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GROWTH AND PLANT PHYSIOLOGICAL PARAMETERS AS MARKERS FOR SELECTION OF POPLAR CLONES FOR CRUDE OIL PHYTOREMEDIATION

RAST I FIZIOLOŠKI PARAMETRI KAO MARKERI PRI SELEKCIJI KLONOVA TOPOLA ZA FITOREMEDIJACIJU NAFTE

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Summary

Phytoremediation is an emerging technology where plants are used for environmental cleanup. Crude oil contaminated soils are one of the most challenging tasks for phytoremediation applications due to the complexity of the process affected by variability in chemical composition of oil, plant-microorganism interactions and phytotoxicity of contaminants. Although signs of phytotoxicity are very often easily visible, sometimes plant physiological processes can indicate stress in plants due to the presence of xenobiotics in cases without visible signs. This paper presents investigation of the potential of various poplar (Populus sp.) clones for phytoremediation of soils contaminated with crude oil through assessment of physiological parameters. Biomass production together with: (i) nitrate reductase activity; (ii) net photosynthesis/dark respiration, (iii) proline content (iv) chlorophyll fluorescence and (v) pigments contents were studied. Investiagted clones showed various reactions to the different levels of soil contamination.

KEY WORDS: poplars, phytoremediation, crude oil contamination, physiological parameters

Introduction
Uvod

Soil, surface water and groundwater may become contaminated with hazardous compounds as a consequence result of either natural or human activities from different traces with both inorganic and organic compounds (heavy metals, radionuclide, nitrate, phosphate, inorganic acids and organic chemicals) from sources including waste materials, explosives, pesticides, fertilizers, pharmaceuticals, acidic deposition and radioactive fallout (Arthur et al., 2005). The processes of soil remediation with use of mechanical, physical and chemical techniques are very expensive and according to Schnoor (1997) range from $ 100–1500 per ton of soil, depending on the techniques involved in treatment. As contrast to these methods, alternatives can be found in the application of phytoremediation with a ten times less cost. Phytoremediation is the use of plants and their associated microorganisms in environmental cleanup (Salt et al., 1995, Raskin et al., 1997). This technology makes use of the naturally occurring processes by which plants and their micro-

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bial rhizosphere flora degrade and sequester organic and inorganic pollutants (Pillon-Smits, 2005). Results of various researches (Chen et al. 2003; Johnson et al., 2004; Rentz et al., 2003) showed increase of hydrocarbon degradation and microorganisms abundance with the use of plants for phytoremediation where even combining more than one species showed good results (Palmroth et al, 2002; Maila et al, 2005). Amongst various tree species used for phytoremediation in the Northern Hemisphere, poplars (*Populus* sp.) proved to be the best candidates for this purpose. Due to their biology of pioneer species of emerging alluvial soils, characterized by very rapid growth and highly developed root capable to uptake large amounts of water, it makes them ideal candidates for phytoremediation (Licht and Isebrands, 2005). 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; Xingmao and Burken, 2004) very often linked to biomass production (Licht and Isebrands, 2005).

Petrol refinery situated at the banks of Danube in Novi Sad was severely damaged in 1999, where according to Nježić and Ačanski (2009) from 73 569 tons of oils stored in the tanks, 90 % was burned, 9.9 % leaked to the surface while 0.1 % leaked to the Danube, where contaminated area was more than 1.5 ha. This leakage caused substantial ecological problem due to vicinity of the Danube and wells that are used as sources for city's water supply. Considering above mentioned research results, together with the ecological impact of crude oil contamination and site specific soil conditions for growing of plants, the aim of this study was to investigate potential of different poplar clones for phytoremediation through assessment of the crude oil contamination effects on the poplar growth and physiology.

