m. soil. According to Kabata-Pendias and Pendias [67], natural total cadmium content (the so-called background) in Polish soils is 0.3 mg Cd kg⁻¹ d.m.

Lead content ranged from 0.79 to 42.96 mg Pb dm⁻³, at the same time being highly variable depending on the location (CV=110.78%). As many as 84.4% tested soils had low contents of this heavy metal (up to 9.22 mg Pb dm⁻³), while samples with 42.96 mg Pb dm⁻³ accounted for 2.2.% (Figure 2). A low lead content (max. up to 4.3 mg Pb dm⁻³) was reported in his study by Kleiber [74]. Lead content (soluble forms) in soils adjacent to transportation routes in the city of Krakow ranged from 11.1 up to 142.8 mg kg⁻¹ d.m. soil, of which the highest proportion (53%) comprised soils with its contents from 40 to 80 mg Pb kg⁻¹ d.m. soil. Mean content of bioavailable Pb forms in soils of allotment gardens located in the right-bank Warsaw was 332.7 mg kg⁻¹ d.m. [41].

The primary source of lead pollution in soils (adjacent to transportation routes) up to 2005 was connected with tetraethyl lead, commonly added to gasoline as an antiknock agent [62]. In turn, the authors of this chapter in their analyses recorded a low content of soluble lead content in the surface soil layer in areas adjacent to transportation routes in the city of Poznań, Poland.

No lead pollution in soils adjacent to exit routes leading from Poznań was detected by Hofman and Wachowski [62].

In turn, chromium content ranged from 0.26 to 0.67 mg Cr dm⁻³. The coefficient of variation for chromium was similar to that calculated for nickel, amounting to CV 22.34%. The greatest proportion (44.4%) of soils was found within the range of chromium contents from < 0.36 to 0.46 mg Cr dm⁻³ (Figure 2). Results of analyses conducted by Bach [9] indicate that the content of chromium (soluble forms) in soils in areas adjacent to transportation routes in the city of Krakow ranged from trace values to 10.23 mg Cr kg⁻¹ d.m.

The recorded nickel content ranged from 0.43 to 1.25 mg Ni dm⁻³. The variability of levels for this metal was relatively medium-ranged (CV=23.19%). Most of soil samples (84.4%) were characterized by nickel contents falling within the range of values from < 0.43 to 0.63 mg Ni dm⁻³ (Figure 2). In soils found in green areas adjacent to transportation routes in Krakow, Poland the content of nickel soluble forms ranged from 1.07 to 6.38 mg kg⁻¹ d.m. soil [9]. In contrast, in soils collected from allotment gardens located in right-bank Warsaw the mean content of bioavailable nickel forms was 28.4 mg kg⁻¹ d.m. [41].

Environmental pollution with heavy metals constitutes a serious problem in some regions of Poland. According to Dmochowski et al. [41], high emissions of heavy metals originating from a dense network of transportation routes with high intensity vehicle traffic causes their accumulation in soils and crops produced in allotment gardens located in Praga Południe, the right-bank district of Warsaw, Poland.

In the opinion of Heck et al. [59], introduction of advanced catalytic systems, containing platinum, rhodium and palladium and constituting a source of environmental pollution also for soils in areas adjacent to transportation routes makes their monitoring a necessary practice. In the nearest future it will be required to create an effective system of environmental pollution monitoring.

No.	Street	pH (H ₂ O)	Contents (mg kg ⁻¹ d.m.)			
			Ni	Cd	Pb	Cr
1	28 czerwca 56 r.	7.67	0, 49	0, 23	14, 81	0, 31
2	Aleje Niepodległości	7.58	0, 51	0, 28	10, 74	0, 33
3	Aleje Solidarności	7.37	0, 58	0, 27	3, 36	0, 49
4	Arciszewskiego	7.30	0, 48	0, 20	6, 55	0, 41
5	Armii Poznań	7.72	0, 59	0, 21	42, 96	0, 55
6	Biskupińska	7.76	0, 52	0, 23	2, 18	0, 46
7	Dolna Wilda	8.26	0, 44	0, 18	4, 00	0, 38
8	Droga Dębińska	7.37	0, 48	0, 23	6, 76	0, 34

No.	Street	pH (H ₂ O)	Contents (mg kg ⁻¹ d.m.)			
			Ni	Cd	Pb	Cr
9	Fredry	7.30	0, 62	0, 33	6, 83	0, 60
10	Głogowska	6.86	0, 62	0, 31	3, 56	0, 57
11	Hercena	7.00	0, 58	0, 21	1, 91	0, 46
12	Hetmańska	7.45	0, 43	0, 19	3, 92	0, 40
13	Jarochowskiego	7.60	0, 55	0, 24	3, 24	0, 46
14	Kopanina	6.64	0, 73	0, 26	7, 45	0, 39
15	Królowej Jadwigi	6.55	0, 86	0, 22	8, 70	0, 35
16	Krzywoustego	6.98	0, 58	0, 28	4, 31	0, 57
17	Księcia Mieszka I	7.17	0, 50	0, 23	18, 89	0, 39
18	Kuźnicza	6.29	0, 70	0, 28	6, 17	0, 43
19	Lechicka	8.18	0, 55	0, 25	2, 89	0, 52
20	Leszczyńska	7.63	0, 57	0, 28	3, 46	0, 67
21	Lodowa	7.72	0, 51	0, 21	3, 10	0, 48
22	Maczka	7.48	0, 60	0, 30	6, 07	0, 56
23	Małopolska	7.47	0, 62	0, 29	2, 97	0, 64
24	Marcelińska	7.89	0, 55	0, 26	2, 78	0, 59
25	Niestachowska	5.84	0, 71	0, 25	10, 97	0, 38
26	Nowowiejskiego	7.14	0, 49	0, 19	3, 02	0, 36
27	Opieńskiego	7.32	0, 47	0, 22	0, 79	0, 39
28	Opolska	7.76	0, 62	0, 28	3, 65	0, 64
29	Ożarowska	7.50	0, 51	0, 24	3, 76	0, 41
30	Piątkowska	7.40	0, 50	0, 26	2, 64	0, 46
31	Piłsudskiego	7.50	0, 60	0, 26	2, 10	0, 57
32	Polanka	6.45	0, 60	0, 24	5, 73	0, 41
33	Poznańska	6.99	0, 44	0, 16	2, 49	0, 26
34	Przemysłowa	7.36	0, 60	0, 28	2, 54	0, 60
35	Serbska	7.79	0, 60	0, 24	2, 34	0, 63

No.	Street	pH (H ₂ O)	Contents (mg kg ⁻¹ d.m.)			
			Ni	Cd	Pb	Cr
36	Słowiańska	6.87	0, 63	0, 23	5, 31	0, 29
37	Stablewskiego	7.55	0, 57	0, 23	3, 28	0, 46
38	Starołęcka	7.12	0, 63	0, 29	6, 01	0, 53
39	Stróżyńskiego	7.53	0, 49	0, 23	2, 71	0, 46
40	Szpitalna	4.32	0, 82	0, 23	11, 84	0, 50
41	Umultowska	6.79	1, 25	0, 42	3, 03	0, 65
42	Widłakowa	7.41	0, 50	0, 22	2, 07	0, 45
43	Witosa	7.09	0, 48	0, 21	6, 07	0, 43
44	Wojska Polskiego	6.53	0, 69	0, 26	9, 48	0, 39
45	Zwierzyniecka	7.64	0, 52	0, 23	2, 49	0, 40
Content	mean	7.22	0.59	0.25	6.00	0, 47
	minimum	4.32	0.43	0.16	0.79	0, 26
	maximum	8.26	1.25	0.42	42.96	0, 67
Standard deviation		0, 65	0.14	0.04	6.64	0.10
Coefficient of variation (%)		9, 0	23.19	17.50	110.78	22.32

