Original Research

Effect of the Reclamation of Heavy Metal-Contaminated Soil on Growth of Energy Willow

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Received: 22 October 2010
Accepted: 27 June 2011

Abstract

In a two-year microplot experiment conducted on sandy and loess soils contaminated with Cd and Pb, the response of energy willow to these metals has been analyzed as well as the results of soil reclamation using two rates of peat. Differences have been observed between the soils, both in terms of the response of plants to pollutants and the effect of the applied peat. Contamination of sandy soil with Cd and Pb have led to complete necrosis of plants, whereas the yield of willow plants obtained on loess was comparable to the control. The application of peat to sandy soil limited the transport of metals by willow plants to aerial parts, restoring the intensity of photosynthesis to a comparable level, as in the control treatment.

Keywords: soil contamination, cadmium, lead, reclamation, energy willow

Introduction

Contamination of soils with heavy metals is not a big problem in Poland. According to the classification prepared by Kabata-Pendias et al. [1], and data published by Oleszek et al. [2], in total – weak and moderate – 1.52% of farmland is contaminated with Zn, and 1.35% with Cd, while 0.65%, 0.4%, and 0.32% are polluted with Pb, Ni and Cu, respectively. Heavily contaminated soils constitute from 0.01% (Ni) to 0.17% (Cd) of the total area of agriculturally used land. Soils that are heavily contaminated occur locally, near non-ferrous metal smelters or mines, and are polluted with Cd (0.08%) or Zn (0.01%).

Reclamation of these areas by traditional physical or chemical methods is costly and often has a destructive impact on the soil environment [3-5]. It seems that only phytoremediation techniques can be helpful in this case. Many research reports are concerned with the so-called phytoextraction. They mainly deal with the attempts to find new plant species that can remove excess heavy metals from soil by taking up large amounts of such elements [6]. For the process of phytoextraction to run successfully, such plant should be characterized by a high concentration of a contaminant in aerial tissues, i.e. at the level of the so-called hyperaccumulation, and to produce a significant amount of biomass. Unfortunately, finding such plants is not easy. Another problem that arises is how to handle contaminated plant material derived from a phytoremediation treatment.

Among the phytoremediation techniques, there is a phtyostabilization method that relies on cropping contaminated land with plants tolerant to high concentrations of toxic substances, e.g. heavy metals. The purpose of this method is to decrease the bioavailability of metals present in soil, to prevent contaminated soil from further degradation caused by erosion, and to reduce the risk of direct contact of animals and humans with the contaminants. Plants used for phytostabilization should be characterized by predominant accumulation of metals in roots and only weak transport of these elements to aerial organs. Storage of heavy metals in roots is helpful because it favors the plant's

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Table 1. Physical and chemical properties of topsoil (0-20 cm).

Soil	$C_{org.}$	SF I	SF II	SF III	Cation exchange capacity mmol(+) kg^{-1}	pH (KCl)	P_2O_5	K_2O	Mg	Total form (mg·kg^{-1})			
	%									initial		metals treatment	
							mg·kg^{-1}			Cd	Pb	Cd	Pb
Sand	0.53	90	8	2	6.8	5.6	135	34	33	0.20	19.9	1.91	338
Loess	0.78	27	63	10	32.4	5.4	144	96	77	0.22	28.0	8.46	1083

SF I – soil fraction 2.0-0.05 mm, SF II – soil fraction 0.05-0.002 mm, SF III – soil fraction <0.002 mm.

increased tolerance to excessive quantities of metals in substrate [7]. Poor transport of heavy metals to aerial parts is associated with protection of the photosynthetic apparatus of plants in which the parameters of photosynthesis appear to be changed under heavy metal stress [8]. Low concentrations of metals in twigs and leaves is also desirable as it reduces the risk of pollutants being carried away by winds and their spreading over new areas. The phytostabilization process can be made more successful when soil is amended with substances blocking heavy metals and stimulating the growth and development of plants, such as lime or peat.

