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Nickel and lead uptake by willows (*Salix viminalis* L.)

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Abstract. Phytoextraction of heavy metals by willow can be a promising technology for remediation of low- and medium-contaminated soil. Willows are especially effective in taking up Cd and Pb from soil and in transporting them to the aboveground parts of plant. *Salix* is characterized by high capacity for biomass production. Willow cultivated on a soil contaminated with heavy metals produces 13–17 Mg·ha^{-1} dry mass of wood per year. The study presents the influence of municipal sewage sludge on the content of Ni and Pb in leaves, bark and wood. The main aim of the experiment was to select a clone that accumulates the highest amount of Ni and Pb in the leaves, bark and wood. The research with the seedlings of willow was established in 2003 and with sewage sludge in 2008–2010 in Experimental Station in Wrocław (Pawłowice). The experiment was established using split-plot design with two changeable factors in three replications: the first factor was related to various doses of sewage sludge: 75 and 150 Mg·ha^{-1} fresh mass (14.3 Mg·ha^{-1} and 28.6 Mg·ha^{-1} dry mass) and the second factor was four selected clones of willow (*Salix viminalis* L). Analysis of variance showed significant influence of various doses of sewage sludge and genetic properties of clones on the content of nickel and lead in plant material. The greatest amount of heavy metals was determined in the leaves, less in the bark and the least in the wood.

key words: clones, heavy metals, lead, nickel, sewage sludge, willow

INTRODUCTION

Sewage sludge is a waste but contains high organic matter and nutrients content, therefore it may be used for remediation of degraded land or for energetic or industrial purposes. Willow may accumulate in the aboveground biomass heavy metals from sewage sludge especially cadmium (Cd), zinc (Zn) and lead (Pb) and thus it may be used for cleaning contaminated soils from heavy metals. Various species and forms of *Salix viminalis* L. react differently to sewage sludge (Kalembasa et al., 2006; Labrecque and Teodorescu, 2003) and this must be taken into account in order to select the appropriate species for using them for remediation of contaminated soils. The intensive growth of shoots takes place in summer months, especially in older plantations (Jóźwiakowscy, 2001). The main aim of present study was to select a clone that may accumulate the greatest amount of Ni and Pb in the leaves, bark and wood from the soil.

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MATERIALS AND METHODS

A field experiment was carried out as a split-plot design in the years 2008–2010. Two doses of sewage sludge: 75 and 150 Mg ha^{-1} fresh sewage sludge (14.3 and 28.56 Mg ha^{-1} DM sewage sludge respectively) and four selected clones of willow (1001, 1047, 1053 and 1054) were used. Sewage sludge was applied at once before starting the experiment in 2008. Sewage sludge was spread in February 2008 on the soil surface using available hand-held facilities. Willow were planted in 2003 at a distance of 0.7 m x 0.4 m (35 714 seedlings per ha). Sewage sludge was coming from Wastewater Treatment Plant Janówek near Wrocław. The moisture of sewage sludge was about 78%. The total content of nutrient and heavy metals in sewage sludge is given in Table 1. Sewage sludge was strongly alkaline and had high content of macronutrients but low content of heavy metals which did not exceed the permissible concentrations imposed by Polish rules on sewage sludge use.

Each year at the end of the growing season plant samples were collected. Leaves were collected in August from the middle part of shoots from ten randomly chosen plants. The bark was scraped off the wood that came from ten randomly chosen plants. Plant material was dried in special dryer for dry mass (two days temp. 70°C, and one

Table 1. Chemical composition of sewage sludge used in the study.

Chemical composition		Value
The ratio of water and sewage sludge		12
Dry mass	[g kg^{-1}]	220
Organic matter	[g kg^{-1} DM]	450
N	[g kg^{-1} DM]	36
P	[g kg^{-1} DM]	28
Ca	[g kg^{-1} DM]	100
Mg	[g kg^{-1} DM]	7
Pb	[mg kg^{-1} DM]	59
Cd	[mg kg^{-1} DM]	2.00
Cr	[mg kg^{-1} DM]	108
Cu	[mg kg^{-1} DM]	415
Ni	[mg kg^{-1} DM]	50
Hg	[mg kg^{-1} DM]	0.05

day 105°C), minced and transported to laboratory. Soil material was dried (room temperature 20–25°C for five days), minced and chemical analysis was performed. The content of heavy metals (Pb and Ni) was determined using AAS (Atomic Absorption Spectometry) method (Rajfur et al., 2010). Statistical data were done by AWA programme (variance analysis, significance level $\alpha = 0.05$).

