SEWAGE SLUDGE APPLICATION EFFECTS TO FIRST YEAR WILLOWS (*SALIX VIMINALIS* L.) GROWTH AND HEAVY METAL BIOACCUMULATION

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ABSTRACT

Management of sewage sludge is a growing problem worldwide. The use of sewage sludge as fertilizer is increasing. The main aim of the study was to investigate the effects of sewage sludge application on first year willows (*Salix viminalis* L.) and determine the accumulation of heavy metals in plants. Plants were exposed to 1; 2.5; 5; 10; 15 kg m-2 of sewage sludge under field conditions. Willows (*Salix viminalis*) were grown in the field for six months. Effects on plant growth (stem height, underground and aboveground biomass) and the heavy metal (Cu, Cd, Zn, Pb) accumulation in the roots, stems, leaves and bark was determined. Sewage sludge application enhanced the growth of willows (*Salix viminalis*). Stem height of willows grown in soil treated with sludge were by 15 – 31% higher than those grown in control plots. Sludge application has led to higher biomass production. The biomass of stems and roots were by 26 – 61 % and 36 – 62%, respectively, higher than that of control plants. Sludge application resulted in increases heavy metals concentrations in soil and willows. Metals concentrations were plants tissue and sludge application rate dependent. The highest bioconcentration factor was detected for Cd. Due to uptake of heavy metals by willows, the concentrations of Cu, Zn, Cd in soil (at 20 and 40 cm depth) after experiment were significantly reduced.

Keywords: bioaccumulation, heavy metals, remediation, sewage sludge, willows
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1. Introduction

The use of renewable energy resources is one of the high priority areas in the EU policy in order to decrease greenhouse gas emissions and to increase energy independence. Short rotation energy forestry, i.e. the production of fast growing tree species, is one of the alternatives for energy production. The most popular are the short rotation plantation of willows and poplar. Moreover, the willows can be used not only for the energy demand but for artisanal works as well. It is estimated that approximately one third of the total willow chip production costs are costs for fertilizers and irrigation (Hasselgren, 1998).

Sewage sludge can be applied as fertilizer to soils in order to improve and maintain productive soils and stimulate plant growth. Sewage sludge application on willows plantation is a common practice. This is of particular concern because the disposal of sewage sludge on soils is a growing trend. Nutrients found in sewage sludge, such as nitrogen, phosphorus and potassium and trace elements such as calcium, copper, iron, magnesium, manganese, sulfur and zinc, are necessary for plant production and growth. However, sewage sludge has an extensive range of heavy metals and persistent organic pollutants that may accumulate in the soil, be transferred from soil to plants and be incorporated into the food chain and pose a risk to human health (Passuello *et al*., 2010). 16-years systematic and periodical sewage sludge application in study plot study in Spain has led to an increase in organic matter content, soil nitrogen, microbial activity, improvement of carbon and nitrogen mineralization processes and some enzymatic functions and induced some negative toxic effects on soil biota (Roig *et al*., 2012). Field study in Sweden has showed that wastewater and sewage sludge application resulted in higher willows biomass, promoted water and nutrient uptake though it may lead to increased heavy metals concentrations in superficial groundwater (Hasselgren, 1998).

The majority of studies with willows plantations focus on the 2-7 years analysis, while first year willows are especially important for artisanal works and the heavy metal content may pose a human health risk. The aim of this study was to investigate the effects of sewage sludge application on first year willows (*Salix viminalis* L.) and determine the accumulation of heavy metals in plants.

