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Andrej PILIPOVIĆ, Saša ORLOVIĆ, Srđan RONČEVIĆ, Nataša NIKOLIĆ, Milan ŽUPUNSKI, Jelena SPASOJEVIĆ [1]

RESULTS OF SELECTION OF POPLARS AND WILLOWS FOR WATER AND SEDIMENT PHYTOREMEDIATION

SUMMARY

Efficiency of water and soil phytoremediation often depends upon the proper selection of species and in some cases even adequate cultivar within selected species. This paper presents researches conducted during selection of poplar and willow clones for phytoremedation of water and sediments. Selection focused on two types of contamination: (1) nitrate groundwater and freshwater and (2) river sediments contamination. Results of selection for nitrate phytoremediation showed potential of investigated clones through accumulation and assimilation. Sediments in waters with high amount of wastewater efflux can pose severe threat to the water ecosystems and environment, therefore, its removal and safe disposal or remediation is needed. Authors investigated the use of extracted sediment from Great Bačka canal for fertilization of one poplar and one willow clone in two concentrations in the experiment performed as greenhouse pot experiment with application of 0.5 and 1 kg sediment per pot. During the experiment, growth and physiological parameters were measured in order to estimate the effect of sediment applications and preliminary results did not show negative effect on all investigated parameters.

Key words: Poplars, willows, phytoremediation, water, sediment

INTRODUCTION

The main sources of water pollution in Serbia are settlements, industry and agriculture. In total, 3,500,000 m^3 of wastewater per day are discharged, of which 70% originates from industry. The total emission from point sources is 13,500,000 PE (1,700,000 kg of suspended solids per day, 120,000 kg of total N per day, 39,000 kg of total P per day) (Dalmacija et al., 2013). The wastewater discharged has led to some watercourses having very bad quality. It is estimated that annually in north Serbia (Vojvodina), about 2,000,000 m^3 of sediment needs dredging, some of which must be treated. The treatment of dredged sediments can be done either in mechanical-physical-chemical way which requires significant amounts of money, or by environmentally friendly phytoremediation

[1] Andrej Pilipović, (corresponding author: andrejp@uns.ac.rs), Saša Orlović, Institute of Lowland Forestry and Environment, University of Novi Sad, 21000 Novi Sad, SERBIA; Srđan Rončević, Nataša Nikolić, Milan Župunski, Jelena Spasojević, Faculty of Sciences, University of Novi Sad, 21000 Novi Sad, SERBIA

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technology where plants and their associated microorganisms cleanup contaminated soils and waters.

The most often used tree species in phytoremediation are poplars due to their high biomass production, rapid growth, easy vegetative propagation, high transpiration rate and dependence on the groundwater levels. Their highly adaptable roots in vadose zone above groundwater obtain high transpiration rate, and enables permanent "communication" with the groundwater. In this way, poplars act as some sort of "water pumps" and transpire enormous amounts of water up to 200 liters per day for a 5-year old tree (Newmann et al., 1997). These characteristics of poplars result in their capability of lowering the groundwater table what can be used for contaminant plume reduction. Also, they uptake contaminants present in groundwater (like pesticides, fertilizers and TCE) and their phytodegradation occurs. Numerous researches show their potential for phytoremediation of different types of contaminants from heavy metals (Banuelos et al., 1997; Di Baccio et al., 2003; Pilipović et al., 2005), to nutrients (Fraser et al., 2004) and organics (Wittig et al., 2003; Zalesny et al., 2005; Pilipović et al., 2012) very often linked to biomass production (Licht and Isebrands, 2005).

Due to the more than 50-year experience in poplar and willow breeding, which resulted in registration of more than 20 various cultivars of poplars and willows, researchers at the Institute of lowland Forestry and Environment at the University of Novi Sad started selection of poplars and willows for environmental uses (phytoremediation) in the first decade of the 21st century, including contaminants such are heavy metals, nutrients and crude oil.

Considering all these, this paper presents some of results obtained during selection of poplars and willows for phytoremediation contaminated groundwater, surface waters, wastewaters, sediments and sludges.

MATERIAL AND METHODS

Experiments for selection of clones for phytoremediation were conducted as greenhouse experiments both in hydroponics and in soil pots in the greenhouses of the University of Novi Sad. Hydroponics experiments included three poplar genotypes: *Populus x euramericana* "Pannonia", *Populus deltoides* cl. B-81 and hybrid (*P. nigra x maximowiczi*) x *P. nigra* "Italica" cl. 9111/93 (inter-section hybrid). Cuttings were placed in hydroponic pots of 40 litres in volume and grown in pure water for 45 days, till beginning of root development. Plants were grown in semi-controlled greenhouse at temperature of approx. 21^{o}C and day-night photoperiod. After root initiation, plants were treated to grow under three different concentrations of nitrates in Hoagland solutions with 2 mM, 10 mM and 30 mM NO_3^- concentration. During growth of plants, investigated parameters included total biomass (M), leaf area (LA) and relative growth rate (RGR), nitrate reductase activity (NRA) and nitrate accumulation in leaves and roots. Thorough explanation of experimental design and obtained results was already published (Pilipović 2005; Pilipović et al., 2006) therefore results of

these publications are used for review and discussion.

