Investigating potential of some poplar (*Populus sp.*) clones for phytoremediation of nitrates through biomass production

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Introduction

Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain or immobilize contaminants from soil and water (EPA, 2000). Mechanisms of phytoremediation can be very diverse depending upon the characteristics of contaminants, media and plant species. For instance, to clean up sites contaminated with heavy metals, mechanism called phytoextraction should be obtained using hyperaccumulator plants, while to immobilize contaminants physically or transform them to less available form phytostabilization mechanism is needed. Some plants stimulate development of microbes that can degrade contaminants (organic origin mostly) in their rhizosphere, while other plants uptake and metabolize contaminants. The former mechanism is called rhizodegradation, while the latter is phytodegradation. Contaminants very often migrate from their sources via groundwater. In order to control contaminant plume and prevent further contamination buffer strips or hydraulic control is used. Hydraulic control prevents groundwater from coming into contact with contaminated sites, while buffer strips are used for phytoremediation of already contaminated groundwater. In both cases, for control of groundwater level and flux tree species are planted in strips opposite to direction of groundwater flow. Sometimes contaminants present in groundwater are taken up by plants and transpired to the atmosphere, where they are degraded by sun. This mechanism is called phytovolatilization, and is used for phytoremediation of VOCs (Volatile Organic Compounds) such is trichlorethene (TCE).

Poplars in phytoremediation

As one of the most often used tree species in phytoremediation, poplar play significant role in remediation of contaminated sites. Their advantages refer to high biomass production and rapid growth, easily vegetative propagation, high transpiration rate and dependence upon the groundwater levels. Poplars are well known as early successional tree species that inhabits new formed alluvial soils. During their growth the "connection" with groundwater maintains constant due to plasticity of their roots. The genus name "*Populus*" came from Latin "*arbor populi*" (peoples tree), because Romans knew that on the places where poplars grew natively it is very certain that you can dig a well and build houses. Roots of poplar trees form a vadose zone above groundwater level in order to obtain high transpiration rate. In this way, poplars act as some sort of "water pumps" and transpire enormous amounts of water up to 200 liters for 5 year old tree (Newmann *et all.*, 1997). Due to above mentioned characteristics poplars are capable of lowering the groundwater table what can be used for contaminant plume reduction. Also the uptake of contaminants present in groundwater (like pesticides, fertilizers and TCE) and their phytodegradation occurs. In order to establish contact between poplar seedling and groundwater in a shorter period than traditional way of planting requires, special techniques of deep rooted planting can be applied in phytoremediation.

Due to the above mentioned advantages, poplars are used in different systems of phytoremediation. Most often use of poplars is to clean up sites and groundwater contaminated with VOCs such is trichloroethylene (TCE), where mechanisms of TCE phytoremediation are combinations of phytodegradation, phytovolatilization and rhizodegradation. Some greenhouse and field researches showed potential of poplars for phytoextraction of some heavy metals such are As, Cd, Ni and Zn. Other researches showed positive impact of poplar roots on development of microorganisms that degrade PAHs like Atrazine, Benzene and Toluene. Phytoremediation of groundwater contaminated with mineral fertilizers that contains large amounts of nitrates and pesticides plays significant role in use of poplars for phytoremediation. Poplars planted as shelterbelts along watercourses prevent migration of contaminants from agricultural sites to rivers and other surface waters via groundwater. Researches conducted by Licht and Schnoor (1993) showed that concentration of nitrates in the groundwater decreased from 150mg/l to 3 mg/l m measuring in front and behind the rows of poplar trees.

Nitrates in environment

Amongst various numbers of contaminants in the environment, nitrates play significant role in ground and surface water contamination. Intensive agriculture and increased use of fertilizers and pesticides enlarges presence of nitrates in food, soils and waters. Excess of nitrates in drinking water can cause methemoglobinemia in infants where nitrates present in digestive tract converts to nitrites and bonds with hemoglobin instead of oxygen. Together with phosphorus nitrates cause eutrophication of surface waters, while their denitrification in the soil and emission in a form of N_2O or other nitrous oxides to the atmosphere cause up to 300 times more harmful effect than CO_2.

Opposite to these facts, nitrogen is most important element for plants. Plants can use nitrogen on various ways and in different forms (figure 1). Most often way is uptake of nitrate and ammonia forms by plant roots, although newer researches showed that nitrogen can get in plants also via diffusion or mass flow through stomata and bark in all available forms (NO_3^-, NH_4^- and NO_x). Besides the pH value of soil, soil properties and plant species, the uptake of nitrates is dependent upon their concentration in substrate. First enzyme in plants related with metabolism of nitrates is nitrate reductase, and its activity (NRA) is related to nitrate concentration in soil. Greater part of NRA is located in leaves of broadleaved trees, while roots mostly serve as container of nitrates that are about to be assimilated.

