Possibility of Using Energy Crops for Phytoremediation of Heavy Metals Contaminated Land—A Three-Year Experience

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Abstract Heavy metal soil contamination is a worldwide problem. The affected sites could be either sites of a former industrial activity or arable land located in their vicinity. The presence of heavy metals in excessive quantities renders these sites idle or underused due to contamination and lack of efficient ways to remediate. Phytoremediation driven energy crops production may be a promising alternative for the management of these sites. A four year field experiment has been established on heavy metal (HM) contaminated sites located in Bytom, Upper Silesia Industrial Region, Southern Poland (arable land) and Leipzig, Germany (post-industrial site). The objective for this experiment was to distinguished energy crop species optimal with respect to both: energy crop yield and phytoremediation potential. The testing involves the following pre-selected plant species: miscanthus (*Miscanthus* x *giganteus*), virginia mallow (*Sida hermaphrodita*), cordgrass (*Spartina pectinata*), and switchgrass (*Panicum virgatum*). The experimental trials were established in May 2014. Both sites were treated as follows: (i) K—Control, no treatment;

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(ii) NPK—NPK standard fertilization, applied to the soil before the experiment; (iii) INC—Commercial microbial inoculum Emfarma Plus®, ProBiotics Poland. The presented data were collected after the third growing season; heavy metal uptake for each of the species and experimental options were determined. Levels of the bioavailable content of heavy metals in the soil seem to be the main factor responsible for the differences in the metal uptake by the plants. Plant species cultivated at the German site were characterized by low metal concentration in shoots, except *P. virgatum* which accumulated a high amount of zinc, even if the bioavailability of this metal in soil was low. The highest lead uptake was observed for *M.* x *giganteus* and *P. virgatum*, while the highest cadmium content was found for *S. hermaphrodita* grown on a contaminated arable soil in Bytom. Cultivation of energy crops on HM contaminated areas could be a solution for remediating these sites while increasing their economic value.

Keywords Phytoremediation · Energy crops · Heavy metals

1 Introduction

Due to the poor sustainability of heavy industries and overexploitation of resources in the second half of the 20th century, a significant proportion of arable land in Poland (~0.9 Mha) is considered contaminated with heavy metals and thus unsuitable for food and feed production [1]. According to the Institute of Soil Science and Plant Cultivation (IUNG) in Poland, it was found that slightly more than ca 2% of the agricultural land in the provinces of the Upper Silesia, Lower Silesia and Lesser Poland are contaminated with heavy metals to a degree greater than 1 (scale 0–5) [2]. The presence of elevated, and sometimes high content of heavy metals in soils [3] has been also observed in the areas where land are used for other purposes than agriculture.

The European Environmental Agency [4] draws attention to “new reclamation techniques” based on plants strongly accumulating metals, which may lead to a reduction of heavy metal content in soils and at the same time help reduce the costs of remediation. However, as indicated in the report, the possibility of applying these methods and their effectiveness are rather limited due to long time of land reclamation by such techniques. Therefore they will rather not deliver a rapid and radical reduction of the surface of the historically contaminated areas. However the attractiveness of phytoremediation can be significantly enhanced if combined for example with energy crops production.

One of the key criteria for success when planning site remediation with the use of plants is the choice of appropriate plants and the goal of the remediation *i.e.* phytoextraction or phytostabilisation. For the soil clean up purpose the species should be characterised by the ability to accumulate high amounts of heavy metals in the above-ground parts. On the contrary, for the phytostabilisation plants with limited ability to extract and accumulate heavy metals from soil should be considered. In both cases however the production of a satisfactory biomass yield is important.

Energy crops seem to demonstrate features making them useful for both phytoextraction and/or phytstabilisation pruposes. Studies on polluted and unpolluted soils conducted by Institute for Ecology of Industrial Areas (IETU) over the last ten years, have revealed that the uptake of metals by energy crops (*e.g. Miscanthus* x *giganteus*, *Panicum virgatum* and *Sida hermaphrodita*) is dependent on the level of HM bioavailable forms in soil [5, 6]. For example, *M.* x *giganteus* and *S. hermaphrodita* grown on contaminated soils can accumulate up to 150 and 4 mg Pb kg^{-1}, 14 mg and 5.2 Cd kg^{-1}, 700 and 1200 mg Zn kg^{-1} respectively [5, 6].

