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# Physiological responses of poplar and willow clones grown in pot trials on soil from landfills

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**Abstract:** Poplars and willows have been cultivated for their environmental benefits for millennia. In the present study, physiological responses were examined in three poplar and three willow clones grown in soil from the landfills near Novi Sad and Belgrade, Serbia. The experiment, conducted under controlled conditions in pots with soil directly from the landfill sites, aimed to identify the most suitable clones based on their physiological performance on examined substrates. Within the process of phyto-recurrent selection, six clones were selected for this experiment: *Populus deltoides* cl. PE 19/6, PE 4/68, S 1-8, and *Salix alba* cl. 107/65-9, 79/64-2, 380. Four physiological parameters (net photosynthesis [A], transpiration [E], water use efficiency [WUE], and stomatal conductance [ $g_s$ ]) were measured throughout the research. Analysis of variance revealed that certain clones exhibited superior physiological responses compared to others. The superior performance of poplar clones PE 4/68 and S 1-8 in key physiological parameters underscores their potential for improving growth, resilience, carbon sequestration, and overall ecological and economic benefits, making them valuable candidates for forestry and environmental management programs.

**Keywords:** phytoremediation, poplar and willow clones, physiology, landfill.

## 1. Introduction

Broad genetic variability of poplars and willows governed the development and creation of cultivars from 1950's onwards on a worldwide scale. Considering the versatility of the climate and soil conditions across the globe, the selection process was directed in the production of the site-specific clones and cultivars (Orlović et al. 2003). Currently, the production of drought-resistant and contamination-tolerant clones and cultivars that can mitigate climate change by sequestering excessive carbon dioxide in their large biomass is essential. This challenge is particularly critical in the breeding of fast-growing species. (Harris et al. 2017; Pecchioni et al. 2020).

Phytoremediation is a complex technology which comprises of several techniques with respect to the specificity in physiological responses of plants to excessive concentrations of different contaminants (Yan et al. 2020). Understanding the physiological responses of poplar and willow clones

to heavy metal induced stress is crucial for optimizing their use in phytoremediation strategies (Zaghloul, 2020). Physiological parameters, including net photosynthesis, transpiration rate, stomatal conductance and water use efficiency, provide insight into the tolerance mechanisms and adaptive capabilities of these clones under stress conditions (Arsenov et al. 2017; Pilipovic et al. 2022). Heavy metals from polluted soils, such as cadmium (Cd), lead (Pb), and zinc (Zn), are prevalent environmental pollutants that pose significant risks to ecosystems and human health due to their toxicity, persistence, and bioaccumulation potential (Tózsér et al. 2023). Thus, phytoextraction of heavy metals from soil and groundwater by fast-growing woody plants could be regarded as useful solution for sites with such contaminations (Polle et al. 2013; Arsenov et al. 2017). The phyto-recurrent selection has been purposed as a breeding method for selecting tree genotypes dedicated for application in phytoremediation projects (Zalesny and Bauer, 2019). In this context, selection based on physiological parameters could be regarded as an important stage of such process (Pilipovic et al. 2022).

By examining these physiological responses, this research seeks to contribute to the broader understanding of plant adaptation and to enhance the practical applications of poplar and willow clones in environmental remediation efforts (Pajević et al. 2009). These findings could be of help in the selection of suitable poplar clones for phytoremediation projects, ultimately aiding in the development of sustainable strategies for managing heavy metal pollution in contaminated soils (Mleczeek et al. 2009; Hou et al. 2020).

This study investigates the physiological responses of different clones of willow (genus *Salix*) and poplar (genus *Populus*) grown in pots with soil sourced from two distinct landfill sites. Our specific objectives were to: (1) assess the differences between willow and poplar clones based on physiological parameters, and (2) analyze interaction between physiological parameters and soil from different sites. Landfills are known repositories of various contaminants, including heavy metals, which can leach into surrounding soils and pose significant environmental challenges (Wang et al. 2022). The use of soils from these sites aims to simulate realistic scenarios and assess the potential of these clones for use in remediation efforts. The findings from this research are anticipated to be valuable in identifying suitable genotypes for landfill sites and/or future afforestation initiatives in such environments.