2. Materials and methods

The sludge was collected from the municipal wastewater treatment plant in Kaunas, Lithuania. A one-year small plot field study was conducted in 2013. Cuttings of *Salix viminalis* (0.20 m length) were planted in 6 plots. The topsoil was characterized as sandy loam. Sewage sludge was applied manually at the beginning of the growing season (May). Application rates were 1, 2.5, 5, 10 and 15 kg m^{-2}. Plant and soil material was collected for analysis in October. The plant samples were subdivided into different compartments and washed with distilled water. The stem height, dry weights of shoots and roots was determined. For shoot dry weight evaluation the upper 1/3 of the shoot was taken and dried to constant weight. Soil samples were collected from two layers: 20 and 40 cm below the soil surface. In the laboratory soil and plants samples were dried and analysed for heavy metals content. Heavy metals content was determined in soil and in plant tissues – roots, leaves, stems and bark. Plant and soil samples were dried for 48 h at 70°C temperature and digested using Milestone Ethos One closed vessel microwave system. For the quantitative determination of metals a Shimadzu AA-6800 atomic absorption spectrometer was used.

Bioconcentration factors (BCF) of heavy metals was calculated as the ratio between heavy metal content in the roots and in the soil. Translocation factor (TF) was calculated as the ratio between heavy metal content in the shoots and in the roots. A one-way analysis of variance (ANOVA) was used to assess the sewage sludge concentration effect on estimated endpoints. Significant differences between control and treatment samples were determined by the Dunnett test and $p<0.05$ were considered to be significant.

3. Results and discussion

Sewage sludge application had a significant effect on the height of willows at the end of the growing season in October ($F=4.42$, $p<0.05$). Sewage sludge application ameliorated the growth of shoots with the exception of the treatment with 0.1 kg m^{-2} of sewage sludge (Fig. 1A). The best growth of stems (by 45 % higher than in control) was observed in the treatment with 5 kg m^{-2}. The better growth of willows after sewage sludge application could be explained by higher amount of available nutrients in soil.

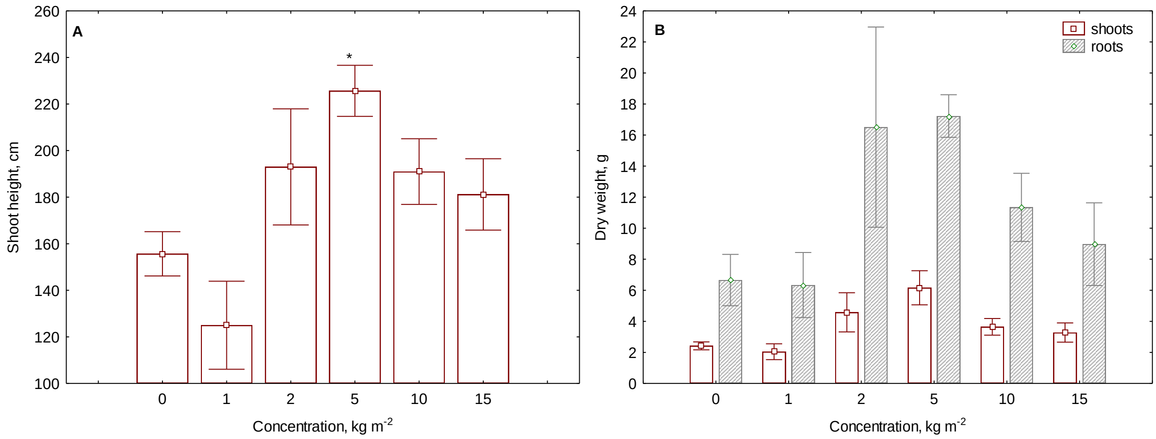

Figure 1: Shoots height (A) and dry shoots and roots weight (B) of willows (Salix viminalis) treated with sewage sludge. An asterisk (*) indicates significant differences (Dunnett, p<0.05) between the treatment and the control.

A significant effect of sewage sludge application was observed (F = 3.42, $p < 0.05$) on the dry weight of shoot, though no significant effect was found on the dry weight of roots (F = 2.08, $p > 0.05$)) (Fig. 1B). The highest dry weight was produced in the treatment with 5 kg m^{-2} of sewage sludge and the dry weight was by 2.6-fold higher than that of control plants. Significant increase in growth and productivity of willows after sewage sludge application was observed in several studies and it was observed that the better fertilization effect could be observed in clay soils than in sandy ones (Labrecque and Teodorescu, 2001).

