UDC 502.174:504.5
582.681.81:669.018.674
Original research paper
doi: 10.5937/AASer1846167D
Acta Agriculturae Serbica, Vol. XXIII, 46 (2018); 167-176

Uptake and distribution of metals in *Populus nigra* and *Populus tremula*

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Abstract: Woody plants are increasingly used in the remediation of contaminated land. The content of metals (Mn, Fe, Cu, Zn, Cr, Ca, Mg) was analyzed in the plant species *Populus nigra* L. and *Populus tremula* L. and in the soil on which they grow. The aim of the research was to determine differences between these two species based on the content of metals in plant organs (sprouts and leaves) and bioaccumulation potential and point out the potential application of these species in phytoremediation. Atomic absorption spectrometry was used to measure these heavy metals in soil and plant material. The results showed that *P. tremula* was a better metal accumulator because it absorbed larger quantities of Mn, Fe, Cu and Zn from *P. nigra*. *P. nigra* absorbed more chromium than *P. tremula*. Both species proved to be good zinc accumulators, and better bioaccumulation potential was found in *P. tremula* of *P. nigra*, although aspen had a strong negative correlation with Zn.
Keywords: *P. nigra* L., *P. tremula* L., phytoremediation, metals

Introduction

The presence of metals/metalloids in toxic amounts is a particularly important ecological problem unfavorably affecting human health. Metals in the soil are primarily derived from the geological substrate (Langer 2011).

Received 29 June 2018   Accepted 22 September 2018

In higher concentrations, Zn is phytotoxic; therefore, studying this metal is very important because it directly affects soil fertility, and the yield, and quality of cultivated plants. The main sources of soil pollution with Zn are: pesticides, fertilizers, mines, iron foundries, use of waste sludges in agriculture, composted materials (Kiekens *et al*., 1995). A high concentration of Zn is toxic to plants. There are plant species that have a distinct ability to accumulate Zn. In such plants, the Zn content in the whole biomass can range from 1.6 to 17% (Kastori, 1997).

Plants that have the ability to absorb large amounts of heavy elements are used in phytoremediation processes. Perennial woody plants are increasingly used to remove metals from soil and transfer them into harmless forms (Miller *et al*., 2011). Plants of the fam. Salicaceae are increasingly used in phytoremediation technologies (Isebrands and Karnosky, 2001) because they are tolerant to floods, rapidly growing and spreading easily, can grow from cuttings, have the ability to tolerate many types of toxic substances (both inorganic and organic compounds) (Schnoor, 1997).

Poplars (*Populus*) are resistant to the presence of metal (Cd, Cu, Zn, Ni, Pb, Fe) and have the ability to accumulate significant amounts of these pollutants in the plant. As documented and suggested in many papers, the use of these plants is very profitable and cost-effective in the extraction of metals from contaminated environments (Schnoor, 1997; Keller *et al*., 2003; Pietrosanti *et al*., 2004).

The aim of this study was to investigate the potential of *P. nigra* L. and *P. tremula* L., growing on unpolluted soils, for phytoextraction of selected elements and the possibility to use these plants for decontamination of areas polluted with Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn. Additionally, the research examined the content and relationship of essential plant elements Ca and Mg in plants and land.

Material and methods

Branches with leaves of *P. nigra* L. (black poplar) and *P. tremula* L. (aspen) were collected for the analysis of the concentration and distribution of metals Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn. *P. tremula* plant material and soil were collected from the Trešnjevica village site (43.135235"N, 19.872544"E) (25 km southwest of Sjenica). *P. nigra* plant material and soil were collected from the Ovčar spa.

Samples of plants, twigs, and leaves were harvested in August and October 2015. After collection, leaves were dried. The plant material was milled. Plant material was digested with concentrated HNO_3 and $HClO_4$. Soil samples were generally taken from a depth of 0–10 cm after removing any loose organic matter from the surface. The soils were air-dried and sieved initially to <2 mm to remove any stony material. Soil samples were prepared by standard procedure (using HCl) for the preparation of samples for chemical analysis (APHA AWWA WPCF 1998).

In the soil and plants samples, the concentrations of Ca, Mg, Fe, Mn, Zn, Cr, Ni, Pb, Co and Cd were determined using a Perkin Elmer Analyst 3300 flame atomic absorption spectrometer at the Faculty of Science in Kragujevac. Each sample was analyzed in six repetitions, followed by calculation of mean value and standard deviation.

A bioaccumulation factor was also calculated. Metal concentration in the plant material and the soil was expressed in mg/kg of dry matter (d.m).

It takes time for plants to reduce the amount of heavy metals in contaminated soils depending on the production of biomass and the bioaccumulation coefficient. The bioaccumulation coefficient is obtained using the next formula:

BAF=Cplantmaterial/Csoil

Cplantmaterial and Csoil are metal concentrations in the plant material ($mgkg^{-1}$) and soil ($mgkg^{-1}$), respectively (Kabata-Pendias, 2011).