Materials and methods
Materijali i metode

Experiment design and plant material

The experiment was established in May 2011 as greenhouse pot experiment in semi controlled conditions with control of irrigation and outer light and temperature. For growing of plants, crude oil contaminated soil from petrol refinery in Novi Sad was added in different volume shares (0, 5, 25, 50, 75 and 100 %) to uncontaminated alluvial soil. Treatments contained from 0.011 to 11.039 g kg^{-1} of total petroleum hydrocarbons (TPH) and 0.005 to 6.839 g kg^{-1} of mineral oils (Table 1). After preparation substrate was transferred to 13 liter pots in which were planted 4 cuttings of poplars in 3 repetitions for each clone/treatment. Three poplar clones: (i) *Populus × euramericana* clone 'Pannonia'; (ii) *Populus deltoides* clone 'Bora'; (iii) *Populus nigra × P. maximowitzii) × P. nigra var. Italica* clone '9111/93' were selected from the clonal archive of the Institute of Lowland Forestry and Environment, Novi Sad, Serbia. After planting of cuttings, pots were irrigated in order to obtain retention water capacity and during the growing of plants pots were irrigated with 0.7 liters of water in the period of 3–7 days depending upon substrate, weather and duration of experiment. During the growth of plants physiological parameters were assessed in August, while after growth cessation and forming of terminal bud plants were harvested for measurement of biomass.

Table 1. Amount of total petroleum hydrocarbons (TPH) and mineral oils (g kg^{-1}) in substrate of treatments
Tablica 1. Sadržaj ukupnih naftnih ugljikovodika (TPH) i mineralnih ulja (g kg^{-1}) u supstratima tretmana u pokusu

Treatment Tretman	TPH TPH	Mineral oils Mineralna ulja
	g kg^{-1}	g kg^{-1}
0%	0,011±0,005	0,005±0,002
5%	0,624±0,347	0,397±0,185
25%	2,588±0,650	1,757±0,395
50%	4,539±1,147	2,823±0,711
75%	7,829±0,928	4,834±0,809
100%	11,039±2,005	6,838±1,027

Investigated parameters – Istraživani parametri

In this experiment following parameters were assessed: (i) net photosynthesis (NPR) and dark respiration rate (DDR) rate; (ii) chlorophyll fluorescence (F_v/F_m); (iii) chlorophyll and carotenoid contents, (iv) nitrate reductase activity (NRA); (v) proline content and (vi) fresh biomass of plants. All physiological parameters were assessed on first fully developed leaf from top with Leaf Plastochron Index value of LPI=5 (Dickmann, 1971). Bulk samples from each repetition were taken for all physiological analysis. Net photosynthesis (NPR) and dark respiration rate (DRR) was assessed polar graphically with use of Clark type electrode according to Walker (1987), while chlorophyll fluorescence was measured by Fluorimeter PSM, BioMonitor, AB and expressed as F_v/F_m ratio. Concentration of acetone extracted leaf pigments of poplar clones was determined by spectrometry (Wettstein, 1957). Nitrate reductase activity (NRA) was assessed *in vivo* in leaves according to Hageman and Reed, (1980). Free proline content was assessed in fresh plant material according to Bates (1973). At the end of experiment, plants were harvested and their biomass was instantaneously weighted on laboratory scale to determine fresh biomass of shoots and roots. Data were analyzed by two-way ANOVA and differences between clones and interactions between clone and treatment were analyzed with Duncan's multiple range test. For statistical analysis was used Statistica 10 software.

Results and discussion
Rezultati i rasprava

Growth of plants – Rast biljaka

Plant growth and establishment at contaminated sites provide useful information defining the species tolerance and phytoremediation potential. Contamination of the environment with crude oil may affect plant performance by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable (Ogbo et al., 2009). To cope with and survive such conditions, plants should enhance the degradation of petroleum hydrocarbons in soil by stimulation of growth and activity of microorganisms capable for degradation of petroleum hydrocarbons in the rhizosphere (Frick et al., 1999; Merkl et al., 2004). One of the most common symptoms of the pollutant phytotoxicity is reduction of plant growth. In the present work, crude oil contamination considerably affected growth of poplars (Fig.1 and Fig.2). Shoot and root fresh mass of clones '9111/93' and 'Pannonia' substantially decreased along with increase of the contamination level. However, low levels of crude oil (5 %) stimulated shoot growth in clone 'Bora', while root growth was not changed in plants exposed to 5 and 25 % treatment.