Many authors have investigated the applicability of willow (*Salix viminalis*) for remediation of soils contaminated with various metals. In general, however, they deal with the process of phytoextraction and often discuss experiments conducted in water solutions or in pots [9-14]. The purpose of this study was to determine the tolerance of willow to soil contamination with Cd and Pb and to evaluate the applicability of willow to soil remediation via phytostabilization.

Experimental Procedures

Our study was conducted in the town of Puławy (5° 25'N, 21° 58'E), where average perennial rainfall is 586 mm and average air temperature is 7.7ºC. During the research, these parameters were at the level of 679 mm and 9.6ºC (2008), and 676 mm and 8.7ºC (2009). Concrete-filled microplots of 1×1.2×1 m filled with sandy soil and loess soil with different physicochemical properties (Table 1) were used in this study. For each of the two soils, the following experimental treatments were tested and compared in four replicates:

1. soil without contamination (control)
2. contaminated soil Cd+Pb
3. soil contaminated with Cd+Pb with a single dose of peat (peat 1)
4. soil contaminated with Cd+Pb with a double dose of peat (peat 2)

The soil contamination was simulated by introducing metals in the form of oxides down to a depth of 20 cm, in doses adjusted to the type of soil so as to obtain an average degree of contamination according to the classification by Kabata-Pendias et al. [1]. Varied levels of concentrations of the metals in soil were obtained, much lower in the sandy than in the loess soil (Table 1). For soil remediation, in treatments 3 and 4, high peat (0.25 kg/l in specific weight) was used in the respective amounts of 20 and 40 l per microplot.

Willow (*Salix viminalis*), clone 1023, was planted in April 2008, eight months after the contaminats had been introduced into the soil. Plant density was 9 units per microplot. Willow was planted more densely compared to standard density on the plantations, because at the too small quantity of plants on the plot, the response of willow to metals can result to a greater degree from individual variability than from the characteristics of species. Furthermore, a greater number of plants per m^2 allowed to remove some of them in the early stages of growth and to determinate the contents of metals in different parts of the willow.

In the first year, four months after the planting, when the willow plants reached the height of 1 m, they were thinned, leaving 5 plants per microplot. The removed plants were weighed, sectioned into leaves, twigs, and roots, and subjected to chemical analysis. The concentration of Cd and Pb was analyzed in the plant material, previously ashed in a muffle oven and diluted with nitric acid, with an application of the AAS method. On the remaining plants, during their growing season, net photosynthetic measures were taken with with apparatus Li-6400 (manufactured by Li-COR) set in the central part of the youngest fully developed leaves, at the pre-set, constant radiation intensity PAR = 1200 μmol m^{2}s^{-1}. The measurements were made in three replicates and on three dates. The final yield of energy willow was harvested in November 2009.

An analysis of variance for complete randomized of experimental system was performed using the software AWAR written at the Department of Applied Informatics, Institute of Soil Science and Plant Cultivation in Puławy, Poland [15]. Differences between treatments were analyzed by means of multiple comparisons using Tukey's differences test at 5% significance level.

Results

The response of willow to heavy metals depended on the type of soil. It was caused by both: a different level of simulated soil contamination and different properties of the soils, such as the sorptive complex and the ability of metals to be translocated to soil solution. In the first year, young

Table 2. Yield of willow.