The differences in the concentrations of metals in the plants, and between the plants and the soil were determined by the analysis of variance (one-way ANOVA) at the level of significance of $p<0.05$. Statistical significance was also determined using Pearson's correlation coefficient. It was determined whether there was a statistically significant difference in the content of metals between soil and plants. The correlation coefficient (r) was measured as: 0–0.3: no correlation; 0.3–0.5: poor correlation; 0.5–0.7: medium correlation; 0.7–0.9: high correlation: 0.9–1.0: very high statistically significant correlation (Ward, 1963; Brereton, 2003; Temple *et al*., 2006). Statistical analysis was performed using the SPSS Statistics software (SPSS 16 for Windows).

Results

The results of the analysis of metal concentrations (Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn) in the soil from which the plant material was collected is shown in Table 1. The amount of Cd in the soil was below the detection limit.

In all tested soils, Fe predominated, whereas the amount of Co was the lowest.

The results of the analysis of metal concentrations (Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn) in the leaf and twig samples of the studied species are shown in Table 2. The concentrations of Ni, Pb, Co and Cd in plant parts were below the detection limit.

Table 1. The concentration [mg kg^{-1} d.m] of Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn in the tested soil

metal	***Populus tremula***	***Populus nigra***	***MPC***
	Soil	**Soil**	
Mn	495.5±5.17	288.8±2.35	2000
Fe	19436.7±39.43	37760.8±39.0	50000
Cu	16.5±0.46	26.9±0.42	100
Zn	46.8±0.55	49.8±0.64	300
Cr	305.5±1.93	505.0±5.55	100
Ca	6630.5±34.67	8054.3±35.71	
Mg	3054.7±26.23	28041±32.41	
Ni	9.4±0.03	89.5±0.69	50
Pb	24.2±0.77	29.9±0.34	
Co	7.1±0.08	20.8±0.60	
Cd	ND	ND	

Table 2. The concentration [mg kg^{-1} d.m] of Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn in the twigs and leaves of the studied plants species

metal	***P. tremula***	***P. nigra***	***P. tremula***	***P. nigra***
	Leaf	**Leaf**	**Twigs**	**Twigs**
Mn	75.7± 0.36	52.5± 1.1	456.6±0.78	16.6±0.42
Fe	418.3 ± 0.69	300.4 ± 1.6	294.5 ± 0.93	318.9±0.66
Cu	4.6 ± 0.07	4.5± 0.06	12.5± 0.28	6.2±0.80
Zn	64.8±0.44	70.8±0.32	117.0±0.57	27.9±0.52
Cr	20.8±0.67	16.4±0.44	20.1 ± 0.58	40.4±0.17
Ca	10967.3±55.52	46542.3±171.41	27181.5 ±78.52	11896.5±77.35
Mg	1567.0±23.96	9570±17.69	3475.2 ±138.84	1591.3±10.42

The mean values of the metal concentrations in branch samples of *P. tremula* were Ca> Mg> Mn> Fe> Zn> Cr> Cu, while those in *P. nigra* branches followed the order: Ca> Mg> Fe> Cr> Zn> Mn> Cu.

The mean values of the metal concentrations in the leaves of *P. nigra* were ordered as follows: Ca> Mg> Fe> Zn> Mn> Cr> Cu. In the leaves of *P. tremula* the mean values of the metal concentrations followed the order: Ca> Mg> Fe> Mn> Zn> Cr> Cu.

The results of the analysis of the bioaccumulation coefficients for the tested metals are shown in Table 3.

Twigs of *P. nigra* and *P. tremula* had bioaccumulation coefficients greater than 1 for Ca, and the bioaccumulation coefficient for Zn in *P. tremula* was larger than 1 . The smallest bioaccumulation coefficient of the branches was found for Fe.

The leaves of all investigated plants had bioaccumulation coefficients greater than 1 for Ca and Zn.

Table 3. Bioconcentration factor values

Plant org./soil	*P. tremula*	*P. nigra*	*P. tremula*	*P. nigra*
	Twigs	Twigs	Leaf	Leaf
Mn	0.92	0.06	0.15	0.18
Fe	0.01	0.008	0.02	0.01
Cu	0.75	0.23	0.28	0.17
Zn	2.5	0.56	1.38	1.42
Cr	0.06	0.08	0.07	0.03
Ca	4.1	1.48	1.65	5.78
Mg	1.14	0.06	0.51	0.34

The analysis of variance was used to compare metal concentrations in the soil and the selected plant species in various regions, to determine the ability of these plants to accumulate the test metals. The results showed statistically very significant differences in the content of metals between the selected species and soil. Also, there was a statistically significant difference in the content of the tested metals in the selected plant species, which were sampled from different locations.