Table 3. Contents of soluble forms of heavy metals and soil reaction in the surface soil layer (0 - 20 cm) in green areas adjacent to selected transportation routes in the city of Poznań, Poland

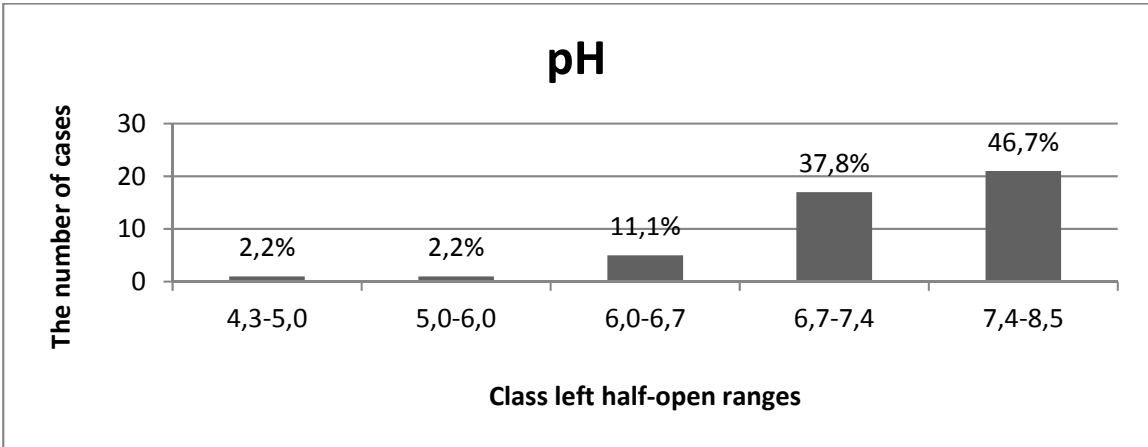


Figure 1. The stemplot for pH (H₂O) of soil in green areas located near to routes in Poznan

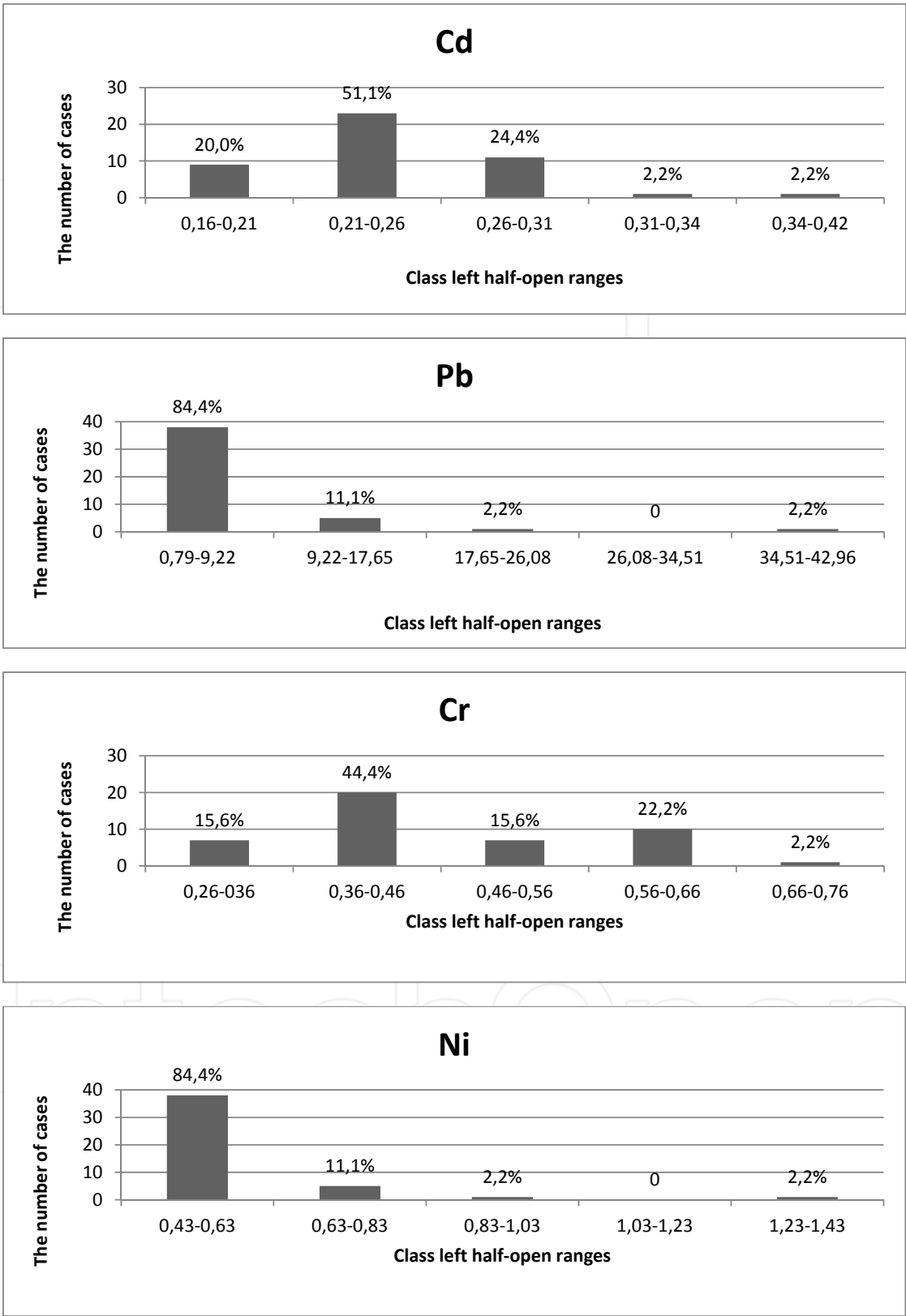


Figure 2. The stemplot for content of heavy metals in soil of green areas located near to routes in Poznan

4.1. Concluding remarks

Based on analyses of soils collected from green areas located in the vicinity of selected transportation routes in the city of Poznań, Poland in most examined locations an alkaline reaction $\text{pH} > 7.4$ (46.7% soil samples) and a neutral reaction (37.8%) were found, which may significantly affect habitat conditions for plants.

Moreover, most tested soil samples contained low amounts of soluble forms of cadmium, lead, chromium and nickel.

5. Heavy metal contents in leaves of selected ornamental plant species growing in green areas adjacent to selected transportation routes in the city of Poznań (western Poland)

Effects of soil pollution with heavy metals may be identified by analyzing their content in plants. Plants may be considered good bioindicators of environmental pollution with heavy metals. Most frequently healthy leaves are indicator parts of plants; however, it depends on the species and occasionally even on the cultivar.

The aim of the analyses conducted by the authors in September 2012 was to determine contents of selected heavy metals in leaves of trees growing in the vicinity of transportation routes in Poznań (western Poland).