RESULTS

Statistical analysis showed that doses of sewage sludge and properties of willow clones significantly affected Pb and Ni content. However the factors under study did not alter Ni content of leaves which fluctuated from 2.84 to 7.83 mg kg^{-1} DM (Table 2).

Research clones did not alter significantly the Ni content of leaves. However, the clones responded differently to various doses of sludge. Clone 1001 reacted positively to the increasing doses of sludge. The double dose caused the highest Ni content in clone 1001. Clone 1047 was characterized with the highest Ni content in the object with a single dose of sludge. The last two clones reacted with the fall of Ni content to the treatment with sludge (Table 2).

Table 2. Influence of sewage sludge rate on the on the willow leaves content of Ni [mg kg^{-1} DM] (mean for 2008–2010).

Dose of sewage sludge	Clone				Mean
	1001	1047	1053	1054	
Control	3.41	6.79	6.55	7.83	6.14
14.3 Mg ha^{-1}	5.38	7.13	5.23	2.84	5.14
28.6 Mg ha^{-1}	5.48	5.32	3.45	4.27	4.63
$LSD_{0.05}$	differences not significant				ns
Mean	4.75	6.41	5.08	4.98	-
$LSD_{0.05}$	differences not significant				-

ns – differences not significant

The study showed significant influence of sludge application on the content of nickel in bark. Application of a single dose of sludge decreased Ni content in clones 1054, 1053 and 1047 compared to the control and had an impact on the level of this element in clone 1001 (Table 3). In clone 1001 single and double dose increased Ni content. This clone seems to be the most tolerant to heavy metals compared to others therefore it may be cultivated on a soil treated with sewage sludge. The fertilization decreased the concentration of Ni compared to that in the control (Table 3). The greatest amount of Ni was found in the bark of the plants grown in the soil without sludge. The content of Ni in bark decreased with increasing dose of sewage sludge. The highest content of nickel in the bark was observed on the treatment without sewage.

Table 3. Influence of sewage sludge rate on the on the willow bark content of Ni [mg kg^{-1} DM] (mean for 2008–2010).

Dose of sewage sludge	Clone				Mean
	1001	1047	1053	1054	
Control	2.60	3.41	3.18	3.98	3.29
14.3 Mg ha^{-1}	2.74	1.26	2.95	1.25	2.05
28.6 Mg ha^{-1}	3.18	2.52	1.12	2.36	2.29
$LSD_{0.05}$	0.71				0.35
Mean	2.84	2.40	2.42	2.53	2.54
$LSD_{0.05}$	differences not significant				-

Interaction between doses of sludge and clones of willow (*Salix viminalis* L.) influenced Ni content in the wood. Research clones did not vary significantly for their Ni content of the wood. Nevertheless, clone 1001 showed the highest Ni content of wood (Table 4). Application of various doses of sludge influenced considerably the Ni content in the studied clones. The double dose of sludge increased Ni content in clones 1047 and 1054 while its effect was opposite in clone 1001. In clone 1053 sludge caused significant changes in Ni content of wood. None of the doses of sludge caused growth of Ni content in clone 1053.

Table 4. Influence of sewage sludge rate on the willow wood content of Ni [mg kg^{-1} DM] (mean for 2008–2010).

Dose of sewage sludge	Clone				Mean
	1001	1047	1053	1054	
Control	1.19	0.16	1.39	0.18	0.73
14.3 Mg ha^{-1}	1.53	0.57	0.15	0.60	0.71
28.6 Mg ha^{-1}	0.13	1.09	0.61	1.41	0.81
$LSD_{0.05}$	0.43				ns
Mean	0.95	0.61	0.71	0.73	-
$LSD_{0.05}$	differences not significant				-

ns – differences not significant

Lead content in leaves was dependent on the interaction between factors: clones and doses of sludge. The higher dose caused the increase of Pb content in the leaves in clones 1001 and 1047 (Table 5). An inverse tendency was observed in clone 1053: decrease of Pb content with the increase of sludge application rate. In clone 1054 the highest Pb content of leaves was found in the treatment without sludge (Table 5).

Table 5. Influence of sewage sludge rate on the willow leaves content of Pb [mg kg^{-1} DM] (mean for 2008–2010).