None of the metals in sewage sludge did not exceed the Lithuanian and EU legislation threshold values for the use of the sewage sludge on land (86/278/EEC). The metal concentrations in the tissue of willows are presented in Fig. 2 A-D.

There was a significant effect of sewage sludge application on copper (Cu) content in different tissues of willows (F>6.71, p<0.0001) with the exception of Cu content in bark (F=1.93, p=0.12). The Cu content in roots and stems increased along with sewage sludge concentration in the soil (roots: r=0.69, stems: r=0.64, p<0.01). While Cu content in the leaves decreased with increasing sewage sludge concentration (r=-0.65, p<0.01). No significant effect (ANOVA, p>0.05) of different sewage sludge concentrations on the Zn and Pb content in different willows tissues was found. No relationship between Pb levels in willows with Pb levels in soil after sewage sludge application was also reported by other researchers (Labrecque *et al*., 1995). Sewage sludge significantly affected Cd content in roots (F=5.16, p<0.01) and the concentration of Cd in roots differed significantly from the control group (p<0.05) and was higher by 49.7-83.6% (r=0. 14, p<0.05).

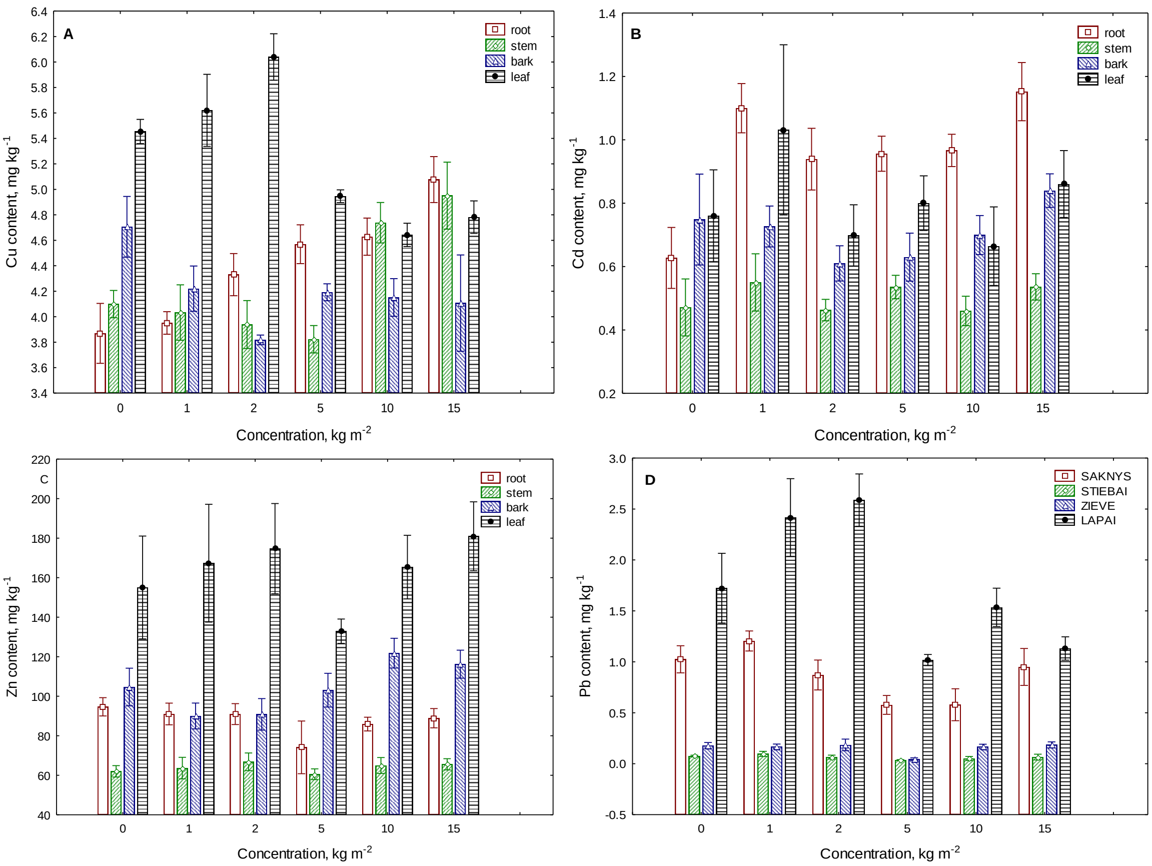

Figure 2: Copper (A), cadmium (B), zinc (C) and lead (D) content in root, stem, bark and leaf of willows (*Salix viminalis*) treated with sewage sludge. An asterisk (*) indicates significant differences (Dunnett, p<0.05) between the treatment and the control.

The highest Cu, Zn and Pb concentrations were observed in the leaves and this phenomenon was also observed in the study in Belgium when willows were planted on contaminated dredged sediments (Meers *et al*., 2005). For the evaluation of willows capability to extract from the soil and accumulate heavy metals, the bioconcentration factor (BCF) was calculated (Table 1). The BCF were ranked in decreasing order as follows: Cd>Zn>Cu>Pb. It indicates that the roots tend to accumulate higher amounts of Cd and the uptake of Pb is very weak. Cd and Zn are mobile in the soil and their phytoavailability is high, on the contrary, Pb is usually tightly bound to soil colloids. High BCF for Cd was detected for different plants species (Vervaeke *et al*., 2003; Žaltauskaitė, Šliumpaitė, 2013). Though, it