Material and methods

It was decided to conduct analyses on healthy plants with no symptoms of damage. Leaves of trees were collected from the central parts of long shoots, distributed at various sides around the circumference of the crown. A total of 15 - 20 leaves were collected from each tree. Leaves were collected from 10 trees of a given genus (1 bulk sample comprised approx. 150 - 200 leaves). The collected material was dried at a temperature of 45 - 50°C and then homogenized. In order to determine total forms of heavy metals they were mineralized in a mixture of nitric and perchloric acids ($v:v=3:1$; $\Sigma 30 \text{ cm}^3$). Following mineralization Cd, Cr, Pb and Ni contents were determined by atomic absorption spectrometry (AAS) in a Carl Zeiss Jena apparatus.

High variation was observed in the contents of heavy metals in leaves of tested trees (*Acer sp.*, *Betula sp.*, *Robinia sp.*, *Salix sp.*, *Tilia sp.*), (Table 4). Mean content of cadmium amounted to 1.18 - 1.74 mg Cd kg⁻¹ (for *Tilia sp.* and *Betula sp.*), for chromium it was 0.46 - 0.82 mg Cr kg⁻¹ for *Tilia sp.* and *Acer sp.* (except for *Betula sp.* – trace content), that of lead ranged from 1.55 to 3.28 mg Pb kg⁻¹ (for *Betula sp.* and *Salix sp.*, respectively). Nickel content ranged from 1.63 mg Ni kg⁻¹ (for *Tilia sp.*) up to 2.23 mg Ni•kg⁻¹ (for *Betula sp.*)

Table (5) present contents of heavy metals in leaves of selected genera of trees reported by Tomašević et al. [117], Lawal et al. [81] and Knezevic et al.[76]. In this study cadmium and nickel contents in leaves did not exceed the levels detected by those authors. In contrast, they found markedly lower chromium contents in leaves of *Tilia sp.*, *Aesculus hippocastanum* and

Azadirachta indica. In this study the content of lead in leaves was markedly lower than that recorded by those researchers for *Tilia* sp. and *Aesculus hippocastanum*, while it was comparable to that in *Azadirachta indica* and *Paulownia elongata* (Tables 4 and 5).

Sample	Content	Heavy metals			
		Cd	Cr	Pb	Ni
<i>Acer</i> sp.	Minimum	0.25	Tr.	0.83	0.80
	Maximum	1.85	1.79	3.19	2.40
	Average	1.31	0.82	2.10	1.81
<i>Betula</i> sp.	Minimum	1.16	Tr.	0.55	1.90
	Maximum	2.87	Tr.	3.49	2.40
	Average	1.74	Tr.	1.55	2.23
<i>Robinia</i> sp.	Minimum	0.47	Tr.	1.06	0.50
	Maximum	2.54	2.13	5.73	2.90
	Average	1.37	0.48	2.78	2.07
<i>Salix</i> sp.	Minimum	1.12	Tr.	2.43	1.60
	Maximum	1.71	1.69	4.61	2.50
	Average	1.39	0.48	3.28	2.10
<i>Tilia</i> sp.	Minimum	0.29	Tr.	2.10	0.80
	Maximum	2.09	1.16	4.28	2.50
	Average	1.18	0.46	3.19	1.63

Table 4. Contents of heavy metals (mg kg⁻¹ d.m.) in leaves of selected genera of trees growing in Poznań (tr. – trace amounts)

Sample	Content	Heavy metals			
		Cd	Cr	Pb	Ni
<i>Tilia</i> sp. ¹	Min	dl	dl	1.88	-
	Max	1.4	0.02	11.4	-
<i>Aesculus hippocastanum</i> ¹	Min	0.4	dl	5.35	-
	Max	4.9	0.33	20.3	-
<i>Azadirachta indica</i> ²	Min	-	0.17	0.77	1.14
	Max	-	0.37	2.25	1.92
<i>Paulownia elongata</i> S.Y. Hu ³	Min	-	-	0.94	3.23
	Max	-	-	3.16	6.63

dl detection limit

¹ Tomašević et al. 2004; ² Lawal et al. 2011; ³ Knezevic et al. 2009

Table 5. Contents of heavy metals (mg kg⁻¹ d.m.) in leaves of selected genera of trees

6. The principle of phytoremediation

Among soil purification methods biological methods are increasingly often focused on as particularly promising [1, 99, 108]. One of these is phytoremediation, based on the activity of living organisms [40]. It is an alternative method, competitive in relation to other technologies extensively applied in pollutant removal from soil. There are methods facilitating deactivation or removal of toxic substances from the substrate. In most cases they are based on methods of physico-chemical extraction, but their application is connected with excessive costs and complete elimination of soil microorganisms. Reconstruction of semi-natural ecosystems in such cases is a lengthy process.

Obvious advantages of this biological method include its applicability at the contamination site, as well as relatively low investment outlays and low operating costs at the simultaneous high effectiveness of the process [95]. In the opinion of Salt et al. [107], other factors promoting its more common use are connected with the fact that it is an environmentally friendly process, which does not disturb soil structure, and that it may use many plant species.

The term phytoremediation originates from Greek *phyton* – a plant and Latin *remediare* – to purify. Salt et al. [108], Blaylock and Huang [14], Schnoor [109], Schwitzguebel et al. [111], McGrath and Zhao [86], Vassilev et al. [122], Vangronsveld et al. [121] and Larcher et al. [80] stated that phytoremediation is a technology using higher plants to stabilize and either remove or reduce amounts of soil pollutants, bottom deposits or surface and underground waters. Cunningham et al. [37] and Salt et al. [107] define a plant as a system of filters and pumps powered by solar energy and extracting from its environment and accumulating specific elements.

As it was reported by Pandolfini et al. [96], this biological method is based on the practical use of three types of physiological response to substances found in the environment, i.e. exclusion, accumulation and hyperaccumulation.

The term phytoremediation refers to the following methods using higher plants to purify environmental matrices [49, 98, 108, 119]:

- phytodegradation – the use of plants and microorganisms to degrade organic pollutants,
- phytostabilization – the use of plants to reduce bioavailability of pollutants in the environment,
- phytoextraction – the use of plants absorbing pollutants and accumulating them in organs removed from fields together with crops in order to purify soil from heavy metals and organic substances,
- phytovolatilization – the use of plants to volatilize pollutants and release them to the atmosphere,
- rhizofiltration – the use of plant roots to absorb pollutants from water and sewage,
- rhizodegradation – the use of plants to supplement the bioremediation process performed by microorganisms colonizing the rhizosphere.

In the opinion of Negri et al. [91], the above mentioned phytoremediation technologies act at three different detoxication levels on pollutants accumulated in the environment, i.e. the pollution-loaded soil matrix (phytostabilization, rhizofiltration and rhizodegradation), plants (phytodegradation, phytoextraction, rhizofiltration) and the atmosphere (phytovolatilization). Studies are also being conducted on the application of plants in the technology to excavate heavy metals, referred to as phytomining [5, 90, 97]. Soils with high heavy metal contents are planted with plants capable of growing under such adverse conditions and accumulating selected elements in their biomass. Such biomass is next treated as the so-called bio-ore. The biomining technology to extract heavy metals from soil, created by R. L. Chaney, J.S. Angle, A.J. Baker and J.M. Li, was patented in 1989 [45].

7. Continuous and induced phytoextraction

Phytoextraction, a phytoremediation method observed in 1885 by Bauman [11], is an excellent concept of soil purification, which is not yet commonly applied. In many centers worldwide research is being conducted on phytoextraction in the search for plant species capable of accumulating heavy metals in their aboveground parts. Within phytoextraction of heavy metals from soil we may distinguish the so-called continuous and induced phytoextraction.