A four year field experiment has been carried out on heavy metal contaminated sites located in Poland (arable land) and in Germany (former sewage sludge dewatering site). It involves testing of 4 preselected plant species: miscanthus (*M.* x *giganteus*), virginia mallow (*S. hermaphrodita*), cordgrass (*Spartina pectinata*), and switchgrass (*P. virgatum*) to find the optimum one with respect to both energy crop yield and phytoremediation capacity. Differences between the test sites as well as the differences between heavy metal concentration in shoots of investigated plant species after the third year of the experiments are presented.

2 Materials and Methods

2.1 Site Description

The Polish test site is located in the Upper Silesian Industrial Region, on the outskirts of Bytom (50° 20′ 43.0″N 18° 57′ 19.6″E)—an industrial city about 15 km from Katowice, in the proximity of a large closed down lead and zinc works, consisting of the ore mining, enriching and smelting facilities. This metallurgical complex was in operation for more than 100 years and contributed significantly to the contamination of the local soils. During the last 30 years the area was used for agricultural purposes. Recently the land has been used for cultivation of grain crops, especially for wheat production. Soil contamination with lead, cadmium and zinc in this area exceeds permissible limits for agricultural soil in Poland.

The German site is a former sewage sludge dewatering site, located in the north of Leipzig (51° 25′ 23.7″N 12° 21′ 56.2″E). The history of this site is directly related to the main sewage plant of the city. The sewage sludge dewatering site and the sewage plant are located in the distance of about 9 km from each other, operated as one unit from 1952 to 1990. During this time the sewage sludge resulting from municipal and industrial wastewater treatment was pumped to the dewatering site. In 1990 the operation of the dewatering site was abandoned and up to this time about 800,000 tons of sewage sludge remained in several basins.

2.2 Experiment Design

Based on the experience of IETU gained from previous investigations with energy crop species, four plant species were selected for the field trials: miscanthus (*M.* x *giganteus*), virginia mallow (*S. hermaphrodita*), cordgrass (*S. pectinata*), and switchgrass (*P. virgatum*). Experimental plots (16 m^2 each) were established in spring 2014 at each of the test sites. Between the plots a 4 m buffer zone was left to avoid interconnection between experimental variants. Due to apprehension of uncontrolled fertilizer application pseudo-replications were performed. On each plot four sections were distinguished: edge plants excluded from further analysis and three sections (pseudo-replication within one plot) from which samples were taken. Plots were treated in a different way as described in Table 1.

2.3 Chemical Analyses of Soil and Plant Samples

Data on soil characteristics were collected before the start of the experiment. For site characterization three composite soil samples per plot (from the depth of 0–20 cm) were collected and analysed. Physical and chemical soil properties such as: soil texture, pH, EC, content of organic matter, total metal concentration (*aqua regia* extraction) and bioavailable fractions of heavy metals ($CaCl_2$ extraction) were analysed.