As first results of phytoremediation were obtained from highly controlled conditions in hydroponics, further research developed to investigation with more close to nature experiments which included soil pots and application of original contaminated sediment. The plant materials for this experiment were cuttings of poplar (*Populus deltoides* Bartr.) clone "Bora" and basket willow (*Salix viminalis* L.). Plants were grown in 10 liter pots with common alluvial soil for 2 months, after different amounts of sediment (Table 3) were added to pots with plants. Treatments included amounts of 0, 0.5 and 1 kg of sediment per pot. After application, plants were irrigated and cultivated on a regular basis, and aboveground biomass was harvested after growth cessation. During growth, photosynthetic parameters and nitrate reductase was assessed.

RESULTS AND DISCUSSION

Selection of poplars for nitrate phytoremediation

Nitrates play a significant role in ground and surface water contamination. This contamination is mostly caused by agricultural practices, where fertilization with the increased application of mineral fertilizers plays a significant role in the increase of nitrate concentrations both in plant tissues and in the environment (Kastori and Petrović, 2003). Also, excess of nitrates in the soil promotes their denitrification and emission of nitrous oxides, such as N_2O, thus causing even up to 300 times more harmful effects than CO_2 (Schepers et al., 2005). Due to their high mobility in soils, nitrates can easily be drained down with irrigation to the depths where roots of agricultural plants cannot grow. Further draining to groundwater makes them available for buffer strips of poplar trees whose roots are in persistent contact with groundwater. Researches conducted by Licht and Schnoor (1993) showed that poplar tree buffer zones along the water bodies significantly contributed to decrease of nitrates concentration in the groundwater from 150 mg/L to 3 mg/L.

Table 1. The effect of nitrate concentration on biomass production (M), leaf area (LA) and reductase activity (NRA) in µmol NO_2 g^{-1} h^{-1} of investigated clones (Pilipović, 2005)

Clone	*Concentration of NO_3^- in the growth medium*								
	2 mM			*10 mM*			*30 mM*		
	M(g)	*LA(cm^2)*	*NRA*	*M(g)*	*LA(cm^2)*	*NRA*	*M(g)*	*LA(cm^2)*	*NRA*
9111/93	12.9e	768e*	2.683a	15.5d	1010d	1.933a	19.5c	1389c	3.474a
Pannonia	15.4d	889de	2.566a	17.5cd	1087d	2.955a	24.3b	1488c	3.631a
B 81	18.2c	1639c	2.172a	25.6b	2341b	2.581a	44.9a	3993a	2.826a

*values with same letter did not differ significantly at p=0.05 regarding each parameter separately

Results obtained during selection of poplars for nitrate phytoremediation (Pilipović, 2005; Pilipović et al., 2006) showed different reaction of poplar clones to given nitrate concentrations. Biomass of investigated clones (Table 1) significantly increased with increase of nitrates in medium. Growth of plants

represented through RGR (Fig. 2) showed superiority of *Populus deltoids* cl. B-81, both in growth and physiological parameters. Stability of physiological processes related to nitrate assimilation was undisturbed, which is confirmed with investigation of parameters related to that process. Nitrate reductase activity (Table 1) did not differ significantly between the treatments. Activity in roots was smaller than in leaves, while on the other side nitrates accumulated in roots up to 8 times more.

Table 2. Relative growth rate (RGR) ($10^{-3}day^{-1}$) of clones after 90 days growing period and nitrate accumulation(mg/g $_{DW}$) in leaves (NO_3^-L) and roots (NO_3^-R) (Pilipović et al., 2006)

Clone	*Concentration of NO_3^- in the growth medium*								
	2 mM			*10 mM*			*30 mM*		
	RGR	*NO_3^-L*	*NO_3^-R*	*RGR*	*NO_3^-L*	*NO_3^-R*	*RGR*	*NO_3^-L*	*NO_3^-R*
9111/93	4,537f	0,839c	3,123f	6,626e	1,302c	6,063d	9,089cd	4,404a	20,195c
Pannonia	4,865f	0,828c	1,357g	6,313e	1,129c	4,289e	9,963c	4,713a	23,641a
B 81	8,265d	1,803c	1,084g	12,250b	1,878c	3,980e	18,56a	2,927b	22,262b

Nitrate accumulation differed only in leaves of plants treated with 30 mM NO_3^-, while accumulation in roots showed differences in all treatments. Results of NRA and nitrate accumulation confirm that main process of nitrate assimilation is located in leaves, while roots serve like nitrate deposition place (O'Neill and Gordon, 1994) and showed no significant effect of nitrate concentrations on enzyme activity. Generally, clone B-81 (*Populus deltiodes*) showed best results of investigated parameters under different treatments, while other two clones ("Pannonia"and 9111/93) also showed stability of investigated physiological parameters, which indicates their ability for nitrate phytoremediation.