Growth reduction is caused by crude oil compounds (aliphatic, aromatic, naphthalic and phenolic like compounds), that may reduce dark respiration, transpiration and photosynthesis of leaves (Trapp et al., 2001). Plant growth on soils contaminated with crude oil affect both physical and chemical soil characteristics, leading to decrease of crude oil phytotoxicity, and these changes may be favourable for plant growth. Njoku et al. (2009) reported that *Glycine max* cultivated on crude oil contaminated soils did not significantly affect the crude oil level in some treatments, but improved physico-chemistry of the soil (pH, moisture, organic matter), which are coherent with plant growth. Previous investigations elucidated that plants may not reduce the concentration of contaminants but can reduce their toxicity (Siciliano and Germida, 1998). However, changes in soil pH may contribute to activity of microorganisms capable for degradation of crude oil (Njoku et al., 2009). Higher degradation of petroleum hydrocarbon in vegetated than in non-vegetated soil was reported by Merkl et al. (2005b). In the present study, a clone specific growth under crude oil contamination may be the consequence of different tolerance of the genotypes to such conditions, and their differential ability to adopt changes in soil characteristics. Trapp et al. (2001) found *Populus nigra* to be more sensitive to diesel fuel than willows, *Salix viminalis* and *S. alba*. Preserved growth at

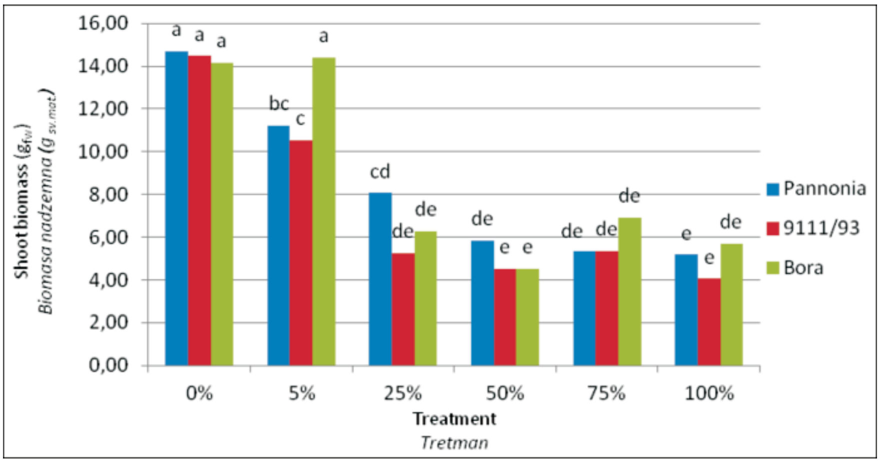

Figure 1 Shoot fresh biomass of poplar clones
Slika 1. Svježa biomasa nadzemnog dijela ispitivanih klonova topola