Table 1 Experimental variants/soil treatments

Variant	Code	Treatment	Description
1	C	No additives	Control plot
2	NPK	NPK standard fertilization	Ammonium sulphate and Polifoska—4% N, 22% P_2O_5, 32% K_2O—calculation based on specific plant requirements, applied once before plant establishment: – *M.* x *giganteus*—nitrogen 70 kg ha^{-1}, phosphorus 30 kg ha^{-1} as P_2O_5 and potassium 45 kg ha^{-1} as K_2O; – *S. hermaphrodita*—nitrogen 100 kg ha^{-1}, phosphorus 80 kg ha^{-1} as P_2O_5 and potassium 120 kg ha^{-1} as K_2O; – *S. pectinata* and *P. virgatum*—nitrogen 80 kg ha^{-1}, phosphorus 50 kg ha^{-1} as P_2O_5 and potassium 75 kg ha^{-1} as K_2O
3	INC	Inoculum addition	Commercial microbial inoculum (EmFarma Plus^{TM}, ProBiotics Polska Magdalena Górska, Poland). Microbial inoculum consisting of: Lactic Acid Bacteria >3.0 × 105 CFU ml^{-1}, Yeast <1.0 × 106 CFU ml^{-1}, and Purple Non-Sulfur Bacteria >1.0 × 104 CFU ml^{-1} in molasses suspension. It was applied on seedlings roots before plantation establishment and on the leaves as aerosol in the middle of every month during the growing seasons (from May to September 2014, 2015, 2016)

Soil pH was measured in H_2O (1:2.5 m/v) with a glass/calomel electrode (OSH 10-10, METRON, Poland) and a pH-meter (CPC-551, Elmetron, Poland) at 20 °C. The conductivity was determined by an ESP 2ZM electrode (EUROSENSOR, Poland) according to the Polish standard [7].

Soil texture was evaluated using a hydrometric method, according to the Polish standard [8]. The content of bioavailable forms of metals was obtained using extraction with 0.01 M $CaCl_2$. Extraction was conducted with 3 g of air-dried and sieved soil and 30 ml 0.01 M $CaCl_2$ for 2 h.

Plant samples for HM concentration in shoots were collected from three randomly selected plants on each plot which was not exposed to the edge effect. Plant samples were washed, cut and oven-dried at 70 °C, milled and digested using concentrated nitric acid in a microwave system (MDS 2000, CEM, USA). Concentrations of metals both in soil and plants were measured with flame atomic absorption spectrophotometry (Varian Spectra AA300).

2.4 Data Analysis

Data reported in this paper were analyzed using a three-way and one-way ANOVA, followed by a post hoc comparison using the Fisher LSD test ($P < 0.05$). Statistical analyses were performed using Statistica 12 (Statsoft, USA). Spider charts were constructed using Excel MS Office (Microsoft, USA) on standardized data. Standardization of HM concentration in shoots was performed using Statistica 12 Software (Statsoft, USA).

3 Results and Discussion

3.1 Soil Characteristics at the Experimental Sites

Soil characteristics at the experimental sites are presented in Table 2. Soil texture on the experimental field at Polish site was classified as silty loam. The pH-value was almost neutral, followed by a moderate content of organic matter and low electric conductivity. Lead and cadmium contamination levels in soil from the Polish site ranged from 362.3 to 639.1 and 13.69 to 26.29 mg kg^{-1} d.w., respectively. For zinc the range was about 1300—2498 mg kg^{-1} d.w. The results showed that the HM content in soil exceeded the limits defined by the government regulation [9]. Total lead and cadmium concentration exceeded the limits set in the regulation 4 to 6-fold, whereas the total zinc concentration exceeded the limits 4 to 7-fold [9]. The level of bioavailable forms of cadmium and zinc were relatively high (about 5 and 2.5% respectively), whereas bioavailability of lead was below the detection limit.

Table 2 Soil characteristics from experimental site

Parameter	Polish site	German site
pH (1: 2.5 soil/KCl ratio)	5.94–6.55	6.19–6.50
Electrical conductivity (µS/cm)	77–117	484–1495
Organic matter content (%)	4.0–7.08	28.3–39.7
Sand (1–0.05 mm) (%)	28	58
Silt (0.05–0.002 mm) (%)	56	19
Clay (< 0.002 mm) (%)	16	23
Total heavy metal concentration (extraction with aqua regia*)*		
Pb (mg kg^{-1})	362.3–639.1	474.0–686.0
Cd (mg kg^{-1})	13.69–26.29	25.70–36.39
Zn (mg kg^{-1})	1300–2498	2974–4044
$CaCl_2$ extractable metal fraction[a]		
Pb (mg kg^{-1})	BDL	BDL
Cd (mg kg^{-1})	0.349–1.928	0.220–0.460
Zn (mg kg^{-1})	9.26–112.47	3.45–25.60