Treatment		Thinning 2008 aerial part		Harvest 2009 twigs	
		g·m^{-2}	%	g·m^{-2}	%
Sand	Control	135.8 a	100	2857 a	100
	(Cd+Pb)	3.3 d	2	1 b	1
	(Cd+Pb) + peat 1	64.5 c	47	2869 a	100
	(Cd+Pb) + peat 2	103.9 b	76	2718 a	95
Loess	Control	115.8 a	100	2499 b	100
	(Cd+Pb)	92.4 b	80	2662 b	107
	(Cd+Pb) + peat 1	98.1 ba	85	3047 a	122
	(Cd+Pb) + peat 2	83.5 b	72	2676 b	107

Identical letters indicate no significant difference according to Tukey's test (P<0.05).

willow plants up to 1 m tall and cultivated on sandy contaminated soil produced just 2% of the aerial mass generated by the control, but when grown on loess soil, the yield was 80% of control (Table 2). In the second year, plants on sandy soil practically died out, whereas on loess soil the yield of twigs on contaminated soil was the same as that obtained on uncontaminated soil.

The addition of peat to contaminated sandy soil considerably limited the depression of yields caused by excess metals. In the first year, young willow plants produced 50% and 75% of the control biomass, respectively, to the dose of peat, which was tens-fold more than on contaminated microplots without peat. Generally, in the first year, peat added to loess soil did not lead to an increase in the biomass yield versus the contaminated treatment. In the second year, however, the yield of twigs obtained from the treatment supplemented with a single dose of peat was 20% higher than the control. A double dose of peat did not cause any further increase in the yield.

The level of the analyzed metals in willow tissues and their distribution in particular parts of plants varied depending on the type of soil. In order to compare the concentration of metals in plants between the soils, the contents are given in relative numbers with respect to the control treatments (Tables 3, 4). Differences in the distribution of the metals in particular parts of willow plants were analyzed according to the ratio of relative concentrations of the elements in roots to their relative concentrations in twigs and leaves (R:T:L ratio).

On sandy soil, in the treatment contaminated with the metals, the concentration of Cd in willow tissues rose by 3- to 10-fold versus the control concentrations. The R:T:L ratio was 1:3.4:0.8, which indicates that Cd accumulated mainly in twigs (Table 3).

Introduction of peat to sandy soil, irrespective of its dose, led to a decrease in the concentration of Cd in twigs and to a beneficial change in its distribution in the plant. The ratio of Cd concentration in roots, twigs and leaves suggests that this element is accumulated mainly in roots (R:T:L ratio = 1:0.6-0.9:0.9).

On loess soil, the concentration of Cd in plants from the contaminated treatment increased approximately 9-fold in aerial parts and about 13-fold in roots compared to the concentration in control plants. Differences in the concentration of this element in particular plant organs were not as significant as on sandy soil, with the highest Cd concentration determined in roots (R:T:L ratio = 1:0.6:0.7). The effect of introducing peat to loess soil was much weaker than on sandy soil. Although a single dose of peat caused a significant decrease in the concentration of Cd in particular plant parts, the distribution of this element in willow plants did not result in considerable changes (R:T:L ratio = 1:0.6:0.6). It was not until a double dose of peat was added to soil that the transfer of Cd from roots to aerial organs decreased considerably (R:T:L rato = 1:0.4:0.4).

Willow grown on sandy soil contaminated with heavy metals contained 6- to 27-fold more Pb in tissues in comparison with the control (Table 4). Lead was mainly accumulated in leaves, followed by twigs and finally roots

Table 3. Concentration of Cd in willow tissues.

Treatment		Leaves		Twigs		Roots		R:T:L-ratio
		mg·kg^{-1}	%	mg·kg^{-1}	%	mg·kg^{-1}	%	
Sand	Control	0.87 b	100	0.35 c	100	0.64 a	100	-
	(Cd+Pb)	2.17 a	249	3.54 a	1011	1.92 a	300	1:3.4:0.8
	(Cd+Pb) + peat 1	2.16 a	248	0.60 bc	171	1.68 a	263	1:0.6:0.9
	(Cd+Pb) + peat 2	2.30 a	264	0.93 b	266	1.80 a	281	1:0.9:0.9
Loess	Control	1.17 d	100	0.46 d	100	0.52 c	100	-
	(Cd+Pb)	11.6 a	991	4.00 a	870	6.98 a	1342	1:0.6:0.7
	(Cd+Pb) + peat 1	8.39 b	717	2.94 b	639	5.96 b	1146	1:0.6:0.6
	(Cd+Pb) + peat 2	4.40 c	376	1.96 c	426	5.52 b	1062	1:0.4:0.4

Identical letters indicate no significant difference according to Tukey's test (P<0.05).