## 2. Material and Methods

### 2.1. Plant material and experimental design

The greenhouse testing of potential poplar and willow clones, sourced from the gene banks of the Institute of Lowland Forestry and Environment (ILFE), was conducted at the ILFE premises (N 45° 14', E 19° 49', 86 m a.s.l., Serbia). The greenhouse testing was conducted from mid-February to the end of June 2023. Six clones were tested in total, comprising three poplar and three willow clones (Table 1). For the experiment, soil was taken from the "Vinča" Landfill (N 44°47', E 20°36', altitude 99 m a.s.l., Serbia) (clay loam) and the "Novi Sad" Landfill (N 45°18', E 19°50', altitude 75 m a.s.l., Serbia) (sandy loam), with soil from the Experimental Site of the Institute for Lowland Forestry and Environment in Kać (N 45°17', E 19°53', 78 m a.s.l., Serbia) used as a control (sand) (Table 2).

**Table 1.** The willow and poplar clones evaluated during the experiment selected to study their physiological responses to contaminated soils.

Measured clones	
Poplar clones	<i>Populus deltoides</i> W.Bartram ex Marshall. cl. PE 19/66
	<i>Populus deltoides</i> W.Bartram ex Marshall. cl. PE 4/68
	<i>Populus deltoides</i> W.Bartram ex Marshall. cl. S 1-8
Willow clones	<i>Salix alba</i> L. cl. 380
	<i>Salix alba</i> L. cl. 107/65-9
	<i>Salix alba</i> L. cl. 79/64-2

The experiment utilized one-year-old cuttings planted in 3-liter pots. During the greenhouse experiment, the temperature ranged from 19°C to 23°C, while air humidity fluctuated between 60% and 80%. Regarding light conditions, plants were exposed to a 16-hour photoperiod followed by 8 hours of darkness. Throughout the experiment, the plants remained in optimal health without the use of pesticides, fungicides, or growth stimulants. Watering intervals for the experimental plants were spaced between 3 to 4 days, and excess water was drained from the trays to prevent anaerobic conditions.

## 2.2. Assessment of the soil physio-chemical properties

Soil samples were collected from landfill sites in Novi Sad and Belgrade, as well as from control soil in Kać, at a depth of 0 to 30 cm, under disturbed conditions. Ten samples were extracted from each site for subsequent analysis. The soil underwent debris removal, sieving, and was subsequently transferred into pots for further experimentation. These soil samples underwent analysis at the laboratory of the Institute for Lowland Forestry and Environment.

The mechanical composition was determined using the Pipette method (Vandecasteele and Vos, 2001), sample preparation for analysis was done with Na-pyrophosphate according to Thun, and the soil texture class was determined using the USDA (United States Department of Agriculture) basic soil texture classification system (USDA, 1999). The calcium carbonate (CaCO<sub>3</sub>) content was determined volumetrically using the Scheibler's calcimeter (Horváth et al. 2005), while the pH value was measured potentiometrically in a soil-water suspension. The humus content was determined according to Tjurin, using the Simakov modification (Simakov and Tsyplakov, 1969).

**Table 2.** Soil physio-chemical properties in a study testing the growth and physiological responses of three poplar and three willows clones grown on soils from landfills.

Treatment	Physical properties				Chemical properties		
	Depth (cm)	Total sand (%)	Total clay (%)	Textural class	CaCO <sub>3</sub> (%)	pH	Hummus (%)
Control	0-30	89.12	10.88	sand	11.12	8.09	1.65
Vinča Landfill	0-30	39.96	60.04	clay loam	6.82	7.95	2.14
Novi Sad Landfill	0-30	63.84	36.16	sandy loam	13.06	8.62	2.71

## 2.3. Assessment of leaf gas exchange

Leaf gas exchange parameters (net photosynthesis ( $A$  [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]), rate of transpiration ( $E$  [ $\text{mmol m}^{-2} \text{s}^{-1}$ ]), stomatal conductance ( $g_s$  [ $\text{mmol m}^{-2} \text{s}^{-1}$ ]), and water use efficiency ( $WUE$  [ $\text{mmol mol}^{-1}$ ])) were measured with a CIRAS-3 portable photosynthesis system (Amesbury, MA, USA) in a time scale between 9:00 AM and 11:00 AM. Gas exchange parameters were measured on June 22 and 23, 2023. The measurements were recorded under photosynthetic active radiation (PAR) of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and CO<sub>2</sub> concentration of 390  $\mu\text{mol mol}^{-1}$ , while humidity and air temperature were assessed during measurements. Three plants per treatment were chosen with five readings from one leaf per plant were taken for every clone.