Plants used in phytoextraction of heavy metals from soil should exhibit:

- good tolerance to high concentrations of heavy metals
- capacity to absorb and accumulate heavy metals in their aboveground parts
- rapid growth
- high increase in biomass
- resistance to diseases, pesticides and adverse atmospheric conditions
- low cultivation requirements
- easy harvesting and processing.

In continuous phytoextraction heavy metals are absorbed by plants and accumulated continuously with plant growth [14, 20, 46, 85, 110].

Apart from it being economically attractive, continuous phytoextraction is also environmentally friendly, leaving the site suitable for cultivation of other plants [37, 63].

In urbanized areas continuous phytoextraction may be used in two types of sites. One comprises degraded soils in post-industrial areas, while the other, highly promising as a future application of phytoextraction, is connected with soils in the vicinity of transportation routes and in urban green areas. The potential of ornamental plant species most frequently planted in urban locations is investigated in many research centers worldwide.

Such species include also *Tagetes erecta* L. It results from studies conducted by Bosiacki [16, 17] that it is a species exhibiting tolerance to high concentrations of cadmium and lead. When comparing several cultivars of this species the author found that cv. 'Hawaii' has the greatest contents of cadmium and lead in individual aboveground organs. The highest cadmium content ($96.06 \text{ mg kg}^{-1} \text{ d.m.}$) was found in leaves of *Tagetes erecta* L. cv. 'Hawaii', while that of lead ($145.00 \text{ mg kg}^{-1} \text{ d.m.}$) was recorded in stems. Accumulation of heavy metals in individual plant organs is not uniform. Bosiacki [15] observed variation in cadmium contents in individual organs of selected ornamental plant species. The highest content of this metal was detected in *Tagetes erecta* 'Inca Yellow' in its roots, in *Salvia splendens* 'Fuego' in leaves and stems, while in *Helianthus annuus* 'Pacino' it was in inflorescences. Liu et al. [82] stated that *Calendula officinalis* L. accumulated more Cd and Pb in roots than in shoots, while *Althaea rosea* L. collected greater amounts of these elements in shoots than in roots.

Zhou and Wang [124] when investigating the effect of cadmium on growth in three ornamental plant species stated that *Salvia splendens* L. is most sensitive to Cd, while *Abelmoschus manihot* Medik and *Tagetes erecta* L. were most resistant.

Phytoremediation of heavy metals from contaminated areas, including urbanized areas, according to Porebska and Gworek [101] may be conducted using many species of ornamental plants and vegetables, e.g. *Salix viminalis* L. (accumulating Zn and Cd), *Alyssum bertolonii* Desv. (Ni), *Brassica pekinensis* Rupr. (Pb), *Thlaspi caerulescens* J. Presl & C. Presl. (Zn). The same authors stated that wild-growing plants, commonly considered weeds in cultivation of agricultural and horticultural crops, may also be used in bioremediation, e.g. *Atriplex nitens* Schkuhr, *Artemisia vulgaris* L., *Chenopodium album* L., (accumulating greatest amounts of Pb among the tested plants) and *Lacuca serriola* L. (accumulating greatest amounts of Zn).

Antonkiewicz and Jasiewicz [6] assessed suitability of *Helianthus tuberosus* L., *Zea mays* L., *Sida hermaphrodita* Rusby, *Amaranthus* sp. L. and *Cannabis sativa* L. in phytoextraction of heavy metals from soil. In their study the highest contents of cadmium and zinc were detected in amaranth, while that of lead and nickel in Virginia fanpetals. Jerusalem artichoke had the greatest content of copper.

Antonkiewicz et al. [7] when investigating phytoextraction of heavy metals (Cd, Pb, Ni, Zn and Cu) from soil using Virginia fanpetals (*Sida hermaphrodita* Rusby) found that this species absorbed the highest rates of nickel from soil and the lowest of copper.

Many authors for phytoextraction of soils polluted with heavy metals recommended sunflower, corn, rape, amaranth, willows, *Miscanthus* and strong growing cereals, while for phytoextraction in urban soils he recommended plants with high tolerance to pollution, e.g. London plane, northern red oak, Japanese larch, poplars, field maple, ashes and dogwoods, desert false indigo, false *Spirea* and forsythia.

Larcher et al. [80] conducted pilot-scale studies in the industrial area of Turin (Italy) using two plant species in phytoremediation of soil to remove heavy metals. They found *Helianthus*

annuus L. "Holeko HO" and *Brassica juncea* Czern. "Red Giant" to be highly suitable for phytoremediation. Those authors stated that further studies in this respect are needed.

In urban green areas the predominant forms are lawns and turf-covered areas in escarpments, embankments, spoil tips, belts separating roadways, parking lots, gas stations, landfills and industrial waste dumps. Depending on the use of turf areas it is highly important to select appropriate species and cultivars of lawn grasses. It results from experiments conducted by Bosiacki and Zieleziński [23] on the potential of three grass species (*Poa pratensis* L. 'Evora', *Festuca arundinacea* Schleb. 'Asterix', *Festuca rubra* L. *sensu lato* 'Jasper') in phytoextraction of nickel that *Poa pratensis* L. 'Evora' and *Festuca arundinacea* Schleb. 'Asterix' are species showing the greatest capacity to accumulate this metal. In turn, *Festuca rubra* L. *sensu lato* 'Jasper' turned out to be the species showing the greatest capacity to accumulate cadmium and lead [21, 22].

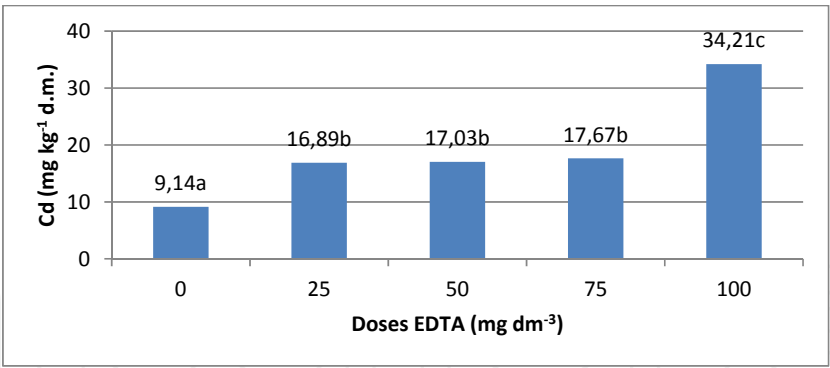
Studies are also conducted on induced phytoextraction using plants producing large amounts of biomass, but additionally such substances as e.g. chelating carriers affecting mobility of individual elements and enhancing pollutant accumulation in plant organs (particularly aboveground parts) are introduced to the environment [35, 92, 123].

Chelating substances are to transform forms of heavy metals sparingly soluble or insoluble in water into forms available to plants. Studies are being conducted using different chelators, both natural and synthetic (e.g. humus substances, low molecular organic acids, citric acid, tartaric acid, amino acids as well as EDTA, EGTA, EDDS, HEDTA, CDTA, EDDHA, DTPA, NTA). Some of them are biodegraded in soil, while others in combination with a heavy metal may be leached to ground waters contaminating them. For this reason it is essential in this technology to determine an appropriate dose and date for the application of a given chelating agent.