The values of the coefficient of correlation between the concentrations of metals in the investigated plant species (Table 4) showed a positive correlation (0–1) for the content of chromium in twigs and leaves of *P. nigra* and *P. tremula*, and a negative correlation (-1–0) for the Ca content of the leaves of both plants and the soil. In Table 4, strong positive and negative correlations are highlighted (*).

Table 4. The values of the coefficients of correlation (r) between the concentrations of metals in the soil and the plant species

		Mn	Fe	Cu	Zn	Cr	Ca	Mg
Plant species	Plant organ	R	R	R	R	R	R	R
P. nigra	Twigs	-0.46	-0.37	-0.03	0.37	0.20	0.22	-0.68
	Leaf	0.17	-0.42	0.15	0.17	0.85*	-0.43	-0.12
P. tremula	Twigs	0.65	-0.58	0.37	-0.78*	0.57	-0.05	0.90*
	Leaf	-0.41	-0.16	-0.59	-0,78*	0.70	-0.31	-0.20

Discussion

The manganese content in the tested soils was below exposure limits (495.5 and 288.8 mg/kg, respectively). *P. tremula* accumulated more manganese (75.7 and 456.6 mg/kg, respectively) compared to *P. nigra* (52.5 and 16.6 mg/kg, respectively). The amount of manganese found in *P. tremula* (Table 2) was above the lower limit of manganese toxicity (80 mg/kg) according to Le Bot *et al*. (1996), which indicates that this species is tolerant to some extent.

The concentration of iron in the tested soils was below permissible levels except at the location of Čačak (51,421 mg/kg) (Table 1). The amount of iron in the examined species was within the limit values (50 to 500 mg/kg, Kabata-Pendias, 2011). Leaf of *P. tremula* absorbed higher Fe concentrations (418.3 mg/kg) compared to *P. nigra* (300.4 mg/kg), while *P. nigra* twig absorbed higher Fe concentrations (318.9 mg/kg) compared to *P. tremula* twig (294.5 mg/kg).

The amount of copper in the analyzed soil was lower than the average value (16.5 mg/kg and 26.9 mg/kg, respectively) (Table 1) according to Kabata-Pendias (2011) (about 30 mg/kg). Research results (Table 2) showed that the test species absorbed a small amount of copper (Greater concentrations of copper in the leaves and twigs were uptaken by aspen (4.6 and 12.5 mg/kg, respectively) than by black poplar (4.5 and 6.2 mg/kg, respectively).

The analysis of the chromium content in the soil from which the plant material was taken showed that the soil contained 3 to 5 times higher concentrations than the maximum allowed quantity (Table 1). Our results showed much higher chromium concentrations than average values (0.1–0.4 mg/kg) in twigs and leaves (Table 2) of the investigated plants (Salt *et al*., 1999), which indicates that the tested types were tolerant of this metal, but are not good accumulators, given their low bioaccumulation coefficients. *P. tremula* leaf accumulated more chromium (20.8 mg/kg) compared to *P. nigra* leaf (16.4 mg/kg), while the twig of *P. nigra* accumulated twice more Cr (40.4 mg/kg) compared to the twig of *P. tremula* (20.1 mg/kg) (Table 2). The content of Cr in

P. nigra was far above the concentration recorded by Kacálková *et al*. (2014) (*Populus nigra* L. x *P. maximowiczii* 0.78 mg/kg in leaf).

In the soil from the Ovčar Spa site, the concentration of Ca was lower in relation to Mg and at the Trešnjevica site it was higher (Table 1). Comparison with Mg is given because the ratio of the amount of Ca:Mg in the soil is important for normal plant development.

The concentration of Ca in the leaf was four times higher in P. *nigra* (46,542 mg/kg) than in *P. tremula* (10,967 mg/kg), while the concentration in twigs was 228% greater in *P. tremula* (27,181.5 mg/kg) than in *P. nigra* (11,896.5 mg/kg). Bioaccumulation coefficients in the investigated species were high (Table 3). Better bioaccumulation properties were obtained for *P. nigra* (1.48 per yield and 5.78 for leaf) than for *P. tremula* (4.1 for sprouting and 1.65 for leaf).

The uptake of magnesium by *P. nigra* leaf was 6 times higher (9570 mg/kg) than that of *P. tremula* (1567 mg/kg), while *P. tremula* twigs absorbed 218% higher concentration of Mg (3475.2 mg/kg) relative to *P. nigra* twigs (1591.3 mg/kg). Bioaccumulation coefficient was low (Table 3) (except for the twigs of *P. tremula* 1.14).