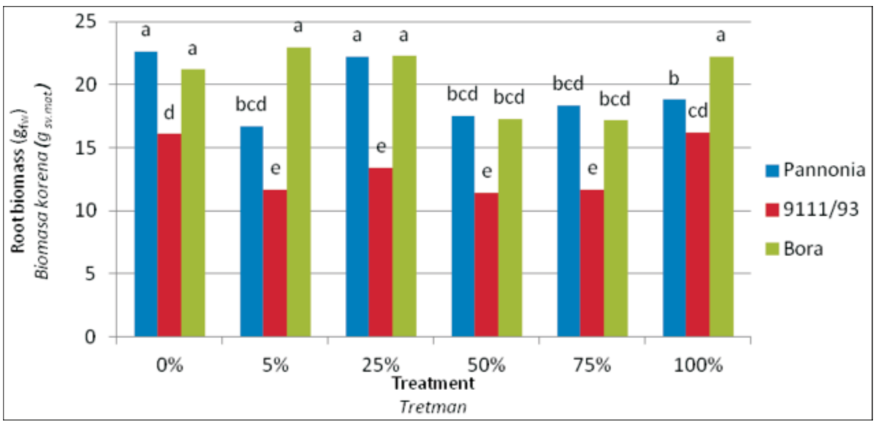

Figure 2. Root fresh biomass of poplar clones
Slika 2. Svježa masa korijena ispitivanih klonova topola

Table 2. Proline content (mg/g) and Nitrate reductase activity (NRA) (mmol NO_2 $g^{-1}_{fw}.h^{-1}$) in leaves of poplar clones
Tablica 2. Sadržaj prolina (mg/g) i aktivnost nitrat-reduktaze (ANR) (mmol NO_2 $g^{-1}_{fw}.h^{-1}$) u listovima ispitivanih klonova topola

	Clone Klon	Treatment Tretman					
		0%	5%	25%	50%	75%	100%
Proline Prolin	Pannonia	10.31 f	12.30 f	10.62 f	33.49 c	10.63 f	33.28 cd
	9111/93	11.23 f	30.73 cd	10.22 f	27.13 cd	9.90 f	28.99 cd
	Bora	18.29 e	50.44 b	11.75 f	58.5 a	11.56 f	26.35 d
NRA ANR	Pannonia	0.833 a	0.763 ab	0.353 fghi	0.473 de	0.437 ef	0.498 de
	9111/93	0.647 c	0.715 bc	0.199 j	0.313 hi	0.368 fgh	0.463 de
	Bora	0.493 de	0.547 d	0.279 ij	0.373 fgh	0.343 ghi	0.426 efg

contaminated site may be a reliable criterion for selection of tolerant genotype or species. For instance, using this criterion, *Paspalum scrobiculatum* L. and alfalfa are good candidates for remediation of crude oil contaminated sites (Kirk et al., 2002; Ogbo et al., 2009).

Proline content – Sadržaj prolina

Treatments with increasing crude oil concentrations affected proline accumulation in poplars with respect to control (Table 2). Proline content in poplars varied from 9.90 to 58.50 μg/g. Considerable changes were observed at 50 and 100 % treatment in all clones. Increased accumulation of free proline in plants exposed to crude oil contamination may be the consequence of disturbed water regime. Many organisms, including higher plants, accumulate free proline in response to osmotic stress due to drought, high salinity and chilling (Nanjo et al., 1999). Water availability could be critical since the oil-impregnated soil does not take up water homogeneously. As a consequence, water drains rapidly through the containers, and limiting water supply of the root system (Merkl et al., 2005a).

Nitrate reductase activity (NRA) – Aktivnost nitrat-reduktaze

Negative effect of soil contamination with crude oil on activity of nitrate-reductase was well pronounced in investigated poplar clones (Table 2.). A lack of considerable changes and significant stimulation were observed at 5 % treatment, respectively. Effect of further increase of oil content in soil mainly decreased activity of the enzyme in poplars.

Inhibition of nitrate reductase activity was reported previously in other plant species. In *Amaranthus hybridusplants* treated with engine oil, decrease of the activity was proportional to applied oil concentrations (Odjegba and Atebe, 2007). 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 reducatse 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). Nitrate reductase was the first recognized substrate inducible enzyme in plants (Lexa et al. 2002). Therefore, changes in availability of essential nutrients, particularly nitrate, can also cause changes in gene expression and nitrate reductase activity. Increased oil levels caould decrease total nitrogen and available nitrate due to their temporal immobilization by microbes (Agbogidi et al., 2007). Furthermore, the inhibitory effect of oil could partly be attributed to the toxic effect of some of its constituents on the enzyme activity (Odjegba and Atebe, 2007). For instance, polycyclic aromatic hydrocarbons (PAH) present a toxic and recalcitrant compounds of engine oil (Wang et al., 2000).