Doses of sewage sludge	Clone				Mean
	1001	1047	1053	1054	
Control	8.8	7.8	12.4	12.6	10.4
14.3 Mg ha^{-1}	10.9	8.5	11.6	7.9	9.7
28.5 Mg ha^{-1}	11.9	11.8	7.8	11.5	10.7
$LSD_{0.05}$	2.13				ns
Mean	10.5	9.4	10.6	10.7	-
$LSD_{0.05}$	differences not significant				-

ns – differences not significant

Weather conditions did not influence Pb content of leaves but interaction between years and doses did. Compared to the control, in the first year of experiment (2008) the single and double dose of sludge caused the decrease of Pb concentration in leaves. In the second year of the experiment (2009) the double dose of sludge caused the increase of Pb content. In the last year of the experiment (2010) there was no influence of doses of sludge on Pb content (Table 6).

Table 6. Influence of sewage sludge rate on the willow leaves content of Pb [mg kg^{-1} DM].

Dose of sewage sludge	Year		
	2008	2009	2010
Control	11.3	9.2	10.7
14.3 Mg ha^{-1}	9.2	10.5	9.5
28.5 Mg ha^{-1}	10.1	12.7	9.5
$LSD_{0.05}$	1.84		
Mean	10.2	10.8	9.9
$LSD_{0.05}$	differences not significant		

Interaction between doses of sludge and clones influenced significantly n the Pb content of bark. Compared to the control, a double dose of sludge caused increase of Pb content in clone 1053. The highest uptaking was observed for clones 1047 and 1054 on the plot with single dose. Application of sludge did not increase Pb content in clone 1001 (Table 7). Genetic properties of clones did not cause changes in Pb concentration.

Table 7. Influence of sewage sludge rate on the willow bark content of Pb [mg kg^{-1} DM] (mean for 2008–2010).

Doses of sewage sludge	Clone				Mean
	1001	1047	1053	1054	
Control	7.72	2.30	4.30	2.66	4.24
14.3 Mg ha^{-1}	4.40	5.76	2.46	6.07	4.67
28.5 Mg ha^{-1}	2.66	3.86	5.55	4.26	4.08
$LSD_{0.05}$	1.67				ns
Mean	4.93	3.97	4.10	4.33	-
$LSD_{0.05}$	differences not significant				-

ns – differences not significant

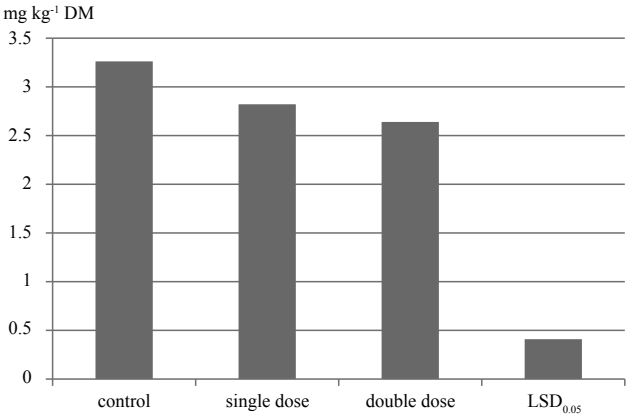

Figure 1. The content of lead in wood (mean for 2008–2010).

None of the doses of sludge caused alternation of Pb content of wood. A Single and double dose of sludge appreciably decreased the concentration of Pb in the wood (Fig. 1).

The amount of Pb in wood was dependent on the interaction between the factors: doses and clones. The clones did not differ appreciably in wood's Pb content. In clone 1001 the increase of the dose of sludge caused an increase and in clone 1047 the highest content was on the control object. Increased doses of sludge compared to control did not change the content of Pb in clone 1047. In clone 1053 the greatest amount of Pb was observed in the treatment with a single dose (Table 8).

Table 8. Influence of sewage sludge rate on the willow wood content of Pb [mg kg^{-1} DM] (mean for 2008–2010).

Dose of sewage sludge	Clone				Mean
	1001	1047	1053	1054	
Control	2.31	3.60	3.23	3.33	3.12
14.3 Mg ha^{-1}	2.66	2.10	4.16	2.14	2.76
28.6 Mg ha^{-1}	3.46	2.90	2.26	2.76	2.84
$LSD_{0.05}$	0.82				ns
Mean	2.81	2.86	3.22	2.74	-
$LSD_{0.05}$	differences not significant				-

ns – differences not significant

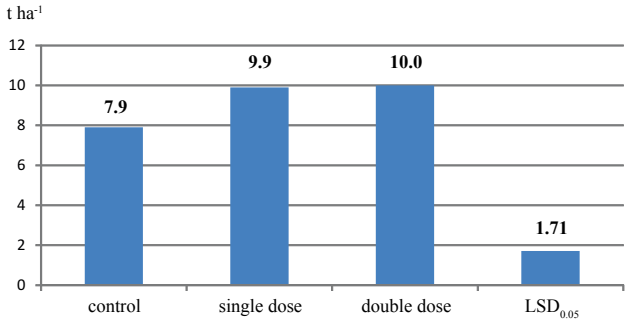

Figure 2. Productivity of selected clones of *Salix viminalis* L. (mean for doses of sewage sludge 2008–2010).

