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# PHYTOREMEDIATION CAPACITY OF POPLAR (*POPULUS* SPP.) AND WILLOW (*SALIX* SPP.) CLONES IN RELATION TO PHOTOSYNTHESIS

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*Abstract* — Good photosynthetic features and a favorable water regimes of woody plants improve their survival and remediation potential under unfavorable ecological conditions. Accordingly, we here present results of testing plant tolerance of Pb, Cd, Ni, and diesel fuel based on gas exchange parameters and WUE of four poplar and two willow clones grown in a greenhouse on soil culture. Photosynthesis and transpiration of plants grown on soils with individually applied heavy metals decreased significantly, but this was less obvious in the case of Cd treatment. A heavy metal mixture in the soil induced significant reduction in photosynthesis (by more than 50%). Diesel fuel as the only pollutant in soil caused very strong and significant inhibition of photosynthesis and transpiration of willow clones. The results indicate genotypic specificity of all investigated physiological parameters and mark poplar clones as very useful in phytoextraction technology for the bio-cleaning of chemically polluted soils.

Key words: Poplar, willow, photosynthesis, transpiration, WUE, phytoremediation, heavy metals

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### INTRODUCTION

As environmental restoration of metal-polluted soils by traditional physical and chemical methods demands large investments of economic and technological resources, efforts are underway to involve in situ methods in environmental protection. Phytoremediation is an emerging technology which utilizes plants and rhizosphere microorganisms to remove and transform toxic chemicals in soils, sediments, ground water, surface water, and the atmosphere (Kumar et al., 1995; Susarla et al., 2002; Ghosh and Singh, 2005; Pajević et al., 2008). Heavy metal accumulation by plants is useful as a phytoextraction technique in phytoremediation, which refers to the use of plants that can extract and move contaminants to their harvestable parts (Marchiol et al., 2004). The efficiency of phytoextraction depends on the metal bioavailability, as well as on several characteristics of the plant-remediator: fast growth, a deep and extended root system, the capability of (hyper)accumulating essential and unessential met-

feasible phytoextraction technology (Unterbrunner et al., 2007).

Pulford, 2005).

parts (Zacchini et al., 2008).

Much attention has been paid to the study of water use efficiency (WUE) in order to define the interdependence of water balance and biomass pro-

als, and the ability to translocate metals to the aerial

woody species have been considered for phytoex-

traction of metal-contaminated sites (Greger and

Landberg, 1999; Rosselli et al., 2003; Dickinson and

advantage of using forest plants in this technol-

ogy is their high production of biomass, which can

eventually be used in producing energy (Laureysens

et al., 2004). Fast growing, high biomass-producing

woody plants that tend to accumulate metals in aer-

ial tissues could be a source for the development of

Over the last few years, metal-accumulating

Apart from cleaning the environment, another

duction and detect genotypes which consume less water during efficient and productive photosynthesis (Orlović et al., 2002; Martin and Stephens, 2006). Genotype adaptive values should be expressed in high photosynthetic potential, relatively high and stabile WUE, and high biomass production with high accumulation of pollutants (heavy metals in particular) in tissue. In this sense, the aim of breeding programs is to produce cultivars (clones) characterized by superior growth and resistance to high levels of pollutants, which have to be extracted from the soil and translocated to aboveground plant parts.

The aim of this investigation was to quantify the photosynthetic capacity of four poplar clones and two willow clones growing on soil contaminated by heavy metals and diesel fuel during the early growing period. A relatively high photosynthesis/transpiration ratio on contaminated soil would distinguish genotypes with high adaptive potential in a contaminated environment, high biomass production, and high phytoextraction capacity.

### MATERIAL AND METHODS

The experimental material consisted of four poplar and two willow genotypes (Table 1).

Genotypes were obtained from the Institute of Lowland Forestry and Environmental Protection, Novi Sad, Serbia. The genotypes chosen for screening are the result of a long period selection of genes that cause high biomass production.