Photosynthetic parameters – Fotosintetski parametri

Rate of photosynthesis (Table 3) in poplar clones affected by oil contamination was either increased or unchanged with respect to control. Contrary to expectations, the 100% treatment stimulated photosynthetic rate in poplars. Light energy absorbed by chlorophyll molecules can be used to drive photosynthesis (photochemistry), while excess energy can be dissipated as heat or it can be re-emitted as light (chlorophyll fluorescence), and these three processes occur in competition (Pellegrini et al., 2010).

The chlorophyll fluorescence parameter Fv/Fm (Table 3.) has been indicator of the maximum quantum efficiency of PSII photochemistry (Butler, 1978). In healthy plants, this value ranges between 0.800 and 0.860 (Björkman and Demming, 1987). Maximum quantum yield of PSII was not considerably affected by crude oil pollution in poplar clone 'Pannonia', while in clone 'Bora' and clone '9111/93' Fv/Fm was significantly changed at 75 and 100% treatment (Table 4.). Chlorophyll fluorescence has been a useful diagnostic tool for the assessment of plant stress and photosynthesis rate (Krause and Weis, 1991). Results of Ralph and Burchett (1998) showed that PSII photochemical efficiency *(Fv/Fm*

Table 3. Net photosynthetic rate (NPR), chlorophyll fluorescence (F_v/F_m) and dark respiration rate (DDR) in leaves of poplar clones
Tablica 3. Neto fotosinteza (NPR), fluorescencija klorofila (F_v/F_m) i disanje (DDR) listova ispitivanih klonova topola

	Clone Klon	Treatment Tretman					
		0%	5%	25%	50%	75%	100%
NPR ($\mu mol\ O_2 cm^2 s^{-1}$)	Pannonia	335.3 fg	471.8 cd	491.8 cd	378.2 efg	377.7 efg	534.3 c
	9111/93	218.9 h	449.7 cde	407.2 def	364.2 efg	300.9 g	715.3 b
	Bora	339.6 fg	493.9 cd	507.1 c	413.3 def	336.4 fg	810.1 a
Fv/Fm	Pannonia	0.721 abcd	0.758 ab	0.750 ab	0.756 ab	0.725 abc	0.694 cd
	9111/93	0.755 ab	0.762 a	0.729 abc	0.745 ab	0.709 bcd	0.639 e
	Bora	0.744 abc	0.715 abcd	0.672 de	0.627 ef	0.625 ef	0.588 f
DDR ($\mu mol\ CO_2 cm^2 s^{-1}$)	Pannonia	108.8 h	174.9 def	245.9 b	182.1 def	159.3 defg	189.6 de
	9111/93	123.6 gh	191.7 de	192.1 de	196.1 cd	159.3 defg	241.3 bc
	Bora	170.0 defg	294.9 a	238.2 bc	140.1 fgh	147.5 efgh	301.6 a

Table 4. Pigments amounts of chlorophyll a (chl a), chlorophyll b (chl b) total chlorophyll (chl a+b) and carotenoids (carot) in leaves of investigated poplar clones
Tablica 4. Sadržaj pigmenata klorofila a (chl a), klorofila b(chl b), ukupnog klorofila (chl a+b) i karotenoida (carot) u listovima ispitivanih klonova topola