Due to their high mobility in soils, nitrates can be easily 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 which roots are in persistent contact with water.

Considering the importance and share of agriculture in Serbia & Montenegro, especially in Northern Province Vojvodina, and log tradition of poplar growing and researches conducted at the Institute of Lowland Forestry and Environment, the aim of this study was to investigate potential of different poplar clones for phytoremediation, through obtaining information about physiological parameters related to nitrate assimilation. Investigation of parameters related to nitrate assimilation could also be suitable in future early selection of poplar genotypes. Researches are obtained in the framework of the project sponsored by Ministry of Science and Environment Protection – Republic of Serbia.

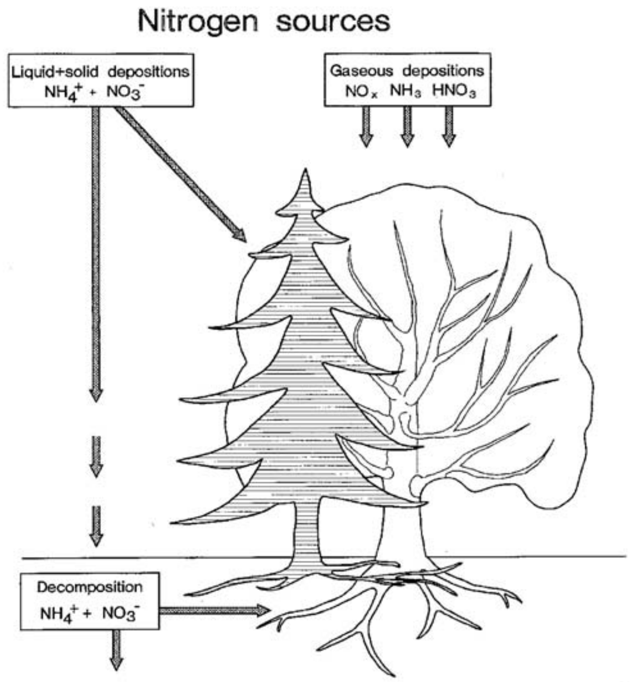

Figure 1 Nitrogen sources for forest trees (Marschner, 1995)

Research and results

Researches conducted at the Institute of Lowland Forestry and Environment involves with ability of different poplar clones for nitrate phytoremediation through measuring an determination of growth and physiological parameters suitable for use in selection of clones for nitrate phytoremediation. Experiment is conducted in greenhouse with semi-controlled conditions where poplar cuttings were grown hydroponically under three different concentration of nitrates: **(1)** in modified Hoagland solution with 2 mM NO_3 , **(2)** in standard Hoagland nutrient solution with 10mM of NO_3, and **(3)** in modified Hoagland solution with 30 mM NO_3. Following clones were selected from spectrum of genus *Populus*: **(1)** *Populus x euramericana* cl. Pannonia, **(2)** *Populus deltoides* cl. B-81 and **(3)** hybrid between *P. nigra x maximowiczii* and *P. nigra* var. *Italica* cl. 9111/93.

Figure 2 Poplar cuttings grown hydroponocally in different nitrate concentrations

During growth of plants, biomass production of plants was measured and represented with parameters such are: total biomass (M), relative growth rate (RGR), net assimilation rate (NAR) and leaf area (LA). Physiology of plants was investigated through measuring parameters related to nitrate assimilation such as nitrate reductase activity (NRA), nitrate accumulation in leaves and roots and chlorophyll fluorescence.