The experiment was set in May of 2008. Plants were grown in a semi-controlled environment (greenhouse) by the soil culture method. Temperature was kept under 30°C. Illumination was natural and dependent on the outside light conditions. Six woody cuttings, 20 cm long with one shoot per cutting, were grown in each of several Mitscherlich pots. Each treatment consisted of 24 poplar and 36 willow cuttings. Pot distribution was randomized. The soil was watered constantly to maintain optimal soil humidity. Every four weeks, 0.21 of full-strength Hoagland solution was added to the pots. The experiment included a control group of plants and six treatments of pollution: Pb, Cd, Ni, Pb+Cd+Ni, diesel fuel, and diesel fuel+Pb+Cd+Ni. The concentrations of metals in the soil in individual metal treatments were: Pb - 360.35 mg/kg, Ni - 205.37 mg/ kg, and Cd - 8.14 mg/kg. In combined metal treatments, the concentrations were: Pb - 382.29 mg/kg, Ni - 185.25 mg/kg, and Cd - 1.34 mg/kg. Total soil hydrocarbon content in diesel treatment was 9.825 g/kg of dry soil. Concentration of metals in the soil was achieved using aqueous metal solutions. The metals used were Cd (10<sup>-4</sup> M and 10<sup>-5</sup> M, supplied as CdCl<sub>2</sub>·H<sub>2</sub>O), Ni (10<sup>-4</sup> M and 10<sup>-5</sup> M, supplied as NiSO<sub>4</sub>·6 H<sub>2</sub>O), and Pb (10<sup>-4</sup> M and 10<sup>-5</sup> M, supplied as Pb-EDTA). Photosynthesis and transpiration were measured in June of 2008, after 42 days of treatment.

Photosynthesis and transpiration were measured using the LCpro+ portable photosynthesis system, manufactured by ADC BioScientific Ltd. Light conditions were set using the LCpro+ light unit, which emitted photosynthetically active radiation (PAR) at 1000  $\mu$ mol  $\cdot$  m<sup>-2</sup>  $\cdot$  s<sup>-1</sup>. The air supply unit provided a flow of ambient air to the leaf chamber at a constant rate of 100  $\mu$ mol  $\cdot$  s<sup>-1</sup>. Temperature, CO<sub>2</sub> concentration, and humidity were at ambient levels.

Parameters of chlorophyll fluorescence induc-

No.	Species	Working name	
1	Populus x euramericana	Panonia	
2	Populus deltoides	PD 3	
3	Populus deltoides	B 81	
4	Populus deltoides	B 229	
5	Salix alba	V 1	
6	Salix alba	V 2	

Table 1. Poplars and willows used in the experiment.

tion kinetics were measured using a PSM fluorimeter (BioMonitor) from middle-leaf parts adapted to darkness (Öquist and Wass, 1988). After illumination by saturated red light, the values of minimal fluorescence (Fo), maximal fluorescence (Fm), and potential quantum yield: (Fm-Fo)/Fm (also defined as Fv/Fm), were determined.

Parameter WUE (water use efficiency) was calculated as the ratio of photosynthesis to transpiration and expressed in µmoles of  $CO_2 \cdot m^{-2} \cdot s^{-1}/mmoles$ of  $H_2O \cdot m^{-2} \cdot s^{-1}$ .

Stomatal conducance of water vapor was measured automatically *in vitro* and expressed in moles of  $H_2O \cdot m^{-2} \cdot s^{-1}$ .

Statistical analyses were conducted using Duncan's multiple range test at a significance level of p<0.05 using one-way factor analyses. The average values shown in tables followed by the same letter did not differ significantly. Values decreased following alphabetical order. The least significant difference (LSD) between average values of treatments is shown at the end of each table.

