

Gas exchange responses of two poplar clones (*Populus euramericana* (Dode) Guinier 561/41 and *Populus nigra* Linnaeus 63/135) to lead toxicity

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ABSTRACT: To evaluate the lead tolerance of *Populus euramericana* (Dode) Guinier 561/41 and *Populus nigra* Linnaeus 63/135 clones in relation to photosynthetic efficiency, we measured physiological parameters of the two poplar clones exposed to Pb treatments. The pot experiment was established in a roofed place in a completely randomized design, with 5 Pb treatments (0, 0.5, 1, 1.5 and 2 g Pb·kg⁻¹) per poplar clone. After 3 months, photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance, intercellular CO₂ concentration (*C_i*), leaf water potential were measured and water use efficiency (WUE) and mesophyll conductance (*g_m*) were computed. Results revealed that increasing Pb concentrations in soil reduced all physiological parameters of the two poplar clones, except *C_i*. At all Pb treatments *A* and *E*, and at a concentration of 0.5 g Pb·kg⁻¹ WUE and *g_m* of *P. euramericana* plants were significantly higher than those of *P. nigra* plants. On the other hand, while a reduction in most physiological parameters of *P. euramericana* plants took place at a concentration of 1 g Pb·kg⁻¹ and at higher concentrations, in *P. nigra* plants these reductions were from a concentration of 0.5 g Pb·kg⁻¹. With respect to physiological parameters, *P. euramericana* 561/41 clone was more tolerant of Pb than *P. nigra* 63/135 clone, therefore it can be considered as a suitable species in phytoremediation of lead-contaminated soils.

Keywords: physiological parameters; phytoremediation; soil contamination; heavy metals; stress

Soil as a component of the biosphere has an important role in food production and environmental sustainability. The pollution of soil with heavy metals has become a serious international problem during recent years (BERNARDINI et al. 2015). Lead is not an essential nutrient in plant metabolism, and it is one of the hazardous heavy metal pollutants of the environment originating from various sources, like mining and smelting activities, burning of coal, effluents from storage battery industries, automobile exhausts, pesticides, and from additives to pigments and gasoline, as well as from the disposal of municipal sewage sludge enriched

with Pb (EICK et al. 1999; VERMA, DUBEY 2003). Lead contaminated soils can cause structural and ultrastructural changes affecting the growth and physiological processes of plants (KUMAR, KUMARI 2015). Generally, toxic concentrations of Pb for plants are described as 30–300 mg·kg⁻¹ dry weight (BAYCU et al. 2006).

Phytoremediation of soils contaminated with heavy metals, which employs plants to remove, degrade or contain contaminants located in soil, has received much consideration as a low-cost and environmentally friendly method, and it has become a promising soil remediation technique compared

to traditional physicochemical methods (HE et al. 2013; SALEHI et al. 2016). As regards, the entrance of heavy metals into the food chain is dangerous, so contaminated soils must be forbidden from agricultural projects and subjected to remediation processes via afforestation (BOJARCZUK, KIELISZEWSKA-ROKICKA 2010). On the other hand, trees have the capability to effectively stabilize the soil with their root system, prevent erosion, and reduce the spread of contaminants. Also, trees can be easily harvested and removed from the area with minimal risk, effectively taking with them a high quantity of the contaminants that were present in the soil (PAZ-ALBERTO, SIGUA 2013; CHANDRA, KANG 2015).

Several studies have reported the effect of heavy metals on the photosynthesis activity of the plants. For example, KALAJI and LOBODA (2007) and HAN et al. (2013) reported that photosynthesis inhibition is a well-recognized symptom of Pb toxicity. In addition, PAJEVIĆ et al. (2009) demonstrated that photosynthesis and transpiration in most of the studied *Populus* Linnaeus and *Salix* Linnaeus clones exposed to Pb, Cd and Ni decreased significantly. Also, the decline in gas exchange parameters of different poplar clones in response to heavy metal stress has been shown by GU et al. (2007), PIETRINI et al. (2010), and CHANDRA and KANG (2015). On the other hand, HE et al. (2013) showed that while Cd exposure caused a decline in the photosynthesis of four poplar species (*Populus alba* Linnaeus × *Populus glandulosa* Ueki, *Populus euramericana* (Dode) Guinier, *Populus cathayana* Rehder, and *Populus deltoides* Bartram ex Marshall), no change was observed on *Populus nigra* Linnaeus and *Populus popularis*. Neither did the soil contaminated with heavy metals have any effect on the photosynthetic rate of poplar clones studied by TOGNETTI et al. (2004) and SALEHI et al. (2014).