Treatment Tretman	Clone Klon	chl a (mg/g_{DW})	chl b (mg/g_{DW})	chl a+b (mg/g_{DW})	carot (mg/g_{DW})
0%	Pannonia	5.046 bc	1.348 bcd	6.394 cd	1.552 bcd
5%		4.586 cde	1.200 cdefg	5.816 cde	1.627 abc
25%		3.898 efgh	0.990 ghij	4.878 fghi	1.259 cdef
50%		6.798 a	1.602 a	8.399 a	2.001 a
75%		4.465 cdef	1.047 fghi	5.511 defg	1.350 bcdef
100%		4.767 cd	1.106 defgh	5.873 cde	1.438 bcde
0%	9111/93	4.824 cd	1.322 bcde	6.146 cd	1.485 bcd
5%		5.228 bc	1.484 ab	6.712 bc	1.658 ab
25%		4.494 cdef	1.243 bcdef	5.737 def	1.478 bcde
50%		4.749 cd	1.394 abc	6.142 cd	1.481 bcd
75%		4.979 c	1.261 bcdef	6.239 cd	1.468 bcde
100%		5.779 b	1.559 a	7.378 b	1.700 ab
0%	Bora	4.092 defg	1.088 efgh	5.180 efgh	1.311 bcdef
5%		3.297 hi	0.822 ij	4.119 ij	1.178 def
25%		2.793 i	0.788 j	3.581 j	1.343 bcdef
50%		3.785 fgh	0.938 hij	4.723 ghi	1.200 def
75%		3.468 ghi	0.837 ij	4.305 hij	1.083 ef
100%		3.252 hi	0.755 j	4.007 ij	1.023 f

ratio) was slightly lower than the control in *Halophila ovalis* exposed to crude oil. The stability of the ratio Fv/Fm indicates preserved efficiency of photosystem II in clones 'Bora' and 'Pannonia'. Furthermore, these clones were characterized by high photosynthetic activity.

Changes in the chlorophylls and carotenoids content in poplars treated with crude oil were clone specific (Table 4.). Chlorophyll a, chlorophyll b and total chlorophylls were not considerably affected by treatments in clones 'Bora' and 'Pannonia' in most cases, while in clone '9111/93' values were considerably decreased, what can be correlated with growth decrease. However, the total carotenoids content was not affected in poplars by treatments. Reduction in chlorophyll content has been an indicator of environmental contamination (Agrawal, 1992) indicating enhanced chlorophyll degradation. That may explain the lower levels of chlorophyll

Table 5. F values of investigated parameters regarding treatment (F_t), clone (F_c) and their interaction (F_{txc})
Tablica 5. F vrijednosti ispitivanih parametara glede tretmana (F_t), klona (F_c) i njihove interakcije (F_{txc})

	Shoot biomass Biomasa nadzemna	Root biomass Biomasa korijena	NRA ANR	Proline Prolin	NPR NPR	DDR DDR	Fv/Fm Fv/Fm	chl a chl a	chl b chl b	chl a+b chl a+b	carot carot
F_t	123.98**	28.34**	134.310**	9.896**	80.665**	36.053**	4.147**	12.420**	8.608**	13.295**	2.190^{ns}
F_c	6.821**	216.81**	61.937**	128.17**	12.111**	16.026**	12.378**	91.394**	87.070**	106.053**	20.470**
F_{txc}	8.175**	8.141**	7.710**	18.273**	5.918**	9.172**	3.297**	7.527**	4.830**	7.921**	2.857*

found in some desert plants exposed to crude oil contaminated soil (Malallah et al., 1998), *Vigna unguiculata* plants (Achuba, 2006), as well as in *Amaranthus hybridus* affected by engine oil (Odjegba and Sadiq, 2002). However, crude oil had no significant effect on the photosynthetic pigments in *Halophyla ovalis* (Ralph and Burchett, 1998).

Dark Respiration Rate (DRR) – Disanje

Dark respiration rate was increased by 5, 25, 50 and 100 % crude oil treatments in poplars with respect to control (Table 3.). Dark respiration plays the crucial role in the regulation of cell metabolism being a basic source of energy resources (Vassilev and Yordanov, 1997). Species growing under stress conditions have higher rates of leaf respiration than species in less stressful environments due to activation of metabolic defense and repair systems (Wright et al. 2006). Old leaves are reported to have lower respiration rates and repair capacities (Di Baccio et al. 2003). The intensity of dark respiration has a significant importance in dry mass accumulation because half of all the photosynthates produced per day are respired in the same period (Lambers, 1985).

