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ARTICLE

Change of root and rhizosphere characters of willow (*Salix* sp) induced by high heavy metal pollution

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ABSTRACT The abandoned Pb/Zn mine causes heavy metal problems in the surrounding area at Gyöngyösoroszi (North-Hungary). The Toka-river transported heavy metal (Cd, Cu, Pb, Zn) ions from several heaps deposited imprudently near a historic lead and zinc-mining site. Willow (*Salix* sp.) is one of the plants often applied for phytoremediation practice, since its high metal accumulation capacity. Proposal of this work was to investigate the change of root and rhizosphere characters of willow plants induced by high heavy metal pollution. The response of root mass/soil samples, fine root mass/other roots and BAF-s (bioaccumulation factors) and also two soil biological properties soil microbial biomass and acid phosphatase activity to heavy metal pollution were studied. All measured biological parameters have proved to indicate soil pollution, root mass decreased, portion of fine root increased, BAF-s values with exception of Cd decreased significantly. Both measured biological parameters of willow rhizosphere therefore could indicate soil pollution, but change was adverse, biomass decreased while phosphatase activity increased. Microbial biomass and phosphatase activity were not correlated, indicating different account of ecological factors that altering biological properties of a soil.
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KEY WORDS

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The mining activity surrounding the historic Pb/Zn mine at Gyöngyösoroszi (Hungary) causes heavy metal problems on the environment through the contamination caused by deposition from stream (Horvath and Gruiz 1996). Element content of plants depends partly on the inherent properties of plants the species, developmental stage and plant part. Willow is one of the plants that often investigated for phytoremediation purposes, having high metal accumulation capacity and easily cultivable characters (Tremela et al. 1997; Pulford and Watson 2003). *Salix* species were reported as Zn-accumulators (Vashegyi et al. 2005), or Cd- and Zn-accumulators (Vandecasteelea et al. 2002; Máthé-Gáspár and Anton 2005). Willow plant is considered that may be the best indicator of elevated Zn and Cd (Pugh et al. 2002).

It is well known that heavy metals causes significant decrease in fresh and dry weight and length of root. Wilkins (1957) and Jowett (1964) introduced a tolerance index (TI), which is a ratio of treated root length to control root length.

Biological and biochemical parameters of the rhizosphere, such as microbial biomass and enzyme activities are considered as indicator of soil quality (Brookes 1995; Szili-Kovács et al. 1998; Simon and Biró 2005; Takács et al. 2005). There are increasing evidences of microbial biomass decrease due to metal contamination in soils (Brookes et al. 1986; Filip 1998). Phosphatase activity showed a sensitive response to metal contamination according to several studies (Máthé and Kovács 1980; Anton et al. 1994; Máthéné Gáspár et al. 2005).

Most investigations are based on the soil metal enrichment in laboratory and very few attempts were made to study it in the field conditions. To study this latter situation is difficult because of the spatial variability of sites and pollutants and it is also critical to find an appropriate non-contaminated control site (Kádár and Németh 2003; Máthé-Gáspár et al. 2004). Szili-Kovács et al. (1999) have investigated experimentally metal polluted field plots seven years after metal loading. All investigated (Cu, Ni, Zn and Cd) polluted soils had lower microbial biomass than the control.

The objective of this paper was to study the change of several root characters, microbial biomass C and phosphomonoesterase activity of willow rhizosphere influencing heavy metal pollution in the field conditions.

Materials and Methods

Experimental site

Experimental site is located at the bank of Toka-river near Gyöngyösoroszi village (North-East Hungary) near an abandoned Pb/Zn mine. The soil type is Fluvisol. The climate of the region is temperate with continental features. The vegetation is heterogeneous containing natural and weedy elements

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Table 1. Main metal accumulation in the topsoil at the polluted and unpolluted experimental sites along the Toka river. Different letter in upper index of the corresponding parameters means significant difference at $p<0.05$.

Element content, mg kg^{-1} soil									
Unpolluted soil					Polluted soil				
samples	Cd	Cu	Pb	Zn	samples	Cd	Cu	Pb	Zn
1	0.360	62.3	35	141	1	19.7	325	1409	3181
2	0.618	84.5	47	211	2	28.8	493	2827	4417
3	0.932	100	65	274	3	11.9	197	724	2190
4	1.05	90.4	64	285	4	18.9	298	1183	3185
5	0.575	87.5	47	199	5	16.9	315	1620	2873
6	0.698	105	56	221	6	15.4	364	1974	2650
mean	0.71^{a}	88^{a}	52^{a}	222^{a}	mean	18.6^{b}	332^{b}	1623^{b}	3083^{b}

as well. A phytoremediation experiment was set up in 2003 year by planting willow trees in rows along the contaminated and uncontaminated sites. The contaminated area is located approximately a 10-metre-wide strip along the river. The site at higher distance from the river is considered to be unpolluted according to the soil chemical data.

Plant and soil sampling and chemical analyses

Plant and rhizosphere soil samples were taken at six unpolluted (UP) and six polluted (P) points around planted willows (*Salix* sp.) in the 6th of October 2004 year from 0-20 cm depth. The moist samples were sieved (2 mm mesh) and stored at 4°C until the analyses has been performed. Root and soil samples were separated. Root samples after mechanical cleaning and washed with deionized water, and then dried at 70°C until the stabilization of weight, soil samples were dried at 105°C.

The metal concentrations of soils and plant roots were determined after standard preparation (soil extraction by HCl/HNO_3 and NH_4-acetate –EDTA and plant by HNO_3) by ICP spectrometry. Basic soil properties like humus content, pH, $CaCO_3$, plasticity index (K_A), salt, moisture content was measured.