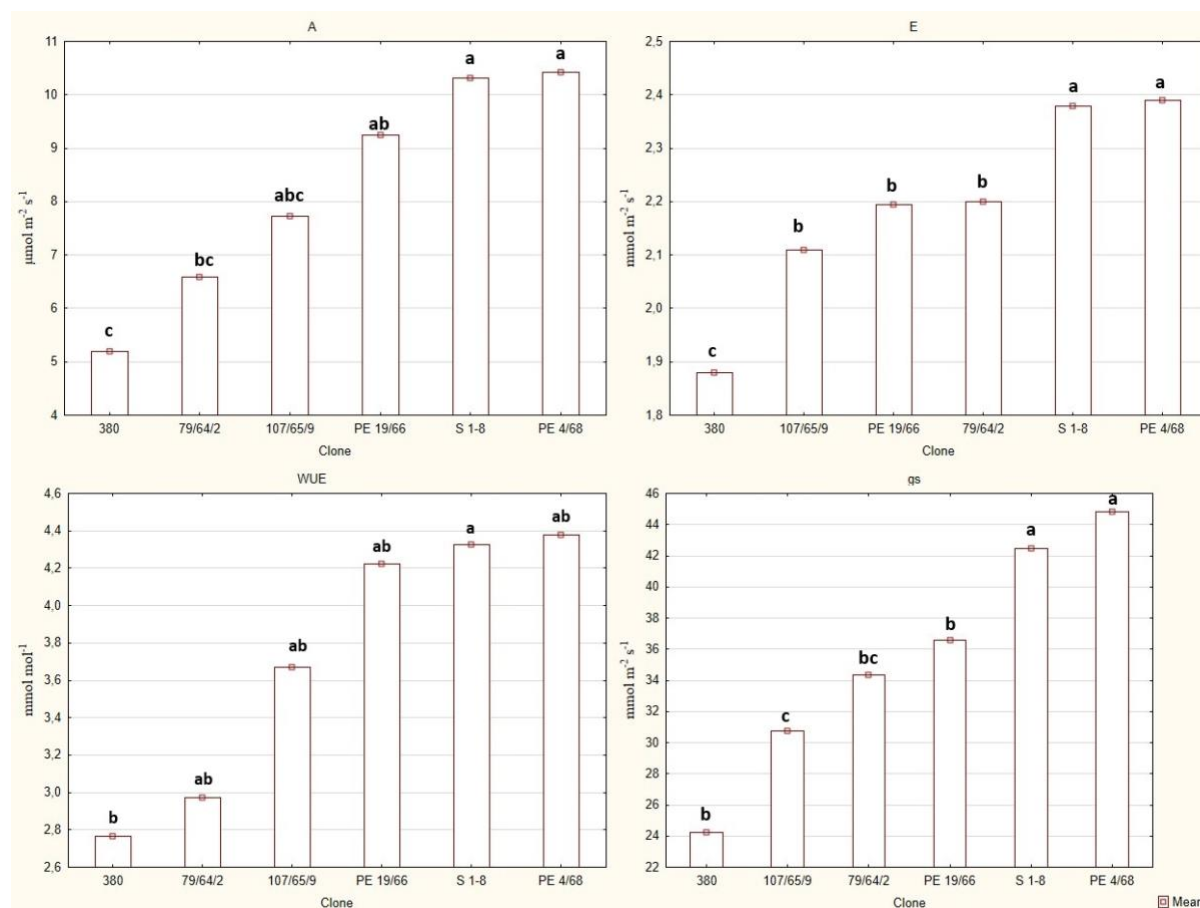
## 2.4. Statistical analysis

Significance of differences between treatments were evaluated using Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons, based on two-way analysis of variance (ANOVA). The analyses focused on identifying significant differences: (i) between clones, and (ii) in the interaction between clones and treatments at the level  $p = 0.05$ . All statistical analyses were carried out with the STATISTICA 13 software package (TIBCO Software Inc, 2020).