It results from preliminary studies (unpublished data) conducted in 2012 by Bosiacki at the Department of Plant Nutrition, the Poznań University of Life Sciences, Poland that EDTA introduced to mineral soil contaminated individually with cadmium at 1.5 mg dm^{-3} in the form of cadmium sulfate ($3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$), lead 100 mg dm^{-3} as lead acetate [$(\text{CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$] and nickel 50 mg dm^{-3} as nickel sulfate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) caused an increase in the contents of these metals in leaves of *Tagetes erecta* 'Taishan Orange'. Doses of EDTA were introduced to soil in the solution form 35 days after plants had been planted to mineral soil contaminated with these metals. Samples of plant material (leaves) were collected 25 days after the application of EDTA to soil. Contents of individual heavy metals in leaves of *Tagetes erecta* 'Taishan Orange' are presented in Figs. 3, 4 and 5. The research found that acid (EDTA) has improved the efficiency of phytoextraction of cadmium, lead and nickel in soil by *Tagetes erecta* L. 'Taishan Orange'.

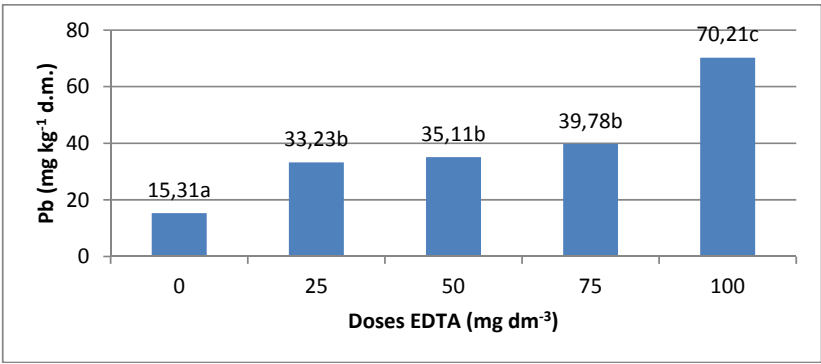
Identification of compounds complexing toxic heavy metals and at the same time biodegradable in the soil medium is crucial for induced phytoremediation.

At present studies are being conducted at the Department of Plant Nutrition, the Poznań University of Life Sciences, Poland on the application of a biodegradable compound in phytoextraction of heavy metals from contaminated soils.



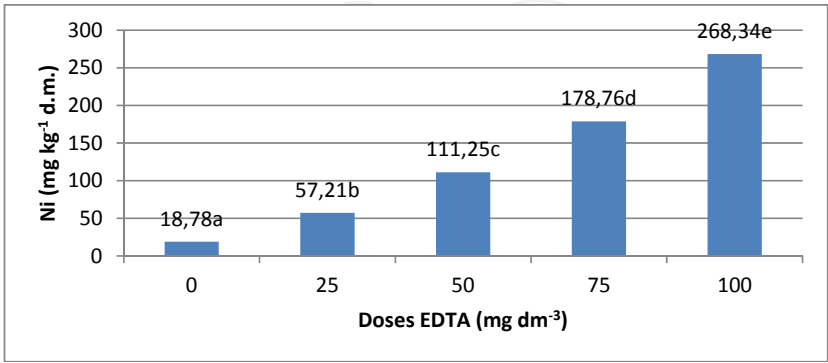
*homogeneous groups were identified using the Duncan test, p = 0.05 (values denoted with identical letters do not differ significantly)

Figure 3. The effect of increasing EDTA doses introduced to soil (contaminated with 1.5 mg Cd dm⁻³) on cadmium content in leaves of *Tagetes erecta* 'Taishan Orange'



*homogeneous groups were identified using the Duncan test, p = 0.05 (values denoted with identical letters do not differ significantly)

Figure 4. The effect of increasing EDTA doses introduced to soil (contaminated with 100 mg Pb dm⁻³) on lead content in leaves of *Tagetes erecta* 'Taishan Orange'



*homogeneous groups were identified using the Duncan test, p = 0.05 (values denoted with identical letters do not differ significantly)

Figure 5. The effect of increasing EDTA doses introduced to soil (contaminated with 50 mg Ni dm⁻³) on nickel content in leaves of *Tagetes erecta* 'Taishan Orange'

8. The phenomenon of hyperaccumulation

As it was stated by Brooks [28] the discovery and description of a phenomenon termed hyperaccumulation has contributed to the practical use of plants to remove metallic pollutants from soil. According to Boyd and Martens [25] and Brown et al. [29], plant species referred to as hyperaccumulators are genetically and physiologically capable of accumulating large amounts of heavy metals with no symptoms of toxicity. Threshold values of metal concentrations have been used to define metal hyperaccumulation, including 100 mg kg⁻¹ dry weight of shoots for Cd, 1000 mg kg⁻¹ for Cu, Ni, Pb and 10 000 mg kg⁻¹ for Zn [10, 28, 86]. Cline et al. [34] stated that concentrations of heavy metals in tissues of hyperaccumulator plants should be 1 - 2%. Van der Ent et al. [120] recommend the following concentration criteria for different metals and metalloids in dried foliage: 100 µg g⁻¹ for Cd, Se and Ti; 300 µg g⁻¹ for Co, Cu and Cr; 1000 µg g⁻¹ for Ni, Pb and As; 3000 µg g⁻¹ for Zn; 10000 µg g⁻¹ for Mn, with plants growing in their natural habitats. There are over 400 known plant species from 45 families classified as hyperaccumulators. Most species belong to the families *Brassicaceae* and *Fabaceae* [103]. Approximate numbers for various elements are as follows: Ni (450), Cu (32), Co (30), Se (20), Pb (14), Zn (12), Mn (12), As (5), Cd (2), Tl (2), [120]. Moreover, hyperaccumulators typically accumulate most efficiently one heavy metal and to date no plant has been identified which would be capable of accumulating in its tissues all toxic metals [37]. According to Vangronsveld et al. [121] successful phytoremediation depends first of all on the selection of an appropriate plant species. For this reason in the opinion of Baker et al. [10] it would be a practical solution to grow many different species at the same time.

Most natural hyperaccumulators are plants characterized by slow growth and production of low amounts of biomass. These traits result in a limited applicability of these plant species in phytoextraction of heavy metals from soil [37]. An example in this respect may be provided by *Thalyspi caerulescens* L., producing only 5 ton dry matter per hectare [32], whereas it is cost-effective to use plants, which yield 20 ton per hectare and the accumulation of metal in aboveground parts is over 1% [63].

Studies are being conducted worldwide on the use of plants producing large amounts of biomass (for energy generation purposes) in the phytoextraction of heavy metals from polluted soils. They include annual plants such as e.g. cereals and rape, and perennials e.g. willows, which capacity for phytoextraction of heavy metals was confirmed by Greger and Landberg [56] and Boyter et al. [26]. Other perennial species of energy crops include *Spartina pectinata*, *Sida Hermaphrodita*, *Rosa multiflora*, *Helianthus tuberosus*, *Populus spp.*, and *Robinia pseudoacacia*. The most significant parameters of these plants include high annual increment of biomass and its high calorific value [116].

Ociepa et al. [94] stated that plants grown for energy purposes may play a considerable role in view of the assumptions of the Common Agricultural Policy and the environmental protection policy of the European Union. One of such plant species is *Miscanthus × giganteus* Greef and Deu.

In the years 2008 – 2011 at the Department of Plant Nutrition, the Poznan University of Life Sciences, Poland, studies were conducted to assess applicability of *Miscanthus × giganteus* Greef

and Deu. in phytoextraction of heavy metals from soil. The aim of the conducted analyses was to determine what amounts of cadmium and lead are transported to aboveground parts of *Miscanthus × giganteus* and whether *Miscanthus × giganteus* would be suitable for rapid phytoextraction of cadmium, lead from polluted soils.