[a]—extraction with 0.01 M $CaCl_2$
BDL—below detection limit

For the German site, the soil is classified as sandy loam, the pH-value is neutral, followed by high (33%) level of organic matter and electric conductivity, due to upper layer of soil build up by dewatered and decomposed sewage sludge. For the German site lead and cadmium levels in soil ranged from 474.0 to 686.0 and 25.70 to 36.39 mg kg^{-1} d.w., respectively. In case of zinc, the range was between 2974 and 4044 mg kg^{-1}. The bioavailability of metals in soil was very low, mainly due to high level of organic matter (Pb below detection limit, Cd 0.25 mg kg^{-1} d.w. and Zn 16 mg kg^{-1} d.w.). Sewage sludge at the former sludge dewatering site was remediated using phytoremediation. One of the best monitored full-scale phytoremediation projects in Europe. Today the heavy metal content is below limits in terms of use as demonstration site with limited access (not open to the public). The total concentration of investigated HM on German site are at the similar level when compare to Polish site and also exceed the limits prescribe in the Polish regulation [9].

With regards to the contents of heavy metals and their bioavailability both Polish and German site should be classified as marginal. Moreover the agricultural production at Polish and German site should be abandoned [10].

3.2 Heavy Metals in Biomass After the Third Growing Season

Heavy metal accumulation in plant organs depends on different factors *e.g.* HM content in soil (site factor), ability of plants to accumulate HM (plant factor), and plant growth which can be improved by the application of soil amendments (treatment factor) [11–13]. As the obtained results showed, beside the mentioned factors, each of the tested plants has a different natural ability to selectively uptake of Pb, Cd and Zn (Table 3). Moreover HM concentration in the soil, especially the level of bioavailable HM has also a high impact on the HM concentration (particularly Pb and Zn) in plant shoots. Fertilization influenced only Zn uptake in plant shoots. In addition, the combined effect of site and treatments did not influence the HM concentrations in the shoots. It had also no influence on Pb concentration in shoots similarly as the combined effect of factors such as site, species and treatment.

Spider charts (Fig. 1) were used as a tool to assess the values pattern changes of the measured parameters in the above ground organs of the tested plant species among different treatments after third growing season for each site separately. The charts can be divided into three representative sections: Pb concentration in shoots of each tested species (*M.* x *giganteus*, *S. hermaphrodita*, *P. virgatum* and *S. pectinata*) (1), Cd concentration in shoots of each tested species (2) and Zn concentration in shoots of each tested species (3). Significant differences in HM concentration in shoots of the tested species and the applied treatment for each site are presented in Tables 4 and 5, respectively.

It was found that for plants grown on the control plot (C) at the Bytom site *P. virgatum* had the highest Pb concentration in shoots. Significantly lower and equal values were found for *S. pectinata* and *M.* x *giganteus* while the lowest value was found for *S. hermaphrodita*. The Pb accumulation in the fertilized experimental variants at the Bytom site showed the same tendencies as in control. The results obtained for the Leipzig site showed no significant differences of Pb concentration in shoots between species, additionally the same tendency was observed for the fertilized plants. In the case of Cd concentration in the shoots of the control plants cultivated at the Bytom site, the highest values were found for *S. hermaphrodita*, other investigated species had lower Cd concentration in shoots and there were no significant differences in those values between them. The same pattern was observed for the inoculum treated plants, however for the NPK fertilized plants *S. pectinata* had lower Cd concentration in shoots when compared to *M.* x *giganteus* and *P. virgatum*. Variations between the Cd concentration in plant shoots cultivated at the Leipzig site were different when compared to those observed in the plants grown at the Bytom site. The highest value of Cd accumulation on control plots was found for *M.* x *giganteus*, while the rest of the plants accumulated comparable amounts of Cd. Fertilization significantly affected the differences between Cd concentration in shoots of the tested species. Chemical fertilization resulted in the same range of Cd accumulation in *M.* x *giganteus*, *S. hermaphrodita* and *P. virgatum* plants, however *S. pecitnata* showed significantly lower values of Cd