Table 4. Concentration of Pb in willow tissues.

Treatment		Leaves		Twigs		Roots		R:T:L-ratio
		mg·kg^{-1}	%	mg·kg^{-1}	%	mg·kg^{-1}	%	
Sand	Control	1.68 c	100	0.57 d	100	3.00 c	100	-
	(Cd+Pb)	45.4 a	2702	9.29 a	1630	19.1 b	637	1: 2.5: 4.2
	(Cd+Pb) + peat 1	6.22 b	370	2.49 c	437	23.4 ab	780	1: 0.6: 0.5
	(Cd+Pb) + peat 2	6.15 b	366	4.25 b	746	26.9 a	897	1: 0.8: 0.4
Loess	Control	1.51 c	100	0.57 b	100	0.84 c	100	-
	(Cd+Pb)	9.39 a	622	1.88 a	330	31.3 a	3726	1: 0.1: 0.2
	(Cd+Pb) + peat 1	6.63 b	439	1.21 ba	212	22.3 b	2655	1: 0.1: 0.2
	(Cd+Pb) + peat 2	6.22 bc	412	1.48 ba	260	23.7 b	2821	1: 0.1: 0.2

Identical letters indicate no significant difference according to Tukey's test ($P<0.05$).

(R:T:L ratio = 1:2.5:4.2). Soil reclamation using a single dose of peat led to a respectively 7-fold and 4-fold decrease in the concentration of Pb in leaves and twigs, and to a small increase in roots. This meant that the R:T:L ratio underwent a positive change (1:0.6:0.5). A double dose of peat was not more successful than a single one.

On contaminated loess soil, the concentration of Pb rose by 3- to 37-fold versus the control. The amounts of Pb accumulated in willow roots were about 10-fold higher than the ones found in twigs and 5-fold higher than in leaves (R:T:L ratio = 1:0.1:02). Soil supplementation with peat, irrespective of the dose, decreased the concentration of Pb in willow tissues, but did not alter the R:T:L ratio.

In willow plants grown on the plot contaminated with metals, the net photosynthetic rate of leaves changed, mainly in the sandy-soil treatments (Table 5). In the first year, the amount of CO_2 produced by leaves of young plants was 15-fold smaller than under the control conditions.

On the plots where soil was enriched with peat, especially with its double dose, the net photosynthetic rate was comparable to the control. In the second year, the net photosynthetic rate on plots with peat supplemented sandy soil was slightly lower than the control.

On loess soil contaminated with metals and reclaimed by introducing peat, willow was characterized by a similar net photosynthetic rate as on the control treatment.

Table 5. Average results of the measurements of the net photosynthetic rate of willow leaves (μmol $CO_2 \cdot m^{2} \cdot s^{-1}$).

Treatment	2008		2009	
	sand	loess	sand	loess
Control	15.8 a	13.3 a	25.4 a	22.5 a
(Cd+Pb)	1.1 b	11.3 a	-	20.0 a
(Cd+Pb) + peat 1	13.5 a	13.3 a	18.1 b	22.0 a
(Cd+Pb) + peat 2	16.4 a	13.6 a	19.1 b	19.0 a

Identical letters indicate no significant difference according to Tukey's test ($P<0.05$).