Plant tolerance to heavy metal stress is an important factor for phytoremediation of contaminated soils (ZALESNY et al. 2005). As regards, some physiological characteristics can be used as indicators of tolerance to contamination, while the analysis of gas exchange could show plant responses to heavy metal stress (PIETRINI et al. 2003). Likewise, in literature it has been reported that tree species belonging to the family Salicaceae are attended as ideal candidates for phytoremediation of soils contaminated with heavy metals, due to the extensive root system, fast growth, large biomass production, metal tolerance and metal accumulation potential (BITTSÁNSZKY et al. 2009; CHANDRA, KANG 2015; HOSSEINI et al. 2015). In general, Salicaceae species exhibit good tolerance to heavy metals, but interspecific and intraspecific dif-

ferences do exist (MRNKA et al. 2012). For this reason, in this study, the lead tolerance of two common species in afforestation practices of northern Iran, *P. euramericana* 561/41 and *P. nigra* 63/135 clones, in relation to physiological parameters was investigated and compared.

MATERIAL AND METHODS

The homogeneously 20 cm long cuttings of *P. euramericana* 561/41 and *P. nigra* 63/135 clones were collected in February and kept at 4°C until planting date (CICATELLI et al. 2010). In late March, the cuttings were removed from cold storage and located overnight under running tap water. Cuttings were planted in 2 l pots filled with soil. The soil used in the pots was supplemented with $\text{Pb}(\text{NO}_3)_2$ equivalent to 0, 0.5, 1, 1.5 and 2 g $\text{Pb}\cdot\text{kg}^{-1}$. Before soil treatment, 3 samples of soil were taken and analysed for physicochemical characteristics. Soil properties are indicated in Table 1. The pot experiment was established in a roofed place in a completely randomized design, with 5 Pb treatments (0, 0.5, 1, 1.5 and 2 g $\text{Pb}\cdot\text{kg}^{-1}$) per poplar clone and with 18 plants in each treatment. Each plant grew in its own pot. The pots were irrigated every other day during the experiment with regard to the field capacity of soil. In the studied site, the mean annual rainfall, mean annual temperature and mean annual relative humidity are 803.4 mm, 17°C and 80%, respectively, and the dry season comes about between May and August (MIRZAEI et al. 2007).

After 3 months, physiological parameters (photosynthetic rate – A , transpiration rate – E , stomatal conductance – g_s , and intercellular CO_2 concentration – C_i) were measured on the fourth and fifth fully expanded leaves from the apex using a portable photosynthesis system (ADC BioScientific, Ltd., Hoddesdon, UK) (BORGHI et al. 2008). All gas exchange measurements were performed between 10:00 and 12:00 h while leaf chamber temperature ($25 \pm 2^\circ\text{C}$) and humidity (50–65%) were adjusted to keep vapour pressure deficit at 2.0 ± 0.2 kPa with light intensity about 400–500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Leaf water potential (LWP) was determined with Plant Moisture Vessel SKPM 1400 (Skye Instruments, Ltd., Powys, UK) using the pressure chamber apparatus (TURNAU 1998). WUE was calculated as the ratio of A to E (PAJEVIĆ et al. 2009). Mesophyll conductance (g_m) was defined as the ratio of A to C_i (BERNARDINI et al. 2015). In each poplar clone, the gas exchange measurements were conducted on three replicate plants for each treatment.

Table 1. Physical and chemical properties of the soil used for the experiment

| Soil texture | pH (in water) | EC (dS·m ⁻¹) | CEC (meq·100 g ⁻¹) | Organic matter (%) | Total N (%) | Assimilable P (mg·kg ⁻¹) | Exchangeable K (mg·kg ⁻¹) |
|--------------|------------------|-----------------------------|-----------------------------------|-----------------------|----------------|---|--|
| Sandy-loam | 7.86 | 0.49 | 24.66 | 0.84 | 0.156 | 48.17 | 264.33 |

EC – electrical conductivity, CEC – cation exchange capacity

The data were analysed using the SPSS software (Version 20.0, 2011). Firstly, the data were subjected to the Shapiro-Wilk test of normality and to Levene’s homoscedasticity test. Since the data exhibited normal distribution, differences between Pb treatments (in each poplar clone) and two poplar clones were assessed by the one-way analysis of variance and *t*-test, respectively. Separation of means was performed using Tukey’s test at $P < 0.05$ significance level.