### RESULTS

The photosynthetic capacity of the youngest group of poplar and willow leaves was significantly influenced by heavy metal and diesel fuel soil contamination. The investigated genotypes showed a specific photosynthetic response under different pollution treatments, which was evident in comparison with photosynthesis of the control plants; there were no significant differences of photosynthesis in the poplar group or in the willow group of genotypes. The obtained values of photosynthetic  $CO_2$  assimilation in control poplar plants ranged from 10.77 µmoles of  $CO_2 \cdot m^{-2} \cdot s^{-1}$  (genotype 1 - Panonia) to 8.22 µmoles of  $CO_2 \cdot m^{-2} \cdot s^{-1}$  (genotype 3 - B 81) (Table 2).

The highest inhibitory effect on photosynthetic assimilation in all of the investigated clones was detected in the case of combined pollution treatment (diesel fuel+Pb+Cd+Ni), and for most genotypes it was impossible to detect any photosynthetic activity in these conditions (except in poplar genotype 1). A very strong inhibitory effect on photosynthesis was also detected in plants grown on soil contaminated with a heavy metal mixture (Cd+Ni+Pb).

This decrease of CO<sub>2</sub> assimilation in plants treated with a heavy metal mixture (Pb+Cd+Ni) was not accompanied by damage to PSII and electron transport, since the chlorophyll fluorescence parameter Fv/Fm remained stable in comparison with the control plants (Table 3). Analyzing individual genotype reactions to pollution treatment, we can conclude that the Panonia poplar genotype had the lowest photosynthesis in soil with a heavy metal mixture (3.32 µmoles of CO<sub>2</sub> • m<sup>-2</sup> • s<sup>-1</sup>), which represents a photosynthetic decrease of about 70% compared to the control plant photosynthesis (10.77 µmoles of CO<sub>2</sub> • m<sup>-2</sup> • s<sup>-1</sup>). A statistically similar decrease of photosynthesis in the same genotype was registered

Table 2. Photosynthetic CO<sub>2</sub> assimilation of poplar and willow leaves (µmoles of CO<sub>2</sub>• m-2 • s-1).

Poplar genotypes				Willow genotypes			
S Treatment	pecies/ Populus x clones euramericand - Panonia	Populus eltoides - PD 3	Populus eltoides - B 81	Populus eltoides - B 229	Salix alba - V 2	Salix alba - V 1	
Pb	5.04 c	4.41 d	3.83 d	4.63 b	3.25 c	2.68 b	
Cd	8.05 b	8.63 a	8.72 a	4.56 b	3.12 c	2.24 b	
Ni	3.76 d	7.47 b	5.44 c	3.49 c	4.88 b	1.44 c	
Pb+Cd+Ni	3.32 d	2.65 e	7.13 b	0.69 d	1.33 d	1.06 c	
Diesel fuel	7.32 d	6.53 c	6.68 b	0.00 e	0.00 e	0.00 d	
D.f.+Metals	4.25 cd	0.00 f	0.09 e	0.00 e	0.00 e	0.00 d	
Control	10.77 a	9.16 a	8.22 a	8.87 a	6.88 a	5.92 a	
LSD 0.05	0.92	0.68	0.73	0.67	0.65	0.44	

for plants grown on Ni and diesel fuel+Pb+Cd+Ni. This genotype of *Populus* x *euramericana* (Panonia) can be singled out on the basis of photosynthetic capacity as suitable for phytoextraction of Cd and diesel fuel in single contamination treatments because in these conditions it had stable and relatively high photosynthesis (Tables 2 and 3). The B 229 *Populus deltoides* genotype showed the lowest photosynthesis and had the lowest remediation potential because all applied treatments induced a decrease of photosynthesis for more than 60%.