Material and methods

The vegetation experiment was conducted in an unheated plastic tunnel with suspended sides, of 6 x 30 m in size. at the Marcellin Experimental Station of the Poznan University of Life Sciences.

Seedlings of *Miscanthus × giganteus* were produced at a tissue culture laboratory of Vitroflora. Plants were planted in the beginning of May in drainless containers (of 7 dm³) filled with previously prepared substrate. The experiment comprised 16 combinations (in each year of the study) and each combination consisted of six replications. A replication comprised one plant growing in pots.

Phytoremediation of cadmium and lead by *Miscanthus × giganteus* was investigated in two years of growth with the plants being grown in two substrates. at four levels of metal contents. Since light soils with low contents of organic matter predominate in Poland. such soil was selected for the experiment. Another substrate was a mixture of this mineral soil with highmoor peat. Highmoor peat was added to increase the amount of organic matter in the mineral soil.

Substrates: mineral soil (sand) and mineral soil with highmoor peat (1:1 v/v)

Doses of cadmium: control (native contents of cadmium), 3, 5 and 10 mg dm⁻³)

Doses of lead: control (native contents of cadmium), 250, 1000 and 5000 mg dm⁻³).

In mineral soil the method according to Mocek and Drzymała [88] was used to determine particle density (which amounted to 2.65 g cm⁻³) and bulk density (1.62 g cm⁻³). Total porosity of mineral soil was 38.9%. Moreover. grain size distribution of mineral soil was determined by the densimetric method according to Prószyński [88]. On the basis of the percentages of fractions the grain size class of soil was identified (according to the guidelines of the Polish Society of Soil Science) - sand.

Prior to the establishment of the experiment, Corg content in mineral soil was determined according to the Tiurin method [51]. Content of organic carbon in sand (the Tiurin method) was 0.55% (0.95% humus). while the percentage of organic matter in the mixture of sand and highmoor peat (from loss on ignition) was 10.05%. In the substrate composed of a mixture of mineral soil with highmoor peat (1:1 v/v) the percentage of organic substance was determined by loss on ignition the substrate by the direct method at high temperature in the presence of oxygen, under the influence of which organic substance is decomposed (carbon is released in the form of CO₂. hydrogen in the form of H₂O and nitrogen as N₂, while the other elements remain in ash).

Experiments were conducted using highmoor peat by Hartmann (sphagnum peat, ground. fractional with acid reaction (pH 4.50). This peat has a high water capacity, at the same time retaining an elastic structure. The weight of 1 dm³ peat was 490 grams.

In order to obtain an appropriate pH for growing of *Miscanthus × giganteus* a neutralization curve was plotted for the analyzed substrates. On its basis the dose of CaCO₃ required for the maintenance of pH within the range of 6.5-7.0 was established. The reaction of the substrate (mineral soil + highmoor peat) was regulated using 3 g dm⁻³ CaCO₃ (chemically pure reagent). The substrate composed of mineral soil did not require reaction control. Despite that fact 1 g dm⁻³ CaCO₃ was applied in order to maintain pH at 6.5-7.0. An adequate amount of calcium carbonate was introduced to each experimental container with the substrate. Cadmium and lead contents in analyzed substrates after liming amounted: in mineral soil Cd 0.27, Pb 27.32 mg dm⁻³ and mineral soil with highmoor peat Cd 0.18, Pb

12.03 mg dm⁻³. Two weeks after liming nutrients cadmium and lead were introduced to the substrate. Cadmium and lead was introduced only in the first year of the experiment in the form of chemically pure reagents (C.P.): cadmium sulfate (3CdSO₄·8H₂O), lead acetate [(CH₃COO)₂Pb·3H₂O]. Pre-vegetation fertilization (in the first year) with macro- and micronutrients was determined taking into consideration initial nutrient contents in substrates. after liming reaching the following levels (in mg dm⁻³): N 200, P 120, K 250, Mg 100, Fe 50, Mn 20, B 1.5 and Mo 1.5. All macro- and micronutrients were introduced in the form of solutions using chemically pure reagents (potassium monophosphate, potassium nitrate, ammonium nitrate, magnesium saltpeter, magnesium sulfate, iron sulfate, copper sulfate, zinc sulfate, manganese sulfate, ammonium molybdate, borax). In the second year of the experiment an identical experimental design was used as in the first year. After plant cutting in the first year of the experiment containers with polluted substrates were stored in an unheated tunnel to the next vegetation year (the second year of the study). In the second year of the study in March - prior to the beginning of vegetation substrate samples were collected and chemical analyses were performed to determine nutrient contents. On this basis nutrient fertilization was established (leading to nutrient contents at the same levels. which were applied in the first year of the experiment). Nutrients in substrates were determined using the "Universal" method [78] in CH₃COOH solution at a concentration of 0.03 mol dm⁻³, pH in water was determined by potentiometry (the substrate to water ratio of 1:2), while conductometry was applied to determine EC (mS cm⁻¹), (the substrate to water ratio of 1:2), [50]. The following nutrient determination techniques were applied: N – NH₄ and N – NO₃ by microdistillation (Bremner modified by Starck), P by colorimetry using the vanadium-molybdenum method, K, Ca and Na by flame photometry, Mg by atomic absorption (AAS), Cl and S – SO₄ by nephelometry [78]. In October in each year of the study prior to harvesting plant height was measured. Dry weight of plants was recorded and samples of plant material were collected for analyses. Harvested plant material (entire aboveground mass) was dried in an extraction drier at a temperature of 105°C for 48 h. Next the material was ground and at 2.5 g from each sample it was digested in a mixture of concentrated HNO₃ (ultra pure) and HClO₄ (analytically pure) at a 3:1 ratio [18]. Content of cadmium and lead in the plant material were determined by flame atomic absorption spectrophotometry (FAAS), AAS-3 spectrophotometer by Zeiss. Moreover, content of metals in the reference material (*Pseudevernia furfuracea* BCR®-482/2009) was determined. In the first and second year of the study samples of substrate were collected after harvest, from which metals were extracted using the Lindsey's solution containing in 1 dm³: 5 g EDTA (ethylenediaminetetraacetic acid), 9 cm³ 25% NH₄OH solution, 4 g citric acid and 2 g Ca(CH₃COO)₂·2H₂O. Next this metal was assayed by flame atomic absorption spectrophotometry (FAAS), AAS-3 spectrophotometer by Zeiss. Results of content of cadmium and lead in substrates and aboveground parts of *Miscanthus × giganteus* were elaborated statistically in the Statobl program applying a one-way analysis of variance for orthogonal factorial experiments, with differences between means determined at a significance level p=0.05.

Miscanthus × giganteus Greef and Deu. (Figure 6) is an interspecies hybrid of a diploid Chinese silver grass [*Miscanthus sinensis* (Thunb.) Anderss.] and a tetraploid Amur silver grass [*Miscanthus sacchariflorus* (Maxim.) Benth], belonging to the family *Poaceae* [54, 55, 61]. After three years of culture the yield of dry matter is 20 to 35 t and plant height is 3 - 4 m [102]. *Miscanthus × giganteus* originates from South-Eastern Asia and it has been grown in Europe, initially as an ornamental plant, for over 50 years. In Poland it still is not a popular crop. In the nearest future interest in growing of this plant in Poland will be increasing. This abundant grass forms new shoots stretching outwards from rhizomes (underground runners), forming an increasingly bigger rounded cluster [102].