RESULTS

As indicated in Table 2, the effect of Pb treatments on physiological parameters (*A*, *E*, g_s , LWP, WUE, and g_m) of two poplar clones (*P. euramericana* 561/41 and *P. nigra* 63/135), except C_i , was significant. As shown in Fig. 1, in *P. nigra* plants, at all Pb treatments, *A*, WUE, g_m , and LWP were significantly lower than those in control plants. At 2 g Pb·kg⁻¹ treatment, *P. nigra* plants indicated 53.7, 23.4, 46.6, and 58.6% reductions in *A*, WUE, g_m , and LWP, respectively, compared to control plants (Figs 1a, e–g). In contrast, significant decreases in *A*, WUE, g_m , and LWP of *P. euramericana* plants were observed as a consequence of 1, 1.5 and 2 g Pb·kg⁻¹ treatments. At the highest Pb concentration, the *A*, WUE, g_m , and LWP of *P. euramericana* plants were 47.7, 29.3, 53.3, and 59.5%, respectively, less than those of control plants (Figs 1a, e–g).

Significant reductions in *E* of *P. euramericana* and *P. nigra* plants exposed to all Pb treatments were observed compared to control plants. These reductions at 2 g Pb·kg⁻¹ treatment for *P. euramericana* and *P. nigra* plants were 46 and 49.3%, respectively (Fig. 1b). On the other hand, the g_s of *P. euramericana* and *P. nigra* plants were significantly reduced by application of 1.5 and 2 g Pb·kg⁻¹ treatments as compared to control plants. At 2 g Pb·kg⁻¹ treatment, the g_s of *P. euramericana* and *P. nigra* plants were 27.2 and 41.6%, respectively, less than those of control plants. The C_i of two poplar clones remained unchanged across Pb treatments.

Based on the results of *t*-test, it was revealed that at all Pb treatments, *A* and *E* of *P. euramericana* plants were significantly higher than those of *P. nigra* plants, except *A* at 0 Pb treatment (Figs 1a, b). In contrast, there were no marked differences in g_s , C_i , and LWP of *P. euramericana* and *P. nigra* plants between Pb treatments (Figs 1c, d, g). Significant decreases in WUE and g_m of *P. nigra* plants in comparison with *P. euramericana* plants just occurred as a consequence of 0.5 g Pb·kg⁻¹ treatment.

DISCUSSION

The analysis of physiological parameters of woody species is valuable in order to choose genotypes suitable for phytoremediation programs (PAJEVIĆ et al. 2009). On the one hand, physiological responses of

Table 2. Significance of Pb treatment effects on physiological parameters of two poplar clones based on one-way analysis of variance

| Physiological parameters | <i>Populus euramericana</i> (Dode) Guinier | | | <i>Populus nigra</i> Linnaeus | | |
|--------------------------|--|-----------------|---------------------|-------------------------------|-----------------|---------------------|
| | <i>df</i> | <i>F</i> -value | <i>P</i> -value | <i>df</i> | <i>F</i> -value | <i>P</i> -value |
| <i>A</i> | 4 | 128.97 | 0.000** | 4 | 187.26 | 0.000** |
| <i>E</i> | 4 | 96.71 | 0.000** | 4 | 106.99 | 0.000** |
| g_s | 4 | 7.53 | 0.000** | 4 | 12.8 | 0.000** |
| C_i | 4 | 1.12 | 0.356 ^{ns} | 4 | 1.07 | 0.376 ^{ns} |
| LWP | 4 | 19.15 | 0.000** | 4 | 23.67 | 0.000** |
| WUE | 4 | 9.13 | 0.000** | 4 | 6.48 | 0.000** |
| g_m | 4 | 153.06 | 0.000** | 4 | 132.26 | 0.000** |

A – photosynthetic rate, *E* – transpiration rate, g_s – stomatal conductance, C_i – intercellular CO₂ concentration, LWP – leaf water potential, WUE – water use efficiency, g_m – mesophyll conductance, ** $P < 0.01$, ns – not significant

plants to contaminated soils are a way to evaluate the plant tolerance to heavy metals (BORGHI et al. 2008), and on the other hand, the high toxicity of heavy metals to overall plant metabolism results in the inhibition of photosynthesis (PAJEVIĆ et al.

2009). Also, heavy metal uptake and translocation from roots to aerial parts of plants are controlled by the transpiration process (ADRIAENSEN et al. 2003).