The registered photosynthetic activities of willow clones were lower than those of poplar clones, average values for the control plants being 6.88 µmoles of  $CO_2 \cdot m^{-2} \cdot s^{-1}(Salix alba - V 1)$  and 5.92 µmoles of  $CO_2 \cdot m^{-2} \cdot s^{-1} (Salix alba - V 2)$ . As in poplar clones, the most obvious inhibitory effect

on photosynthesis in willow clones was detected for diesel fuel+Cd+Ni+Pb and heavy metal mixture treatments. Willow genotypes were defined as unsuitable for phytoextraction of diesel because their photosynthetic rates were very low, below the level of registration. To judge from the results obtained on photosynthetic activity, willow clone V 1 could be used for remediation of Ni from soil polluted with this heavy metal. Excessive concentrations of Cd in the soil had the lowest effect on photosynthesis of almost all of the investigated poplar and willow clones (Table 2).

The relatively stable values of chlorophyll fluorescence parameter Fv/Fm in polluted conditions indicated stability of thylakoid structure and electron flow through the photosystems (Table 3).

Table 3. Chlorophyll fluorescence parameter Fv/Fm in poplar and willow leaves.

Poplar genotypes					Willow genotypes		
Species/ clones Treatment	Populus x euramericana - Panonia	Populus deltoides - PD 3	Populus deltoides - B 81	Populus deltoides - B 229	Salix alba - V 2	Salix alba - V 1	
Pb	0.753 a	0.707 a	0.681 b	0.694 ab	0.732 a	0.742 a	
Cd	0.708 a	0.714 a	0.711 ab	0.747 a	0.745 a	0.741 a	
Ni	0.753 a	0.765 a	0.747 ab	0.731 ab	0.726 a	0.739 a	
Pb+Cd+Ni	0.732 a	0.742 a	0.735 ab	0.739 ab	0.692 a	0.691 ab	
Diesel fuel	0.754 a	0.736 a	0.764 a	0.724 ab	0.660 ab	0.632 b	
D.f.+Metals	0.754 a	0.719 a	0.551 c	0.695 ab	0.587 b	0.628 b	
Control	0.711 a	0.752 a	0.717 ab	0.677 b	0.736 a	0.743 a	
LSD 0.05	0.063	0.063	0.063	0.063	0.089	0.063	

Table 4. Rate of transpiration in poplar and willow leaves (mmoles of H<sub>2</sub>O •m-2 •s-1).

Poplar genotypes					Willow genotypes	
Species/ clones Treatment	Populus x euramericana - Panonia	Populus deltoides - PD 3	Populus deltoides - B 81	Populus deltoides - B 229	Salix alba - V 2	Salix alba - V 1
Pb	1.02 bc	0.74 b	0.73 bc	0.97 b	0.72 c	0.75 b
Cd	1.53 ab	1.62 a	1.62 a	1.15 b	0.83 bc	0.61 bc
Ni	0.84 c	1.56 a	1.39 ab	0.96 b	1.11 ab	0.50 c
Pb+Cd+Ni	0.85 c	0.77 b	1.69 a	0.74 b	0.69 c	0.63 bc
Diesel fuel	1.91 a	1.63 a	1.75 a	0.00 c	0.00 d	0.00 d
D.f.+Metals	1.07 bc	0.00 c	0.25 c	0.00 c	0.00 d	0.00 d
Control	1.78 a	2.21 a	1.95 a	8.87 a	1.41 a	1.11 a
LSD 0.05	0.64	0.62	0.68	0.65	0.32	0.18

Photosynthetic light reactions and electron transport through PSII were disturbed the most in both willow clones and poplar clone B 81 in soil treated with diesel fuel and all metals (Table 3).

Transpiration rates measured on the youngest leaves were similar in poplar clones and ranged from 2.37 mmoles of  $H_2O \cdot m^{-2} \cdot s^{-1}$  (clone B 229) to 1.78 mmoles of  $H_2O \cdot m^{-2} \cdot s^{-1}$  (clone Panonia). Statistical analysis showed a very similar pollution effect on transpiration of different clones (Table 4). As in the case of the photosynthetic rate, clone B 229 exhibited the lowest transpiration rate in polluted conditions, especially pollution with diesel fuel+metals, which distinguished it as the genotype with the lowest remediation potential.