Selection of poplars and willows for river sediment phytoremediation

In order to investigate potential for phytoremediation of river sediments, in year 2014 was established greenhouse pot experiment with application of river sediment for fertilization of poplar and willow clones. For this research, sediment from the Great Backa canal was used, in the 6 km long section at Vrbas city, where 400,000 m^3 contaminated sediment is disposed. Based on metals content, the sediment is classified in accordance with the Regulation on limit values of pollutants in surface and groundwater and sediments, and deadlines for achieving them (Official Gazette RS, 50/2012), as shown in Table 3.

Table 3**.** Sediment characterization and classification

Moisture	*Organic matter*	*Fraction <2 µm*	*pH*	*Total N*	*Total P*	*Cu*	*Cd*	*Cr*	*Pb*	*Ni*	*Zn*
%			-	*g/kg*		*mg/kg*					
72.5	12	15.1	7.8	13.1	7.65	295	1.87	400	61	124	465
Classification						4	1	4	0	3	1

According to the criteria for the assessment of sediment, it was found that the sediment is class 4, i.e., the sediment is highly polluted. Remediation of the sediment is mandatory, or disposal of the dredged materials under controlled conditions with special protection measures to prevent the distribution of hazardous materials into the environment.

Table 4. The effect of application of sediment on fresh (M_{fw}) and dry (M_{dw}) aboveground biomass, phorosynhesis (A), transpiration (E) and nitrate reductase (NRA) on investigated plants

Clone	Treatment	M_{fw} (g)	M_{dw} (g)	LA (cm^2)	A $\mu mol\ CO_2\ m^{-2}\ s^{-1}$	E $\mu mol\ H_2O\ m^{-2}\ s^{-1}$	NRA $\mu mol\ NO_2\ g^{-1}\ h^{-1}$
Bora	*1*	13.95a	6.97b	451a	6.794b	0.946b	0.420b
Bora	*0.5*	15.93a	6.57b	501a	8.205b	1.220b	0.221c
Bora	*Ø*	16.41a	11.05a	651a	8.045b	1.019b	0.619a
S viminalis	*1*	13.48a	5.37b	587a	20.258a	1.665a	0.049d
S viminalis	*0.5*	10.92a	4.49b	505a	18.291a	1.735a	0.128cd
S viminalis	*Ø*	16.13a	6.63b	655a	23.600a	1.657a	0.106cd

Investigated growth parameters (Table 4) did not show decrease in fresh aboveground biomass, while dry biomass was decreased in poplar during both treatments with sediment. Photosynthesis (A) and transpiration (E) did not differ significantly in both investigated species, while assimilation of nitrogen expressed through nitrate reductase activity (NRA) was decreased in treated poplar plants. Nitrate reductase catalyses the first step in nitrate assimilation, the reduction of nitrate to nitrite, which has been considered as the rate-limiting step of this metabolic pathway (Campbell, 1999). Inactivation of nitrate reductase occurs in response to stress conditions including the loss of light, a decrease in CO_2 levels, an increase in cytosolic pH or variations in photosynthetic activity (Kaiser et al., 1999). Obtained results indicate disturbing effect of sediment application on plants processes, although it was not pronounced in photosynthesis and respiration. The negative effect of contamination on NRA was more pronounced in poplars than in willows (Pilipović et al., 2012) which is in accordance with results of this study. Although these results are preliminary, they indicate that willows may have greater potential for remediation of river sediments, wastewater sludge and effluent than recorded in various papers (Vervaeke et al., 2003; Hasselgren, 1999, Dimitriou and Rosenqvist, 2010).

CONCLUSIONS

Selection activities conducted at the University of Novi Sad showed the potential of investigated clones for phytoremediation of contaminated waters and sediments. Clones with greater growth vigor showed higher potential for phytoremediatiom of nitrates which is confirmed by decreased accumulation of

nitrates in tissues. Application of sediments for fertilization of poplars and willows, preliminarily did not show significant negative effect on plants, although sediment was classified as highly polluted. Slight advantage is recorded for willows whose physiological processes were not affected. In order to confirm the potential of both poplars and willows for river sediment phytoremediation, further research is needed, especially due to the increased concentrations of heavy metals in sediments whose cumulative effect on investigated plants could disturb their vitality.

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