Analyses conducted by physiologists classify *Miscanthus* to the group of plants of the C-4 pathway, which are characterised by a highly efficient photosynthesis process, ensuring a rapid and high increase in biomass, at a simultaneous lower transpiration coefficient, i.e. lower water consumption [58, 89].

Proposed methods to manage *Miscanthus* after phytoextraction of heavy metals from soil include combustion (ashes – hazardous waste), bio-ore, paper and pulping industry, production of particleboards as well as chemical industry (packaging plastics).



Figure 6. *Miscanthus x giganteus* Greef and Deu. in the third year of growth

The aim of the conducted analyses was to determine the effect of increasing doses of cadmium, lead introduced to mineral soil (sand) and to mineral soil with an addition of highmoor peat (at a 1:1 ratio, v/v), on the tolerance index of *Miscanthus x giganteus*. The tolerance index (T_i) was calculated, i.e. the ratio of the yield (dry biomass) obtained in metal-polluted soil to the yield (dry biomass) produced in unpolluted soil. This index is considered as the most reliable indicator of the toxic effect of heavy metals contained in soils and substrates exercised on plants.

$T_i < 1$ value lower than one - inhibition of growth or plant death

$T_i = 1$ value equal one - no effect of increased metal contents on yielding

$T_i > 1$ value greater than one - positive effect of metal on yielding.

In the first year of growth of *Miscanthus × giganteus* the tolerance index greater than one was recorded in plants in mineral soil polluted with 3 mg Cd dm⁻³ and 1000 mg Pb dm⁻³ (Table 6). In the second year of growth the tolerance index greater than one was found in plants in mineral soil contaminated with all the used cadmium pollution rates and in soil, to which 250 and 1000 mg Pb dm⁻³ were introduced. The tolerance index greater than one was also recorded in the substrate constituting a mixture of mineral soil with highmoor peat contaminated with 3 mg Cd dm⁻³ (in the first year of growth) and 5 mg Cd dm⁻³ (in the second year of growth).

Metal	Dose of metal (mg dm ⁻³)	Substrates			
		Mineral soil		Mineral soil + highmoor peat	
		The first year of growth	The second year of growth	The first year of growth	The second year of growth
Cd	3	1.02	1.20	1.02	0.88
	5	0.99	1.34	0.83	1.01
	10	0.70	1.24	0.95	0.85
Pb	250	0.80	1.30	0.78	0.99
	1000	1.01	1.28	0.99	0.90
	5000	1.00	0.98	0.93	0.58

Table 6. The tolerance index (Ti) in *Miscanthus × giganteus*

In the opinion of Arduini et al. [8] and Kozak et al. [77], *Miscanthus* is a less tolerant species to high concentrations of heavy metals in comparison to osier and it is necessary to conduct further tests on the applicability of varieties of this species in the phytoextraction of heavy metals.

Kalembasa and Malinowska [72], when testing different clones of *Miscanthus* found differences in their capacity to accumulate individual heavy metals, particularly cadmium. Iżewska [65] in the third year of *Miscanthus* culture recorded a greater content of cadmium and a lower content of lead in its biomass. In the opinion of Kalembasa and Malinowska [71], contents of cadmium and lead in biomass are also dependent on the harvest date of *Miscanthus*. The highest cadmium concentration was recorded in *Miscanthus* biomass at the beginning of June, lead at the beginning of September and October. Those authors stated that the triploid *Miscanthus* species was characterized by higher contents of cadmium and lead in its biomass in comparison to the diploid species. According to those authors, nitrogen, phosphorus and potassium fertilization has an effect on cadmium content in biomass of diploid *Miscanthus* genotypes.

Based on studies conducted by the authors of this chapter it was found that cadmium applied at 3 and 5 mg dm⁻³ mineral soil in the first year of growth of *Miscanthus × giganteus* had no significant effect on the content of this metal in aboveground parts of plants in comparison to those growing in soil with no addition of this metal (Table 7). In the second year of growth in

mineral soil to which 5 mg Cd dm⁻³ were introduced a significantly greater content of this metal was detected in aboveground parts of plants in comparison to plants growing in unpolluted soil. In plants growing in mineral soil the greatest Cd content was found in the second years of growth of *Miscanthus × giganteus* growing in soil polluted with 10 mg Cd dm⁻³.

In a mixture of mineral soil with highmoor peat, to which 3 mg Cd dm⁻³ were introduced both in the first and second year of growth of *Miscanthus × giganteus* no significant differences were observed in the contents of this metal in aboveground parts. The greatest cadmium content was recorded in plants growing in the substrate polluted with 10 mg Cd dm⁻³ in the first and second years of growth.

Substrate	Dose of metal (mg dm ⁻³)	Year of culture							
		1st year				2nd year			
		min.-max. range	range R	SD	mean	min.-max. range	range R	SD	mean
Mineral soil	Control	0.98-1.29	0.31	0.12	1.17 ab	1.29-1.52	0.23	0.10	1.39 ab
	Cd 3	1.31-1.99	0.68	0.24	1.70 bc	1.14-2.59	1.45	0.51	2.06 a-d
	Cd 5	1.33-3.69	2.36	0.78	2.61 bcd	1.78-4.69	2.91	0.97	3.52 d
	Cd 10	3.56-8.34	4.78	1.62	5.51 e	6.16-12.62	6.46	2.81	9.30 g
Mineral soil + highmoor peat	Control	0.64-1.33	0.69	0.25	0.97 a	0.95-1.46	0.51	0.19	1.20 ab
	Cd 3	1.34-1.93	0.59	0.22	1.60 abc	1.38-1.90	0.52	0.20	1.66 abc
	Cd 5	2.39-3.96	1.57	0.67	2.97 cd	2.68-4.78	2.10	0.97	3.41 d
	Cd 10	5.23-9.34	4.11	1.55	7.46 f	5.54-11.17	5.63	2.48	8.66 fg

*homogeneous groups were identified using the Duncan test, p=0.05 (values denoted with identical letters do not differ significantly)

Table 7. Contents of Cd (mg kg⁻¹ dry weight) in aboveground parts of *Miscanthus × giganteus* growing in substrates polluted with cadmium

A significantly greater lead content in aboveground parts of *Miscanthus × giganteus* in comparison to the control was found in plants growing in all tested substrates contaminated with this metal (Table 8). In plants growing in mineral soil contaminated with 10 mg Pb dm⁻³ a higher content of this metal was detected in the first year of growth and it was the highest content recorded in the analyses. No differences were observed in lead contents in aboveground parts of *Miscanthus × giganteus* in the first and second year of growth in a mixture of mineral soil with highmoor peat.