The investigated willow genotypes showed significant metabolic disturbance in conditions of pollution: their photosynthetic and transpiration rates were very low (below the registration level) in soil polluted with diesel fuel and heavy metals (Table 4).

Stomatal conductance of water vapor in leaves of poplar and willow clones was in correlation with transpiration rates. Inhibition of stomatal conductance in plants grown under conditions of soil contamination indicated that depression of photosynthesis and transpiration occurred to a great extent at the stomatal level.

Water use efficiency (WUE) is a good economic indicator of bioproduction per unit of water volume

consumed, and we expressed it as the photosynthesis/transpiration ratio (Table 5).

The results obtained on WUE of poplar clones indicated that diesel fuel and diesel fuel+metals in the soil significantly disturbed water consumption by the plants and consequently their photosynthetic organic production (Table 5). The Panonia and B 81 genotypes showed very stable WUE during growth in polluted soil, and only treatment of soil with diesel fuel and a metal mixture together provoked severe depression of WUE in genotype B 81. Stable WUE values in unfavorable environmental conditions is sign of high organic production and indicates a good remediation potential of those genotypes in which they are recorded.

Willow clone V 2 grown in contaminated soils showed significantly lower WUE in contaminated soil compared to V 1 and other poplar genotypes, which distinguishes it as the least suitable for phytoextraction and remediation.

## DISCUSSION

There are many forest localities that are polluted by heavy metals originating from atmospheric deposition of industrial and traffic emissions. Soil pollution with heavy metals has been found to decrease forest biomass productivity. A very useful ecological solution for cleaning contaminated forest areas and obtaining high biomass (wood) production is growing plant species that have a good heavy metal accu-

Table 5. Rate of transpiration in poplar and willow leaves (mmoles of  $H_2O \cdot m-2 \cdot s-1$ ).

Poplar genotypes					Willow genotypes	
Species/ clones Treatment	Populus x euramericana - Panonia	Populus deltoides - PD 3	Populus deltoides - B 81	Populus deltoides - B 229	Salix alba - V 2	Salix alba - V 1
Pb	5.09 ab	6.16 a	5.36 a	5.32 a	4.69 a	3.59 b
Cd	5.16 ab	5.50 ab	5.25 a	4.42 ab	4.04 a	3.88 b
Ni	4.60 ab	4.97 ab	4.25 a	3.91 b	4.47 a	2.92 b
Pb+Cd+Ni	4.28 ab	3.85 b	4.44 a	1.24 c	1.53 b	1.71 c
Diesel fuel	3.88 b	4.08 b	3.84 a	0.00 c	0.00 c	0.00 d
D.f.+Metals	4.08 ab	0.00 c	0.45 b	0.00 c	0.00 c	0.00 d
Control	6.15 a	4.17 b	4.31 a	3.78 b	4.90 a	5.48 a
LSD <sub>0.05</sub>	1.96	1.68	1.52	1.30	1.19	1.06

mulation capacity on contaminated soils. The heavy metal-accumulating plants *Populus* sp. and *Salix* sp. have been suggested for phytoremediation by many authors (Robinson et al., 2000). Poplar trees represent one vegetation option for phytostabilization of heavy metal-contaminated sites and can be used for the *in situ* decontamination of polluted soils. Rosselli et al. (2003) found that *Salix* and *Betula* transferred zinc and cadmium to leaves and twigs, but *Alnus*, *Fraxinus*, and *Sorbus* excluded them from their aboveground tissues.

As good heavy metal-remediators are needed in order to obtain high biomass production and intensive plant growth, it is of great interest to establish whether their photosynthetic features improve their survival and growing potential under unfavorable (polluted) ecological conditions. Defining CO<sub>2</sub> photosynthetic assimilation of different woody species is important in order to choose genotypes suitable for phytoremediation breeding programs. Heavy metal uptake and translocation from the root zone to stems and leaves of plants are driven by transpiration, and the water status of plant tissues and soil moisture are therefore of crucial importance for photosynthesis and organic assimilation (Marchiol et al., 2004). According to Klang-Westin and Perttu (2002), water availability is a critical factor for growth of Salix, and water availability will probably be the most limiting factor for growth during some periods of the growing season.