Results of analysis of variance and F values (Table 5.) showed high significance ($p>0.01$) of interaction between treatment and clone for all investigated parameters except concentration of carotenoids in leaves of poplar clones. Effect of treatments on carotenoids concentration was not significant, while significance of interaction was evident ($p>0.05$). This may be due to the fact that carotenoids do not play primary role in photosynthesis, compared to the chlorophyll.

Conclusions
Zaključci

Results showed significant differences between investigated clones of poplars exposed to crude oil treatments. The 5% treatment (578 mg g^{-1} of TPH) slightly affected biomass production of poplar clones, although significant differences were recorded at treatment 25% (2812 mg g^{-1} of TPH). The highest decrease of fresh biomass was recorded in clone 9111/93.

The effect of crude oil contamination on physiological processes of poplar clones was observed in all investigated parameters with exception of the carotenoids concentration.

On the basis of these results, poplar clones 'Bora' and 'Pannonia' showed potential for growth on crude oil contaminated soils.

All investigated parameters could be used as markers in selection of poplar clones for crude oil phytoremediation.

Success of phytoremediation is not only result of type of contamination and its level or soil properties and microorganisms' abundance but also the proper selection of clones and cultivars plays significant role. Considering this fact together with obtained results, further research directed to the investigation of plant-microorganisms-soil interaction including clonal selection is required.

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Zahvala

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Sažetak

Fitoremedijacija je korištenje biljaka i s njima povezanih mikroorganizama u čišćenju okoliša. Od različitih vrsta drveća koje se koriste za fitoremedijaciju na sjevernoj hemisferi, topole (Populus sp.) su se pokazale kao najbolji kandidati za tu svrhu. Njihova biologija pionirskih vrsta aluvijalnih tala, koje karakterizira vrlo brzi rast i razvijenost korijenovog sustava sposobnog za apsorpcije velikih količina vode, čini ih idealnim kandidatima za fitoremedijaciju. Rafinerija nafte smještena na obali Dunava u Novom Sadu oštećena je 1999. godine, gdje je, od 73569 tona nafte pohranjene u spremnicima, izgorjelo 90%, 9,9 % se izlilo po tlu, dok je 0,1% iscurilo u Dunav s kontaminiranim područjem od 1,5 ha. Uzimajući u obzir prijašnje rezultate istraživanja, zajedno s ekološkom utjecajem naftne kontaminacije specifičnih uvjeta za uzgoj biljaka na takvome tlu, cilj ove studije bio je istražiti potencijal različitih klonova topola za fitoremedijaciju i procijeniti njihov utjecaj onečišćenja na rast i fiziologiju topola.

U pokusu su korištena tri klona topola iz klonskog arhiva Instituta za nizijsko šumarstvo i životnu sredinu: (i) *Populus × euramericana* klon 'Pannonia'; (ii) *Populus deltoides* klon 'Bora'; (iii) *Populus nigra × P. maximowitzii × P. nigra* var. Italica klon '9111/93' uzgajana u stakleniku u polukontroliranim uvjetima u supstratu koji je sadržavao naftno onečišćenje u vrijednostima od 0,011 do 11,039 g kg^{-1} ukupnih naftnih ugljikovodika i 0,005 do 6,839 g kg^{-1} mineralnih ulja. Tijekom rasta biljaka izvršena su mjerenja intenziteta fotosinteze i disanja, aktivnosti nitrat-reduktaze, fluorescencije klorofila, sadržaja slobodnog prolina i sadržaja pigmenata, a nakon prestanka rasta i oblikovanja terminalnog pupa biljkama je izmjerena svježa biomasa nadzemnog dijela i korijena.