Table 3 Significance of the factor and/or a combination of factors influencing HM uptake to above-ground plant parts of energy crops

	Site	Species	Treatment	Site × Species	Site × Treatment	Species × Treatment	Site × Species × Treatment
Pb	0.0000[*]	0.0000[*]	0.3047	0.0000[*]	0.9156	0.7966	0.2205
Cd	0.3673	0.0000[*]	0.0552	0.000[*]	0.0795	0.0357[*]	0.0080[*]
Zn	0.0000[*]	0.0000[*]	0.0003[*]	0.0000[*]	0.1294	0.0000[*]	0.0000[*]

Values are probabilities that investigated factor or factors combination had significant effect on heavy metals concentration in plants shoots. Probabilities were obtained using two-way ANOVA at $P \leq 0.05$

[*] Significant differences among factors

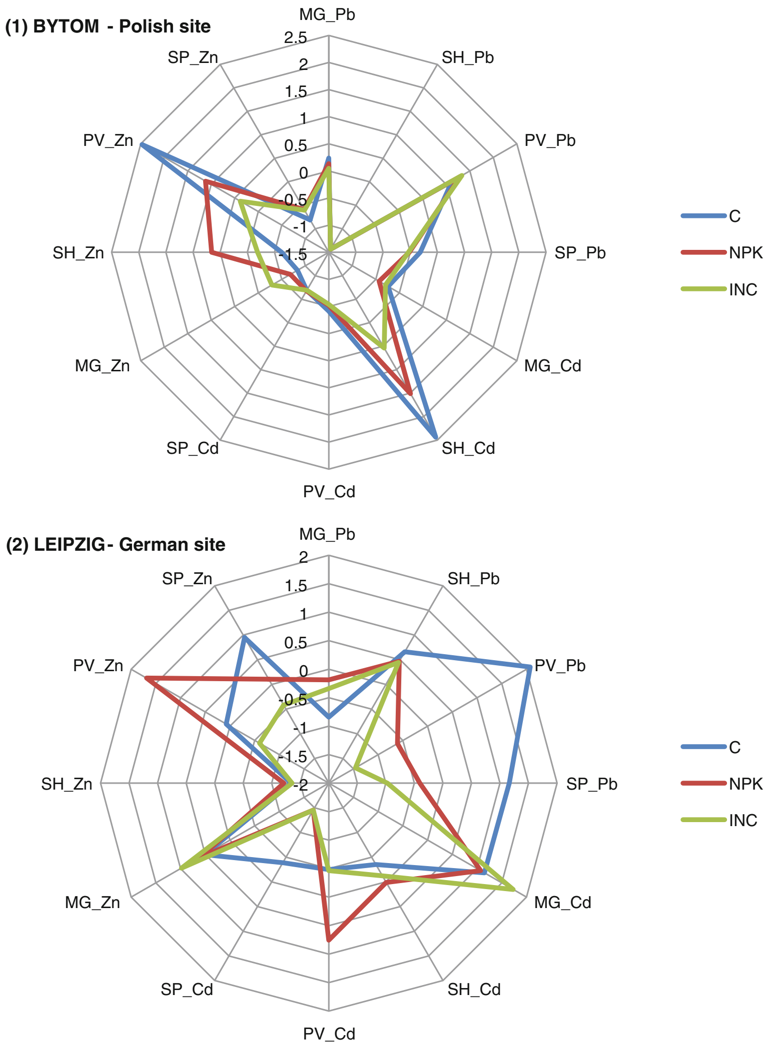

Fig. 1 Spider charts constructed on heavy metals concentrations in shoots show patterns of differences between tested species (MG—*Miscanthus* x *giganteus*; SH—*Sida hermaphrodita*; PV—*Panicum virgatum* and SP—*Spartina pectinata*) and treatments (C—control, NPK—NPK fertilizer treated plant, INC—microbial inoculum treated plant) on Bytom (1) and Leipzig (2) site after third growing season (2016). Pb, Cd, Zn—heavy metal concentration in above ground plant organs. For better data visualization all presented values were standardized. Each measurement was performed in 3 replicates (n = 3)