was shown that plants may accumulateCd in roots in a non-active form and this may reduce Cd negative impact to roots (Sandalio *et al*., 2001). Sewage sludge application rate had significant impact on the Cd and Zn bioconcentration factors ($F>3.98$, $p<0.01$). BCF of Zn significantly decreased with sewage sludge application rate ($r=-0.56$, $p<0.05$), indicating decreasing uptake ability.

Table 1: Bioconcentration factors and translocation factors of heavy metals for willows (*Salix viminalis*) treated with sewage sludge

Sewage sludge concentration, kg m^{-2}	Cu		Cd		Zn		Pb	
	BCF	TF	BCF	TF	BCF	TF	BCF	TF
0	0.61	1,07	7,62	0,87	1,55	0,67	0,04	0,08
1	0,58	1,02	13,83 *	0,49	1,54	0,71	0,06	0,08
2	0,62	0,92	10,72	0,55	1,63	0,75	0,04	0,08
5	0,60	0,84 *	11,70 *	0,57	1,18	1,53	0,03	0,08
10	0,57	1,03	11,79 *	0,44 *	1,24	0,76	0,03	0,09
15	0,56	0,98	11,50 *	0,47 *	1,08 *	0,74	0.06	0,09

* – significant differences (Dunnett, $p<0.05$) between the treatment and the control

Ability of willows to translocate heavy metals from roots to shoots was further confirmed by calculating the translocation factor (TF). TF values less than 1 imply higher accumulation of heavy metals in roots while a value more than 1 indicates heavy metal transportation from roots to shoots. TF values for Cd and Pb were less than 1, implying that the roots are primary target of these metals accumulation and the transportation to the shoots is limited. No clear relationship between TF and external Cd and Pb concentration was detected, though the tendency of decreasing TF with external Cd concentration could be observed. The TF value of Cu were above 1 or very close to 1, indicating that Cu is mobile and is rather easily translocated in the plant.

4. Conclusions

Results indicate that sewage sludge application does not pose toxicity to willows (*Salix viminalis*) growth. Application of sewage sludge resulted in a significant increase in the growth and biomass production. Willows did not exhibit high Pb and Cu accumulation potential, though significant accumulation of Cd and Zn was observed. The study indicates that first-year willows do not pose a risk due to high heavy metal bioconcentrations and they may be safety used for different purposes.

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