Rezultati su pokazali znatni utjecaj naftnog onečišćenja na rast topola. Svježa biomasa nadzemnog (Graf 1.) i podzemnog (Graf 2.) dijela klonova '9111/93' i 'Panonnia' bili su znatno smanjeni s povećanjem stupnja kontaminacije. Međutim, niska razina sirove nafte (tretman 5 %) stimulirala je rast nadzemnog dijela klona 'Bora', dok je rast korijena bio nepromijenjen u biljkama izloženim tretmanima 5 i 25 %.

Tretmani s povećanom koncentracijom nafte utjecali su na nakupljanje prolina u listovima topola (Tablica 2.) u odnosu na kontrolu. Sadržaj prolina topola varirao je od 9,90 do 58.50 μg/g. Značajne su promjene primijećene pri tretmanima 50 i 100 % kod svih klonova. Povećana akumulacija slobodnog prolina u biljkama izloženim naftnom onečišćenju može biti posljedica poremećenog vodnog režima, jer mnogi organizmi, uključujući i više biljke, nakupljaju prolin kao odgovor na osmotski stres zbog suše, visokog saliniteta i hladnoće.

Negativan utjecaj kontaminacije nafte na aktivnost nitrate-reduktaze (Tablica 2.) je bio dobro izražen kod ispitivanih klonova topola. Nedostatak značajnih promjena i značajni poticaj zabilježen je pri tretmanu 5 %, dok je utjecaj daljnjeg povećanja sadržaja nafte u tlu uglavnom utjecao na smanjenu aktivnost enzima.

Naftno zagađenje nije značajno utjecalo na smanjenje intenziteta fotosinteze i intenziteta disanja (Tablica 3.), čak je zabilježeno povećanje pri tretmanu 100%. Fluorescencija klorofila bila je značajno smanjenja samo kod klonova 'Bora' i '9111/93', dok kod klona 'Pannonia' nije zabilježen utjecaj naftnog zagađenja.

Promjene sadržaja klorofila i karotenoida u listovima topola (Tablica 4.) u prisutnosti nafte pokazala su klonsku specifičnost. Sadržaj klorofila a, klorofila b i ukupnog klorofila u listovima klonova "Bora" i "Pannonia" nije bio znatno promijenjen pod utjecajem tretmana, dok su kod klona '9111/93' te vrijednosti bile znatno smanjene, što može biti u vezi s njegovim smanjenjem biomase. Međutim,tretmani nisu imali značajan utjecaj na sadržaj karotenoida u listovima topola.

Rezultati analize varijance i F vrijednosti za ispitivane parametre (Tablica 5.) pokazali su visok utjecaj tretmana, klonova i njihove interakcije ($p<0,01$) za sve ispitivane parametre, osim sadržaja karotenoida,što se može objasniti činjenicom da karotenoidi nisu primarni pigmenti fotosinteze i nemaju važnost kao klorofil.

Kao najbolji kandidat za fitoremedijaciju naftom zagađenih tala pokazao se klon "Bora", koji je pokazao najmanji utjecaj tretmana na ispitivane parametre, dok je najveći utjecaj naftnog zagađenja zabilježen kod klona 9111/93. Rezultati ovog pokusa pokazali su značajne razlike između ispitivanih klonova topola te potvrdili da se parametri rasta i fiziološki parametri mogu koristiti kao markeri za selekciju klonova na fitoremedijaciju naftom zagađenih tala, kao i da uspjeh fitoremedijacije nije samo rezultat vrste onečišćenja i njegove razine, svojstva tla i brojnost mikroorganizama, već i pravilanog izbora sorti i klonova. S obzirom na navedene činjenice, i uvažavajući dobivene rezultate, neophodna su daljnja istraživanja usmjerena na analize interakcije biljke-mikroorganizmi-tlo, uključujući i selekciju klonova.

KLJUČNE RIJEČI: topole, fitoremedijacija, naftna kontaminacija, fiziološki parametri