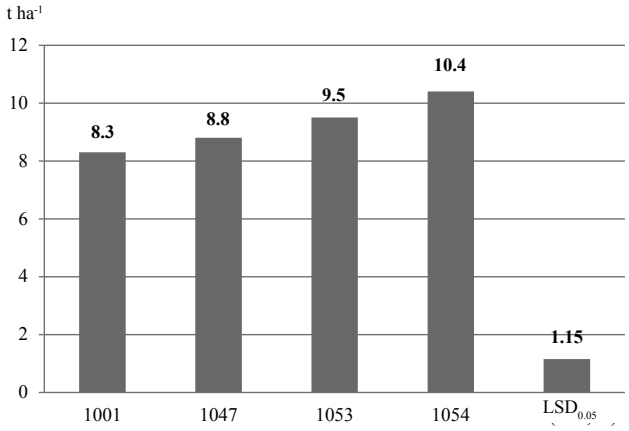

Figure 3. Productivity of selected clones of *Salix viminalis* L. (mean for clones 2008–2010).

The level of accumulation of heavy metals was dependent on the biomass yield of clones. Productivity of clones of *Salix viminalis* was dependent mainly on the doses of sewage sludge and properties of clones (Fig. 2, 3). The highest yield was found in the treatment with a double dose of sludge. Clone 1054 was characterized with the highest yield. Clone 1001 was characterized with the best phytoremediation properties. The amounts of heavy metals which were taken up by clones were calculated in small quantities. Pb was absorbed with higher effectiveness than Ni by the clones.

DISCUSSION

Biomass of willow is characterized by different contents of micronutrients which usually follow the order: leaves>bark>wood (Labrecque et al., 1995; Landberg and Greger, 1996, 2002; Kaniuczak et al., 2000, 2003; Meers et al., 2005, 2007; Maxted et al., 2007; Keller et al., 2003). According to Kalembasa et al. (2009) the greatest amount of lead and nickel was observed in the treatment with the double dose of sewage sludge. Many scientists detected the interaction between clones of willow and the soil. The content of nickel varied in organs of willow. Nickel is easily available for plants and dislocates quickly to their above-ground parts (Jakubiak and Śliwka, 2010). In Kaniuczak et al. (2000) research the greatest amount of nickel was determined in the leaves, bark and wood of clone 1015 that belongs to basket willow. The greatest concentration of Ni was determined in bark, and lower in wood and in leaves. The inverse tendency is observed in this study. The greatest amount of Ni was accumulated in the leaves of clone 1047 and in the bark and wood of clone 1001. According to Kalembasa et al. (2009) the greatest amount of Ni was accumulated in the first year of cultivation. The content of Ni in some parts of willow exceeded the physiological range (0.1–5 mg kg^{-1}) but did not reach the toxic level.

Lead is taken up by the root system in inert way. The quantity of Pb was dependent on genetic properties of clones and soil conditions (Jakubiak and Śliwka, 2010). According to Kaniuczak et al. (2000) the highest concentration of lead was determined in bark, lower in wood and the lowest in leaves. In Michałowski and Gołaś (2001) experiment lead was lowest in roots and shoots. According to Kaniuczak et al. (2000) clones 1012 and 1033 have greater ability to accumulate heavy metals compared to other clones. The amount of Pb did not exceed the toxic level of 5–10 mg kg^{-1} (Jakubiak and Śliwka, 2010). In this study the greatest concentration of Pb was determined in clone 1054 (leaves), 1001 (bark) and 1053 (wood).

CONCLUSIONS

1. Doses of sewage sludge and the properties of selected clones of *Salix viminalis* L. modified the content of Ni and Pb in leaves, bark and the wood.
2. The greatest content of Ni and Pb was determined in leaves, less in bark and the lowest was found in wood.
3. Generally, double dose of sludge caused an increase of Ni and Pb content in plant material compared to single dose.
4. The highest ability for heavy metals accumulation was found in clone 1001.

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