concentration in shoots compared to the other experimental variants. Microbial inoculation of plants cultivated at the Leipzig site showed similar pattern to this obtained for control plants with the exception of *S. pectinata* which had lower Cd concentration in shoot compared to *S. hermaphrodita* and *P. virgatum*.

Table 4 Matrix of statistical significant differences among analyzed parameters presented on spider charts: differences between tested plant species (Fig. 1)

		Bytom site				Leipzig site			
		MG	SH	PV	SP	MG	SH	PV	SP
C	Pb	b	c	a	b	a	a	a	a
	Cd	b	a	b	b	a	b	b	b
	Zn	b	b	a	b	ab	c	b	a
NPK	Pb	b	c	a	b	a	a	a	a
	Cd	b	a	bc	c	a	a	a	b
	Zn	b	a	a	b	b	c	a	b
INC	Pb	b	c	a	b	a	a	a	a
	Cd	b	a	b	b	a	b	b	c
	Zn	b	b	a	b	a	c	b	b

MG—*Miscanthus* x *giganteus*; SH—*Sida hermaphrodita*; PV—*Panicum virgatum*; SP—*Spartina pectinata*

C—control; NPK—NPK fertilized plants, INC—microbial inoculated plants

A lower case letter (a, b, c—where "a" corresponds to the highest value and "c" to the lowest) denotes significant differences among heavy metals concentration in plant shoots taken from different plots at $P \leq 0.05$ according to Fisher LSD test. Each measurement was performed in 3 replicate (n = 3)

Table 5 Matrix of statistical significant differences among analyzed parameters presented on spider charts: differences between applied treatments (Fig. 1)

		Bytom site			Leipzig site		
		C	NPK	INC	C	NPK	INC
MG	Pb	a	a	a	a	a	a
	Cd	a	a	a	a	a	a
	Zn	b	ab	a	a	a	a
SH	Pb	a	a	a	a	a	a
	Cd	a	a	a	a	a	a
	Zn	b	a	b	a	a	a
PV	Pb	a	a	a	a	b	b
	Cd	a	a	a	a	a	a
	Zn	a	ab	b	b	a	a
SP	Pb	a	a	a	a	a	a
	Cd	a	a	a	a	b	b
	Zn	a	a	a	a	b	b

MG—*Miscanthus* x *giganteus*; SH—*Sida hermaphrodita*; PV—*Panicum virgatum*; SP—*Spartina pectinata*

C—control; NPK—NPK fertilized plants, INC—microbial inoculated plants

A lower case letter (a, b,—where "a" corresponds to the highest value and "b" to the lowest) denotes significant differences among heavy metals concentrations in plant shoots taken from different plots at $P \leq 0.05$ according to Fisher LSD test. Each measurement was performed in 3 replicate (n = 3)