Our results suggest that morphological and ecological characteristics of clones and species were affected by heavy metal and diesel fuel treatment in different ways. The high toxicity of bivalent heavy metal ions for overall plant metabolism is most obvious in inhibition of photosynthesis. Multiple inhibitory effects were manifested as leaf chlorosis, decrease of leaf area, and reduced biomass production. The most depressive effect on the rate of photosynthetic CO<sub>2</sub> assimilation in all of the investigated poplar and willow genotypes was evident in plants grown on diesel fuel and a metal mixture in the soil. Also, diesel fuel alone caused very strong and significant inhibition of photosynthesis and transpiration of willow clones, but the same treatment did not provoke drastic decrease of the photosynthetic rate in poplar clones, except in the case of clone B 229. Relatively stable values of fluorescence parameter Fv/Fm in polluted conditions indicated stability of thylakoid structure and electron flow through the photosystems, but disturbance of photosynthesis occurred on the level of carboxylation. This observation is contrary to the findings of Küpper et al. (2007), who reported that during excessive Cdinduced stress, a few mesophyll cells became more inhibited and accumulated more Cd than the majority, while this heterogeneity disappeared during acclimation in good remediator-plants. Chlorophyll fluorescence parameters related to photochemistry were more strongly affected by Cd stress than nonphotochemical parameters, indicating that Cd inhibits photosynthetic light reactions more than the Calvin-Benson cycle.

Treatment with combined excessive Pb, Cd, and Ni was herein found to be a very strong stress factor for photosynthetic productivity, especially that of willow clones and poplar clone B 229. The negative effects of a mix of contaminating metals on the physiology (assimilation and growth) of some other plant species (Brassica napus and Raphanus sativus) in the presence of metals was previously verified by Marchiol et al. (2004). Substitution of heavy metals in chlorophyll leads to a breakdown in photosynthesis. The concomitant presence of metals exerted strong influence on growth of the plants and also on their phytoextraction efficiency, probably due to additive effects of the metals on plant metabolism. Also, vacuolar compartmentalization appears to be an important source of hypertolerance of natural hyperaccumulator plants (Chaney et al., 1997). The presence of heavy metals in plant tissue can reduce photosynthetic CO<sub>2</sub> fixation through partial closure of stomata in developed leaves, which is in agree with our results.

Our results indicated that a combination of metals (Pb+Cd+Ni) in the soil had a stronger inhibitory effect on photosynthesis in comparison with the effect of single metals.

Among the poplar genotypes, clone B 229 exhibited the lowest photosynthetic potential in a contaminated environment, which distinguishes it as the least suitable for good biomass production in polluted soils. Also, comparison of the results referring to photosynthetic potential indicates that *Salix* species are characterized by significantly lower biomass production and remediation ability in a chemically loaded environment. Comparing remediation potentials of the two species rape (*Brassica napus* L.) and willow (*Salix* spp.), Máthé-Gáspár and Anton (2005) found that uptake and translocation of metals were generally higher in willow than in rape. Their results indicated that increased uptake and translocation of Cd and Zn from root to shoot make possible phytoextraction technology, while high Cu and Pb concentrations in roots with a low rate of translocation suggested the phytostabilization method.

In well-adapted species, higher adaptability to stress is accomplished through a high rate of photosynthesis per unit of water loss, i.e., high WUE. In our study, WUE of poplar clones did not decrease in pollution treatments as photosynthesis did. This is because the transpiration rate was also affected by heavy metal stress, and plants that are better adapted to a polluted environment expend less water for nutrient absorption. Plant populations which suffer strong selection pressure from contaminated conditions have lower WUE and higher N content, suggesting that the plants may be "wasting water" to increase N delivery for photosynthetic apparatuses via the transpiration stream (Donovan et al., 2007).