Since heavy metals can affect plant photosynthetic efficiency (LUNÁČKOVÁ et al. 2003), in this

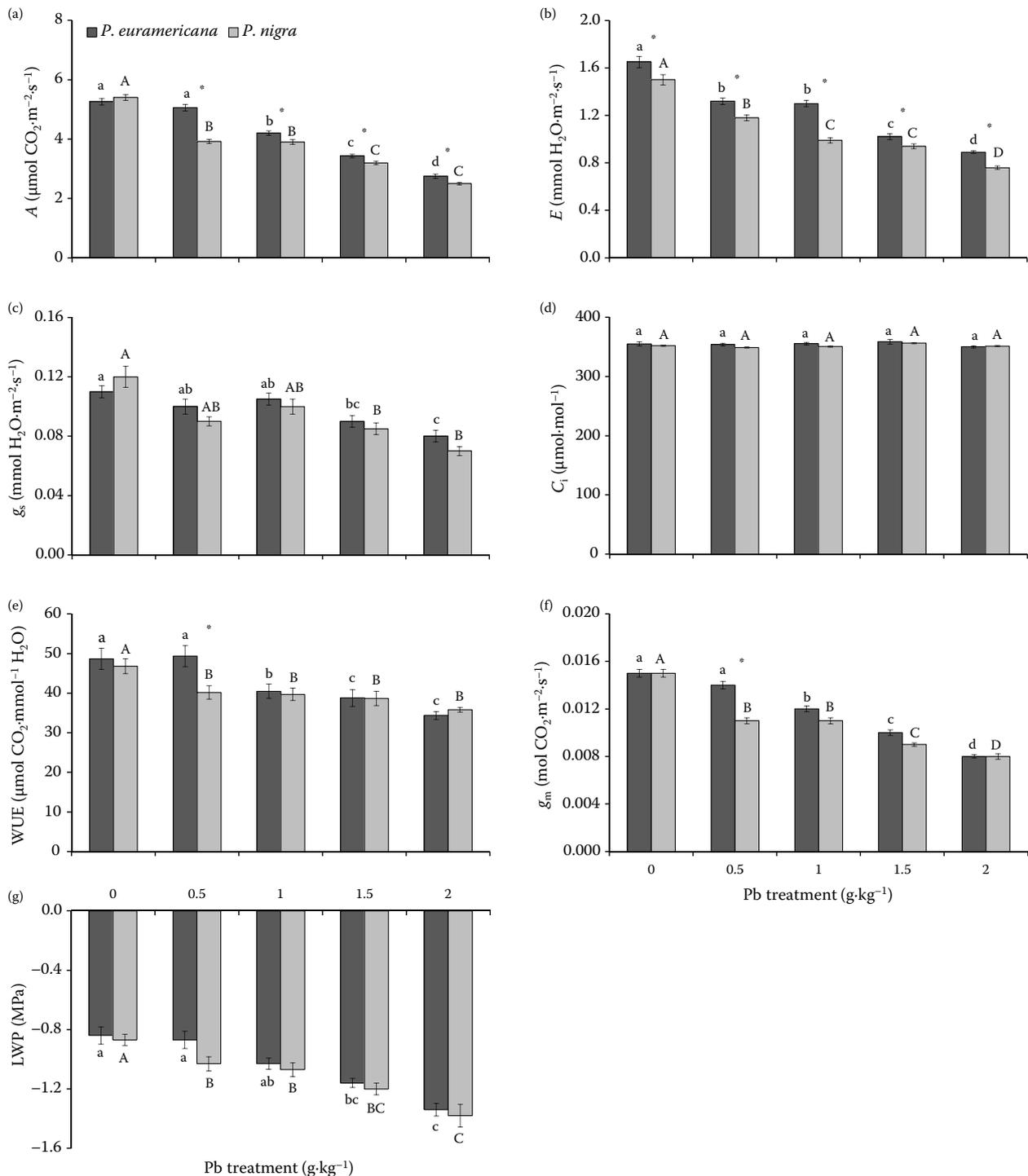


Fig. 1. Physiological parameters of *Populus euramericana* (Dode) Guinier and *Populus nigra* Linnaeus plants on Pb contaminated soil bars – standard errors, different small letters – significant difference between Pb treatments in *P. euramericana*, different capital letters – significant difference between Pb treatments in *P. nigra* (P < 0.05), *significant differences between two poplar clones in each Pb treatment (P < 0.05), A – photosynthetic rate, E – transpiration rate, g_s – stomatal conductance, C_i – intercellular CO₂ concentration, WUE – water use efficiency, g_m – mesophyll conductance, LWP – leaf water potential

study the lead tolerance of *P. euramericana* 561/41 and *P. nigra* 63/135 clones was evaluated in relation to physiological parameters. Based on the results, in the two poplar clones, with increasing Pb concentration in soil A , E , g_s , WUE, g_m , and LWP were diminished. In contrast, at all Pb treatments, C_i of the two poplars remained constant. Similarly, PAJEVIĆ et al. (2009) showed that A and E were significantly decreased in most of the *Populus* and *Salix* clones exposed to Pb, Cd, Ni. In addition, the reduction of A , E , g_s in different poplar clones under heavy metal stress has been reported by GU et al. (2007), PIETRINI et al. (2010), and CHANDRA and KANG (2015).