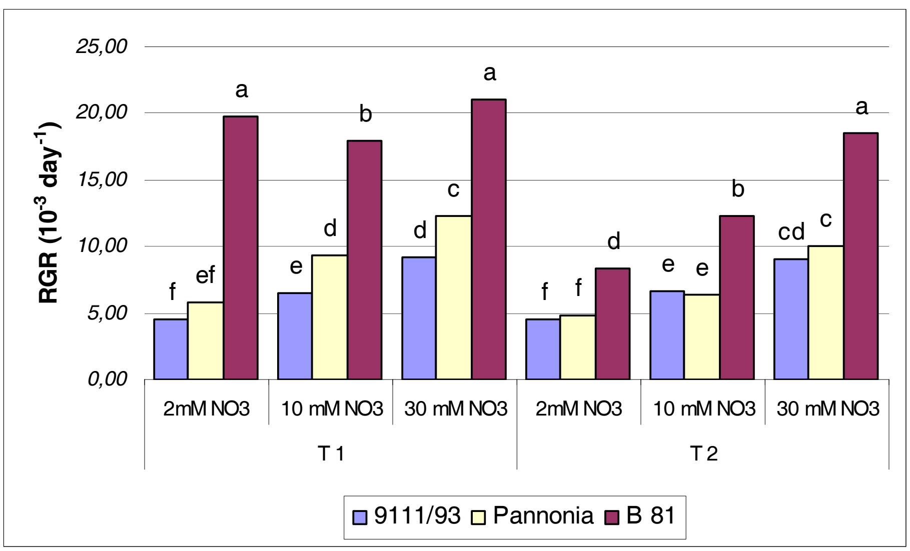

Figure 3 Relative growth rate (RGR) clones after 45 (T_1) and 90 (T_2) days growing period

Results showed different reaction of poplar clones to given nitrate concentrations. Biomass of investigated clones significantly increased with increase of nitrates in medium. Growth of plants represented through RGR (fig.4) and NAR showed superiority of *Populus deltoids* cl. B-81, both in growth and physiological parameters.

Stability of physiological processes related to nitrate assimilation was undisturbed, what is confirmed with investigation of parameters related to that process. Both nitrate reductase and chlorophyll fluorescence did not differ significantly between the treatments. Nitrate reductase activity in roots was smaller than in leaves, while on the other side nitrates accumulated in roots up to 8 times more. 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. Measurements of NRA 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, what indicates their ability for nitrate phytoremediation.

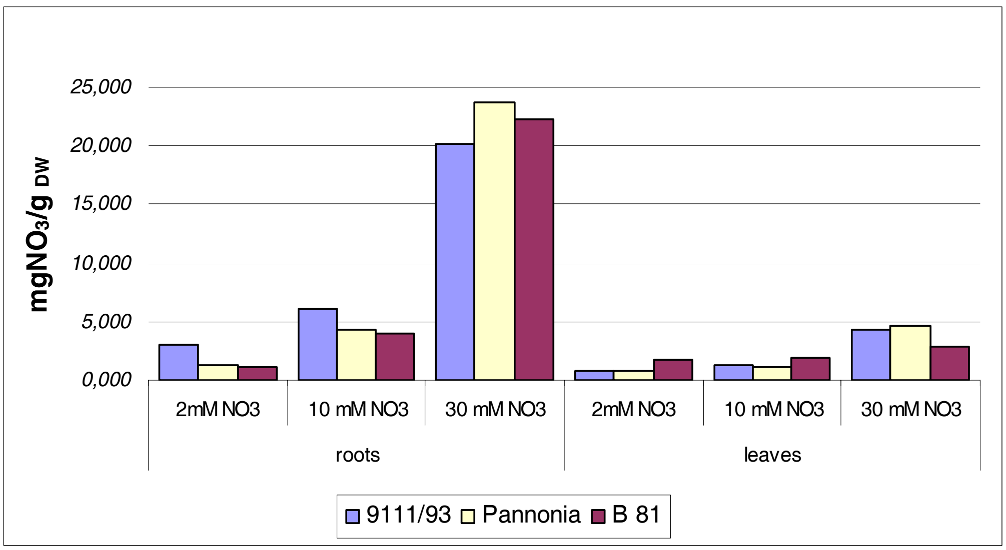

Figure 4 **Nitrate accumulation in leaves and roots of investigated clones**

Conclusion

Results in this research showed various potential of investigated clones for nitrate phytoremediation. Stability of investigated parameters was most expressed by clone B-81, which reacted with highest growth and lowered accumulation of nitrates at treatment with 30mM NO_3. Data indicates that larger amount of nitrates is assimilated due to clone's vigorous growth. Other two clones also showed potential for nitrate phytoremediation which is represented with stability of processes in plants.

If we consider that results are obtained in optimal conditions for plant growth, where other biotic and abiotic factors are excluded or controlled, potential of all three investigated clones could be considered because due to difference in their abilities of rooting, requests for site properties, management techniques and biological characteristics are various. For instance, clone B-81 is high yield and intensive cultivation request clone, clone "Pannonia" has modest site requests while clone 9111/93 has good rooting ability and all of these characteristics would be stressed at various phytoremediation sites.

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