A good potential for adaptation exists in plants that retain high WUE and good distribution of nitrogen in photosynthesis during heavy metal induced-stress, in other words ones characterized by good resource investment in bioproduction. Our results confirmed that all of the genotypes are partially tolerant to heavy metals and indicated poplar clones Panonia, PD 3, and B 81 and willow clone V 1 as genotypes with high bioproduction potentials in contaminated environmental conditions.

Becerril et al. (1989) found that different metals may have different effects on transpiration and growth in the same plant, Pb caused a drastic reduction of water use efficiency, while Cd inhibited transpiration and carbon assimilation to a similar degree and thus did not change WUE. The study of Menon et al. (2007) showed that young trees of different species can respond to heavy metal soil contamination differently, and that these responses may depend on subsoil properties and their variation from year to year.

Our results indicated genotypic specificity of all the investigated physiological parameters and marked poplar clones as very useful in phytoextraction technology for bio-cleaning of chemically polluted soils.

More extensive characterization of genotypes under a variety of conditions, including heavy metal soil pollution, is likely to reveal more about the suitability of each hybrid for site-specific remediation.

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# КАПАЦИТЕТ ЗА ФИТОРЕМЕДИЈАЦИЈУ КЛОНОВА ТОПОЛЕ (*POPULUS* SPP.) И ВРБЕ (*SALIX* SPP.) У ЗАВИСНОСТИ ОД ФОТОСИНТЕЗЕ

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Добре фотосинтетичке карактеристике и водни режим дрвенастих биљака побољшавају њихово преживљавање и потенцијал за ремедијацију у условима загађене животне средине. У вези са тим, у раду су презентовани резултати истраживања фотосинтезе (СО<sub>2</sub> фиксације и Fv/Fm), транспирације и степена ефикасности коришћења воде за биопродукцију - WUE (Water Use Efficiency) четири клона тополе (*Populus* spp.) и два клона врбе (*Salix* spp.). Физиолошка база биљне толеранције на повишене концентрације полутаната је комбинација изражене фотосинтезе и високе ефикасности коришћења воде, која представља однос интензитета фотосинтезе и транспирације. Биљке су гајене у стаклари методом земљишних култура, а постојала је контролна група биљака и шест третмана: Pb, Ni, Cd, комбинација Pb+Cd+Ni, дизел гориво и комбинација дизел гориво+Pb+Cd+Ni.

Интензитет фотосинтетичке асимилације  $CO_2$  (µmol • m<sup>-2</sup> • s<sup>-1</sup>) најмлађих листова значајно је зависио од типа полутанта и генотипске варијабилности клонова топола и кретао се у распону од 10.77 до 8.22 µmol  $CO_2 • m^{-2} • s^{-1}$ . Испитивани клонови врба имали су сличне вредности (просечно око 6.5 µmol  $CO_2 • m^{-2} • s^{-1}$ ). Фотосинтеза и транспирација биљака гајених на појединачним третманима тешких метала значајно се смањила, а најмање на третману Cd. Смеша тешких метала у земљишту изазвала је редукцију интензитета фотосинтезе и до 50 %. Инхибиција интензитета транспирације (mmol  $H_2O \cdot m^{-2} \cdot s^{-1}$ ) је под утицајем тешких метала била много мање изражена. Дизел гориво је изазвало јаку инхибицију фотосинтезе и транспирације клонова врба. Третман који је у комбинацији садржао све примењене полутанте се потврдио као најјачи фактор стреса са негативним деловањем на продуктивност фотосинтезе. Релативно висок интензитет фотосинтезе, у комбинацији са добром економијом коришћења воде имао је за последицу високе вредности WUE, што указује на велики потенцијал неких клонова у фиторемедијацији. По добијеним резултатима, три клона топола и један клон врба су потенцијално задовољавајући "алати" у процесу ремедијације.