The inhibition of plant photosynthetic parameters under heavy metal stress results in damage to photosynthetic enzymes and chloroplast membranes, decrease in chlorophyll synthesis, replacement of heavy metals in the chlorophyll molecule, accumulation of heavy metals in the photosynthetic organs of plants (leaves) and interference of metal ions with the uptake of essential elements of the photosynthetic process (CHANDRA, KANG 2015). Of course, regarding the medium or growing conditions, the plant responses may be different. For example, BORGHİ et al. (2008), monitoring two poplars in a hydroponic culture for 30 and 80 days, observed that the photosynthetic rate stayed stable across Cu treatments in *Populus × canadensis* Moench, while a decrease of about 28% occurred with increasing Cu concentration in *P. alba*. Also, TOGNETTI et al. (2004), RENNİNGER et al. (2013), and SALEHI et al. (2014) found that the studied heavy metals had no toxic effect on photosynthesis in the studied poplar clones. In reality, it can be stated that various physiological responses of poplars exposed to heavy metals can be due to genotype, different growth substrates, plant physiological conditions, type of heavy metal and time periods of exposure to heavy metals (MÈNON et al. 2007; BORGHİ et al. 2008). KIEFFER et al. (2009) demonstrated that a short-term Cd treatment (14 days) had a strong negative effect on the photosynthetic process, but for a long-term Cd treatment (up to 56 days), this effect was lower. In fact, in long-term treatments, some poplars can adapt to heavy metals to minimize the negative impacts on photosynthesis and growth (HE et al. 2013).

In this study, the WUE of two poplar clones was reduced ($P \leq 0.01$) by increasing Pb in soil. This is in agreement with the findings of PAJEVIĆ et al. (2009) on *Salix alba* Linnaeus, ETEMADI et al. (2013) on *P. nigra* and *P. alba*, and HAN et al. (2013) on *P. cathayana*. Generally, WUE is a good economic indicator of biomass production per unit

of water volume consumed (PAJEVIĆ et al. 2009). In reality, under heavy metal stress, some plants may benefit from wasting water to increase N delivery for the photosynthetic process via the transpiration stream (DONOVAN et al. 2007).

It has already been reported that the reduction of LWP under Pb stress is due to diminishing the level of compounds that are associated with maintaining cell turgor and cell wall plasticity (SHARMA, DUBEY 2005), restricted root growth, reducing the absorption of water by root, hydraulic conductivity reduction and loss of plant available water (AKINCI et al. 2010). Similarly, in the present study we observed in both clones that LWP was reduced with increasing Pb concentration in soil.

The same as the finding of VELIKOVA et al. (2011) on *P. nigra* where g_m was largely decreased by Ni stress, in our investigations the g_m of two poplar clones was negatively affected by increasing Pb concentration in soil. In fact, mesophyll conductance influences photosynthetic capacity (CENTRITTO et al. 2009). It can be stated that in plants grown in the presence of excessive concentrations of heavy metals photosynthesis is impaired due to a depletion of CO₂ at the rubisco carboxylation site, as a consequence of major decreases in stomatal and mesophyll conductances to CO₂ (SAGARDOY et al. 2010).

Comparison of two poplar clones demonstrated that these poplars differ in their response to Pb treatments in terms of physiological parameters. While a reduction in most physiological parameters of *P. euramericana* plants took place at a concentration of 1 g Pb·kg⁻¹ and at higher concentrations, in *P. nigra* plants these reductions were from a concentration of 0.5 g Pb·kg⁻¹. On the other hand, A and E at all Pb treatments, and WUE and g_m of *P. euramericana* plants at a concentration of 0.5 g Pb·kg⁻¹ were significantly higher than those of *P. nigra* plants. These results show that *P. nigra* plants are more sensitive to lead stress than *P. euramericana* plants in terms of physiological parameters.

In conclusion, Pb treatments induced the reduction of most photosynthetic parameters in the two poplar clones, but these clones exhibited varying responses to Pb treatments. Based on the results, *P. euramericana* plants are more tolerant to Pb than *P. nigra* plants in terms of physiological parameters at least at the doses applied in our experiment. Since the phytoremediation process may be facilitated through selection of more contaminant-tolerant tree species, therefore the attention to plant physiological responses as stress indicators can be important in phytoremediation programs.

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