Substrate	Dose of metal (mg dm ⁻³)	Year of culture							
		1st year				2nd year			
		min.-max. range	range R	SD	mean	min.-max. range	range R	SD	mean
Mineral soil	Control	1.23-1.77	0.54	0.21	1.54 a	1.76-2.74	0.98	0.38	2.31 a
	Pb 250	29.87-48.25	18.38	7.44	39.29 b	27.37-46.78	19.41	7.71	36.83 b
	Pb 1000	56.89-75.43	18.54	6.92	62.17 b	53.45-73.56	20.11	7.58	64.88 bc
	Pb 5000	71.46-280.62	209.16	85.24	210.20 e	58.56-131.34	72.78	29.19	104.49 d
Mineral soil + highmoor peat	Control	1.14-1.62	0.48	0.18	1.47 a	1.12-1.67	0.55	0.21	1.38 a
	Pb 250	27.89-40.99	13.10	5.65	34.29 b	21.67-45.62	23.95	8.00	31.92 b
	Pb 1000	48.67-61.78	13.11	4.74	53.48 b	40.56-62.38	21.82	7.80	49.30 b
	Pb 5000	73.56-110.34	36.78	12.56	92.98 cd	53.56-137.89	84.33	31.89	96.46 d

*homogeneous groups were identified using the Duncan test, p=0.05 (values denoted with identical letters do not differ significantly)

Table 8. Contents of Pb (mg kg⁻¹ dry weight) in aboveground parts of *Miscanthus × giganteus* growing in lead-polluted substrates

In the conducted analyses the cadmium and lead concentration indexes were calculated for aboveground parts of *Miscanthus × giganteus*. The metal concentration index was calculated from the formula

$$C = a : b$$

a - content in a plant growing in polluted substrate

b - content in a plant growing in unpolluted substrate.

The greatest concentration index was recorded for lead in the first year of growth in the case of plants growing in mineral soil (Table 9). Plants growing in mineral soil were characterized by a greater concentration index for cadmium and lead in the first year of growth. An identical dependence was found in plants growing in a mixture of soil and peat, except for plants growing in a substrate contaminated with 5000 mg Pb dm⁻³, in which a higher lead concentration index was found in the second year of growth.

Miscanthus sp. was tested on heavy metal contaminated arable soil in Southern Poland [110]. The authors concluded that this species accumulates high amounts of metals what may cause high emission of contaminants during biomass combustion.

According to Kalembas [70] in ash of *Miscanthus sinensis* Thumb. the content of individual heavy metals ranks in the following decreasing levels: Zn>Cd>Pb>Ni>Cu>Cr.

Metal	Dose of metal (mg dm ⁻³)	Substrates			
		Mineral soil		Mineral soil + highmoor peat	
		The first year of growth	The second year of growth	The first year of growth	The second year of growth
Cd	3	1.45	1.48	1.65	1.38
	5	3.06	2.53	3.06	2.84
	10	7.69	6.69	7.69	7.22
Pb	250	25.51	15.94	23.33	23.13
	1000	40.37	28.09	36.38	35.72
	5000	136.49	45.23	63.25	69.90

Table 9. Metal concentration indexes in aboveground parts of *Miscanthus x giganteus*

Both in soil and in a mixture of soil and peat a lower cadmium content was recorded after the second year of culture except for substrates contaminated with 10 mg Cd dm⁻³, in which this dependence was not observed (Table 10).

When analyzing lead content in tested substrates after the completion of growth a lower Pb content was found also in the second year except for a mixture of mineral soil with peat, to which lead was not introduced (table 11).

In the substrate being a mixture of soil with peat lower contents of cadmium and lead were observed in comparison to those recoded in mineral soil in all the experimental variants (Tables 10 and 11).

Type of pollution	Substrate	Year of growth	
		1st year	2nd year
Control (native content of Cd mg dm ⁻³)	mineral soil	0.09 c	0.07 b
	soil + peat	0.07 b	0.05 a
Weak pollution (Cd 3 mg dm ⁻³)	mineral soil	2.23 d	1.09 b
	soil + peat	1.79 c	0.77 a
Medium pollution (Cd 5 mg dm ⁻³)	mineral soil	4.12 d	2.65 c
	soil + peat	2.51 b	1.32 a
Strong pollution (Cd 10 mg dm ⁻³)	mineral soil	6.37 b	6.39 b
	soil + peat	5.43 a	5.22 a

*homogeneous categories were identified with the Duncan test, p=0.05 (values denoted with identical letters do not differ significantly)

Table 10. Cadmium contents (extracted with Lindsey’s solution) in substrates (in mg dm⁻³) after the completion of plant growth in the first and the second year of analyses

Type of pollution	Substrate	Year of growth	
		1st year	2nd year
Control (native content of Pb mg dm ⁻³)	mineral soil	24.84 c	15.18 b
	soil + peat	6.95 a	4.79 a
Weak pollution (Pb 250 mg dm ⁻³)	mineral soil	227.71 d	192.68 c
	soil + peat	130.31 b	104.74 a
Medium pollution (Pb 1000 mg dm ⁻³)	mineral soil	837.70 d	575.34 b
	soil + peat	665.30 c	236.88 a
Strong pollution (Pb 5000 mg dm ⁻³)	mineral soil	3755.06 d	3304.71 c
	soil + peat	2431.14 b	1401.27 a

*homogeneous groups were identified with the Duncan test, p=0.05 (values denoted with identical letters do not differ significantly)

Table 11. Contents of lead (extracted with Lindsey's solution) in substrates (in mg dm⁻³) after the completion of plant growth in the first and the second years of analyses

9. The amount of ash (%) after incineration of aboveground parts of *Miscanthus × giganteus*

Aboveground parts of *Miscanthus × giganteus* were incinerated at a temperature of 450°C in a Linn Elektro Therm furnace. Incineration was performed in two stages:

- stage I - preliminary carbonization at a temperature of 100°C for 1 h
- stage II – combustion at 450°C for 5 h.

Incineration was performed on 20 randomly selected samples of aboveground dry matter of *Miscanthus × giganteus*. Assuming dry matter used in the incineration process as 100%. after combustion a mean 8.20% ash was obtained (SD 0.55), (Table 12).

The amount of ash left after combustion of aboveground parts of *Miscanthus × giganteus* was analyzed in terms of empirical probability distribution. Mean observations of the amount of ash were ordered in the so-called stemplot. in which separate intervals called classes were identified. Five classes were determined for the amount of ash left after incineration and next the number of observations (the so-called class frequencies) belonging to them were specified for each class. In order to ensure uniqueness of classification of observations it was assumed that classes are left side open intervals and right side closed intervals. Moreover, frequencies were added which when multiplied by 100 determine the percentage of observations of the amount of ash left after combustion of plants as classified to individual classes.

Among all observations after the combustion process the highest percentage (40%) was found for the amount of ash within the range of 8.44 to 8.88%. The amount of ash within the range of 8.00 to 8.44% ranked second constituting 30% observations.

In a study conducted by Kalembasa [70] largest content of raw ash was obtained from the *Sida hermaphrodita* Rusby. (59.5 kg t⁻¹), less *Miscanthus sinensis* Thumb. (52.5 kg t⁻¹) and at less *Salix* sp. (31.5 kg t⁻¹).

Miscanthus x giganteus	Classes	Number f _i	Frequencies f _i /n	Cumulative frequencies
Amount of ash after combustion in (%)	V = (6.68-7.12]	1	1/20	1/20
	IV = (7.12-7.56]	1	1/20	2/20
	III = (7.56-8.00]	4	4/20	6/20
	II = (8.00-8.44]	6	6/20	12/20
	I = (8.44-8.88]	8	8/20	20/20
Total		20	1	

Table 12. The stemplot for the amount of ash (%) obtained after combustion of aboveground pars of *Miscanthus x giganteus*

Concluding remarks

Miscanthus x giganteus may be used in phytoextraction of cadmium and lead from polluted soils; however, when assessing its potential for phytoextraction of these metals from soil it was found not to be considerable. This plant need to be further tested in terms of cadmium and lead phytoextraction capacity from contaminated soil in natural *environment*.

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