Concentration of Zn in shoots for control plants cultivated at the Bytom site was the highest for *P. virgatum*. The values of this parameter obtained for other tested species were not significant. Similar results were found for the inoculated plants cultivated at the Bytom site. Plant exposed to the fertilizer showed the highest values for *P. virgatum* as in control and for *S. hermaphrodita*. Other tested species had equal and significantly lower values of Zn concentration in shoots. Data obtained for the Leipzig site concerning Zn concentration in shoots showed different effects for each of the treatments. For control plots, the highest Zn concentration in shoots was found for *S. pectinata* and *M.* x *giganteus*, in addition it was found that there were no significant differences between the accumulation of Zn in *M.* x *giganteus* and *P. virgatum*. The lowest values on control plots were found for *S. hermaphrodita*. In the case of fertilization, the highest Zn accumulation was found for *P. virgatum* shoots. The values obtained for *M.* x *giganteus* and *S. pectinata* were equal and lower compared to *P. virgatum*. The lowest Zn concentration in shoots was found for *S. hermaphrodita*. Microbial inoculation caused the highest Zn concentration in shoots of *M.* x *giganteus* while the lowest was found for *S. hermaphrodita*. Accumulation of Zn in *P. virgatum* and *S. pectinata* shoots was significantly higher compared to *S. hermaphrodita* and much lower compared to *M.* x *giganteus*. In addition, the results presented on the spider chart constructed for plants cultivated at the Leipzig site (Fig. 1) show that the changes between the accumulation of Zn in shoots of *P. virgatum* and *S. pectinata* were mostly driven by the applied fertilizer and the response of those plants to the treatment considering the fact that the Zn concentration in shoots was species-specific.

The applied treatment had no influence on Pb concentration in shoot of *M.* x *giganteus*, *S. hermaphrodita* and *S. pectinata* at both investigated sites. However, data on *P. virgatum* plants cultivated at the Leipzig site show that both fertilizers caused lower Pb concentration in shoots compared to control. There were no differences in the Cd accumulation in shots between control and the plants treated with the fertilizers observed for each of the tested species cultivated at the Bytom site. Application of the fertilizers resulted in lower Cd concentration in *S. pectinata* shoots when cultivated on Leipzig site. All the tested species with exception of *S. pectinata* cultivated on Bytom site showed differences in Zn concentration in shoots under different fertilization treatment. *M.* x *giganteus* demonstrated the highest Zn concentrations in shoots when treated with the microbial inoculum, in addition the lowest Cd accumulation was found for control plants. There were no differences between Cd concentrations in shoots of the fertilized plants. In the case of *S. hermaphrodita* NPK fertilizer caused higher Zn concentration in shoots compared to the other experimental variants. Lower values of Zn concentration in *P. virgatum* shoots were found when plants were treated with fertilizers. In the case of plants cultivated at the Leipzig site fertilization did not affect Zn accumulation in *M.* x *giganteus* and *S. hermaphrodita*, however significantly lower and significantly higher values of this parameter were found for *S. pectinata* and *P. virgatum* treated with fertilizers, respectively. There is a dearth of information comparing those four energy crop species on HM contaminated sites, especially under field conditions. Korzeniowska and Stanisławska-Głubiak [14] performed plot experiment where

HM were introduced artificially. They compared accumulation of Cu, Ni and Zn in *M.* x *giganteus* and *S. pectinata* after the first and second growing season in above and below ground plant organs. *S. pectinata* showed higher suitability for phytoextraction of Zn compared to *M.* x *giganteus*. Our results indicate that after the third growing season *S. pectinata* accumulated more Zn than *M.* x *giganteus,* however in comparison to other plant species those differences are insignificant.

4 Conclusions

Among the tested plant species the highest Cd concentration in shoots was found for *S. hermaphrodita* and *M.* x *giganteus* grown at the Bytom and Leipzig site, respectively. The highest Pb concentration in shoots was found for *P. virgatum* originating from the Bytom site, while there were no differences in Pb accumulation among the investigated species grown at the Leipzig site. The highest Zn concentration in shoots was found for *P. virgatum* from the Bytom site, while for Leipzig site the highest values were found for *S. pectinata* and *M.* x *giganteus*. Treatments turned out to have no significant influence on Pb and Cd concentration in the shoots of the tested species. The Zn concentration in shoots of the tested species was differently affected by the treatments. *P. virgatum* and *S. pectinata* showed to be species which were the most sensitive to treatments, while considering HM concentration in shoots. The presented evaluations allow to identify the most suitable species, among investigated, for phytoextraction purposes, simultaneously indicating that the choice of proper species for phytoextraction or phytostabilisation is strongly site specific. When properly planned remediation approach on HM contaminated land using energy crops can be an effective solution for the environmental and economic restoration of such areas.

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