Discussion

Tolerance of willow to a high rate of heavy metals in substrate depended on the type of soil and the time elapsed from the planting of willow shrubs. On sandy soil, which is characterized by a small sorptive complex, excessive levels of Cd and Pb caused a large loss in the yield of aerial biomass produced by young plants and led to a nearly complete disappearance of willow plants in the second year of cultivation. On loess soil, with good sorptive properties, the yield of willow was only 20% lower than under the control conditions. In the second year, the yield from contaminated loess soil was as high as from the control treatment. Older plants probably reached with their roots further down where no contaminants appeared, or became more resistant to the effect produced by metals. Hydroponic experiments conducted by Mleczek et al. [16] on the accumulation of heavy metals by willow suggest that 2- and 3-year-old plants are more stress-resistant and adaptable than 1-year-old plants.

A large yield loss of willow plants grown on contaminated sandy soil was attributed to a much higher concentration of metals in aerial organs of young willow plants than in plants on the control plot and in comparison with their concentration in roots. This difference was particularly significant in the case of Pb. Due to such a high accumulation of metals in green parts of willow plants, the intensity of photosynthesis that they conduct is much weaker, which means that the ability to produce biomass is also negatively affected. On loess soil, willow accumulated metals, particularly Pb, mainly in roots. Therefore, the photosynthesis process was less disturbed, as well as the production of biomass. Many authors describe the effect of heavy metals on physiological processes in plants. When heavy metals are present, the activity of many enzymes changes [17, 18], the content of chlorophyll and organic acids in leaves decreases [19], and the activity of photosystem I and II is depressed [17, 20]. Plekhanov and Chemeris [21] found out that an early effect of the toxicity of heavy metals such as Cd and Zn consists of sudden disorders in the release of photoinduced oxygen by cells and inactivation of photosystem II. Ci et al. [22] determined that a cultivar of wheat tolerant to Cd was characterized by lower translocation of Cd from roots to shoots and better parameters of photosynthesis than a cultivar susceptible to excess amounts of this metal. Analogously, various clones of willow are characterized by a different degree of tolerance to heavy metals [18, 23].

Large concentrations of metals in willow aerial part, especially in leaves, is an unfavourable characteristic not only because of the certain disorder it causes in the photosynthesis process and depressed yields of willow, but also because willow, which is affected by such changes, does not fulfill the basic requirement for a plant to be suitable for phytostabilisation, i.e. ability to arrest metals in roots with only a slight translocation of these elements to aerial organs. Moreover, leaves that contain a great amount of heavy metals, when airborne, can spread the contamination to other areas.

Application of peat as a substance enhancing the soil's sorptive complex and improving conditions for the growth and development of plants has led to a decrease in the concentration of metals in particular plant organs and to changes in the R:T:L ratio. Owing to soil supplementation with peat, the toxic effect of metals decreased, especially on sandy soil, where willow-accumulated metals lie mainly in roots and not in aerial parts. As a result, the net phytosynthetic rate was much higher than on the contaminated plots without peat amendement and not much lower than on the control plot. The addition of peat distinctly limited the yield depression caused by excessive quantities of metals in soil.

On loess soil, the effectiveness of peat addition was much lower than on sandy soil. It was not until the double dose of peat had been introduced to soil that the transfer of Cd from roots to aerial organs became limited. As for Pb, the ratio of its concentration on different plant organs was identical on contaminated soil and reclaimed with either dose of peat. The net photosynthetic rate on both treatments was likewise similar.

Conclusions

1. Willow (*Salix viminalis*), clone 1023, grown on loess soil was more tolerant to concomitant contamination of soil with Cd and Pb than when grown in sandy soil, where it responded to the contamination by depressing yields and accumulating the metals mainly in aerial parts. This observation indicates that willow is useful for phytostabilization of these metals predominantly on heavier soils.
2. Use of willow for phytostabilization of metals on light soils is possible if the soil is amended with an appropriate rate of peat.
3. The first year after planting willow is essential for phytostabilization to be successful. Young plants are much more vulnerable to the effect produced by Cd and Pb than older ones. In the second year after planting, they adapt well to the unfavorable growth conditions created by excessive quantities of these metals in soil.

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