Microbial biomass C and phosphatase activity

Microbial biomass C of the rhizosphere soil samples of willow was estimated by chloroform fumigation extraction (Vance et al. 1987). Fifteen g of soil was fumigated by chloroform in a desiccator for 2 min then left it overnight. After chloroform removal by repeated vacuum fumigated and unfumigated samples were extracted by 0.1 M K_2SO_4 after shaking filtered and the organic carbon was measured by a combustion TOC analyzer (Shimadzu). Biomass C was calculated as the difference of extracted organic C between the fumigated and unfumigated samples multiplied by a conversion factor, k_{EC} = 2.63 proposed by Vance et al. (1987).

Acid phosphatase activities were determined in the sampling time according to Tabatabai and Bremner (1969). One g of moist fresh soil was incubated in 4 mL modified universal buffer (pH 5.5 for acid phosphatase) and 1mL p-nitrophenyl phosphate (15 mM) for 1h at 37°C. After incubation, 1 mL $CaCl_2$ (0.5 M) and 4 mL NaOH (0.5 M) were added to stop the reaction and to increase the pH. The nitrophenol concentration was determined photometrically at 410 nm.

Data analysis

Root mass characters, chemical data of roots and soil samples, the biomass C and acid phosphomonoesterase activity values between the polluted and unpolluted sites were compared by two-samples *t*-test.

Results and Discussion

Heavy metal pollution of soil

Polluted soil samples were characterized by high content of Cd, Cu, Pb, Zn, (11.9-29.8, 197-493, 724-2827, 2190-4417 mg/kg, respectively (Table 1). The repeated flooding may deposit sediments containing metals in elevated concentration. The humus content and pH was not differed significantly between the polluted and unpolluted sites.

Changes of root characters

Heavy metal pollution induced a decrease in the root mass/soil mass value (in the sample mean), but increased in fine root mass/other root mass value (Table 2). Heavy metals in enhanced concentration caused a reduction in root mass (35.54%) but increased fine root mass (468%). Changes indicate significance in differences of growth affected by high heavy metal concentration of soil.

While the concentration of heavy metals has increased in the soil, their amounts in plant roots have decreased therefore the bioaccumulation factors were also decreased (Table 3). Values of BAF of willow root decreased, with the exception of Cd resulting 2.5, in the order of Zn (to 75.8%), Cu (to 66.4%) and Pb (to 38.9%).

Table 2. Root mass/soil mass, fine root mass/other root mass (g dry weight/ g dry weight, %). Different letter in upper index of the corresponding parameters means significant difference at $p<0.05$.

Unpolluted soil samples	root/soil, %	fine root/other root, %	Polluted soil samples	root/soil, %	fine root/other root, %
1	17.60	0.45	1	7.70	1.20
2	11.50	0.62	2	4.72	2.55
3	17.02	0.10	3	6.20	0.96
4	24.00	0.05	4	5.25	1.85
5	16.22	0.58	5	2.34	2.20
6	15.76	0.26	6	11.10	0.89
mean	17.02 [a]	0.34 [a]	mean	6.22 [b]	1.61 [b]

Table 3. Bioaccumulation Factors of willow root.

Bioaccumulation Factors Unpolluted soil samples	Cd	Cu	Pb	Zn	Polluted soil samples	Cd	Cu	Pb	Zn
1	4.363	0.14	0.0092	0.9434	1	2.2386	0.042	0.0029	0.4041
2	2.199	0.128	0.0068	0.5806	2	1.5346	0.039	0.0024	0.3672
3	2.134	0.111	0.0155	0.6477	3	3.2484	0.121	0.0067	0.7307
4	2.3163	0.104	-	0.6232	4	2.6224	0.089	0.0038	0.4439
5	2.5001	0.103	0.0121	0.6499	5	3.5803	0.119	0.004	0.667
6	2.5957	0.106	-	0.6944	6	2.8196	0.081	0.0068	0.5915
mean	2.5041	0.114	0.0106	0.6713	mean	2.513	0.075	0.0041	0.5085

Table 4. Mean values of phosphorus (total, LE-soluble), water content, phosphatase activity and microbiological biomass of the rhizosphere soil samples. Different letter in upper index of the corresponding parameters means significant difference at $p<0.05$.

Soil samples	'Total' P content mg kg^{-1} d soil	'LE –P content mg kg^{-1} d soil	Soil water content, %	Phosphatase activity, µmol pNP g^{-1} d soil h^{-1}	Biomass C µg g^{-1} d soil
UP	1112 [a]	653 [a]	18.74 [a]	0.8482 [a]	186.2[a]
P	783 [a]	71 [b]	20.66 [b]	1.3242 [b]	71.3[b]

Rhizosphere response. Microbial biomass and phosphomonoesterase activity

Microbial biomass C was significantly higher in unpolluted soils comparing with polluted ones (Table 4). The standard errors were higher in unpolluted soils, which might be attributed to the heterogeneous nature of the sample having microsites with various microbial activities. The average of soil microbial biomass C was 186 mg kg^{-1} and 71 mg kg^{-1} for unpolluted and polluted soils respectively.

Phosphomonoesterase activity of unpolluted (UP) soil samples ranged between 0.78 and 0.97 µmol pNP g^{-1} dry soil h^{-1}. A significant increase of acid phosphomonoesterase activity (0.88 and 1.58 µmol pNP g^{-1} dry soil h^{-1}) was determined in the soil due to the pollution. Phosphatase production of living organisms could be stimulated by the higher moisture content and by the significantly lower LE-soluble phosphorus content of the polluted soil samples (Table 4).

All measured biological and biochemical parameters therefore could indicate soil pollution, root mass and microbial biomass decreased, portion of fine root and phosphatase activity increased, BAF-s values with exception of Cd decreased significantly. Changes in observed parameters indicate not only the influence of heavy metals, but also other ecological factors.

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