The content of Zn in the tested soil was below the MPC value (Table 1) (50 mg/kg Ludajić, 2014). *P. nigra* leaf absorbed a higher concentration of Zn (70.8 mg/kg) compared to *P. tremula* leaf (64.8 mg/kg), while the twigs of *P. tremula* absorbed a higher concentration of Zn (117 mg/kg) as compared to *P. nigra* twig (27.9 mg/kg). Both of the investigated plant species proved to be good zinc accumulators (Table 3). Bioaccumulation potential was better in *P. tremula* (2.5 and 1.38) than in *P. nigra* (0.56 and 1.42) although aspen had a strong negative correlation with Zn. In this study, native poplars, grown on uncontaminated soil, showed the ability of phytoremediation.

The amount of zinc in *P. tremula* twig (117 mg/kg) was 44.6% higher than the quantity in the leaf (64.8 mg/kg) (Table 2). *P. tremula* that grows close to mines on contaminated soil can accumulate up to 3600 mg/kg Zn and 45 mg/kg Cd in leaves (Unterbrunner *et al*. 2007, Krpata *et al*., 2009). Zn concentration in the leaf of the hybrid *Populus tremula x Populus tremuloides* that grew on contaminated sites in the south of France was 950 mg/kg Zn (Migeon *et al*., 2009). The concentration of zinc in these studies was much higher than our results because these poplars grew on contaminated soils. Bioaccumulation factor for twigs and leaves in the present study was 2.5 and 1.38, respectively (Table 3), based on which aspen can be classified into hyperaccumulator plants. Migeon *et al*. (2009) obtained the following coefficients for Zn: *Populus tremula* × *Populus tremuloides* 1.22; *Populus tremula* × *Populus alba* 0.41. Bioaccumulation coefficients in this study were lower than ours. The reason is that our poplar grew on land that was not overloaded with zinc.

In *P. nigra* leaf, the amount of zinc (70.8 mg/kg) was 60.6% higher than the amount found in the twig (27.9 mg/kg) (Table 2). Dos Santos Utmazian and Wenzel (2007), in experiments with willows and poplars, which were subjected to the combined action of Cd and Zn (plus Cu and Pb), recorded Zn concentrations of 596 to 935 mg/kg in clones of *P. nigra*. Di Baccio *et al*. (2003) found that *P. nigra* plants have the potential to be used in plantations on soils loaded with zinc. Zinc concentrations in clones were considerably higher than those of the native species that we tested, but *P. nigra* has a great natural potential to uptake zinc, as shown in our research.

The bioaccumulation coefficient of zinc was 0.56 for twig and 1.42 for the leaf. Based on the bioaccumulation coefficient (Table 3) for zinc in the leaf, the species *P. nigra* can be classified into hyperaccumulator plants. The bioaccumulation coefficient was 0.62 for *P. nigra* and 0.78 for the *P. deltoids x P. nigra* clone, (Migeon *et al*. 2009), lower than in our results.

In general, *P. tremula* proved to be a better metal accumulator because it absorbed larger amounts of Mn, Fe, Cu and Zn compared to *P. nigra* (*P. nigra* absorbed more chromium than *P. tremula*). It is interesting to note that the twig of *P. tremula* accumulated larger amounts of Mn, Zn, Ca and Mg in relation to leaves, as opposed to *P. nigra*. The leaf of *P. tremula* accumulated more Fe in relation to the twigs, while the reverse situation was observed in black poplar.

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USVAJANJE I DISTRIBUCIJA METALA U BILJNIM VRSTAMA *POPULUS NIGRA* I *POPULUS TREMULA*

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Rezime

U remedijaciji zagađenih zemljišta sve češće se koriste drvenaste biljke. Analizirali smo sadržaj metala (Mn, Fe, Cu, Zn, Cr, Ca, Mg) u biljnim vrstama *Populus nigra* L. i *Populus tremula* L. i u zemljištu na kome rastu. Cilj istraživanja je da se, na osnovu sadržaja metala u biljnim organima (izdanci i lišće) i bioakumulacionog potencijala, utvrde razlike između ove dve vrste i ukaže na potencijalnu primenu ovih vrsta u fitoremedijaciji. Koristili smo atomsku apsorpcionu sprektrofotometriju za određivanje metala u zemljištu i biljnom materijalu. Rezultati su pokazali da je *P. tremula* bolji akumulator metala jer je usvojila veće količine Mn, Fe, Cu i Zn od *P. nigra*. *P. nigra* je usvojila više hroma u odnosu na *P. tremula*. Obe ispitivane biljne vrste su se pokazale kao dobri akumulatori cinka a bolji bioakumulacioni potenicijal je imala *P. tremula* od *P. nigra* iako je jasika imala jaku negativnu korelaciju prema Zn.

Ključne reči: *P. nigra* L., *P. tremula* L., fitoremedijacija, metali