### 3. Results

#### 3.1. Differences between clones

The analysis of variance revealed statistically significant differences across all examined physiological parameters. Variations between clones concerning net photosynthesis (A) suggest that clones *P. deltoides* cl. S1-8 and PE 4/68 exhibit the highest values, whereas the willow clone *S. alba* cl. 380 demonstrates the lowest value. Similar distinctions were observed for the rate of transpiration (E) and stomatal conductance ( $g_s$ ). Statistically significant variations in water-use efficiency (WUE) were observed in clones *P. deltoides* cl. S1-8 and *S. alba* cl. 380 (Figure 1)



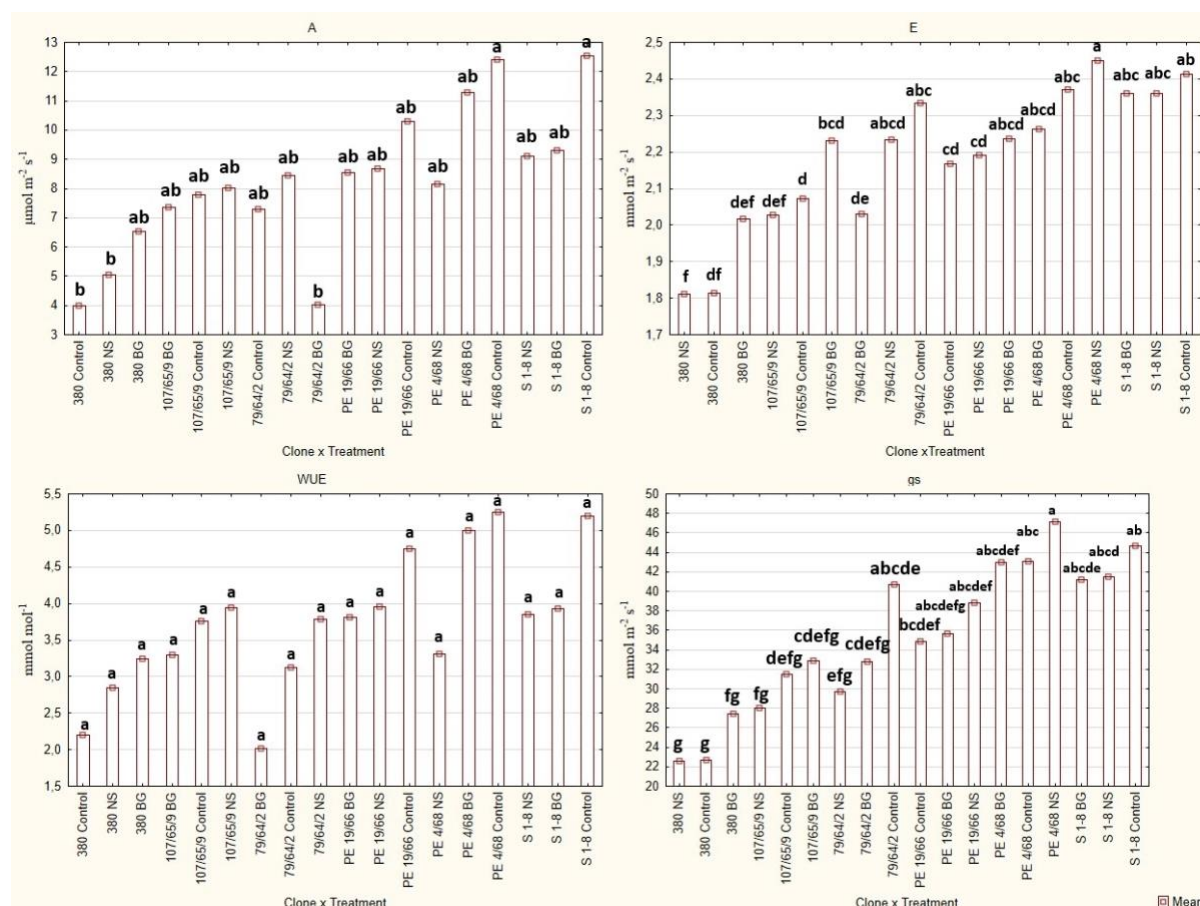
**Figure 1.** Differences between leaf gas exchange (net photosynthesis (A), rate of transpiration (E), stomatal conductance ( $g_s$ ), and water use efficiency (WUE)) of three poplar clones [*P. deltoides* cl. PE 19/66; S 1-8; and PE 4/68] and three willows clones [*S. alba* cl. 380; 107/65/9; and 79/64/2]. Bars with the same letters were not different according to Tukey's test at  $p < 0.05$ .

#### 3.2. Interaction clone x treatment

The effect of clone x treatment interaction was significant only for E and  $g_s$ , but not for A and WUE (Figure 3.). Evaluating total photosynthesis (A), significant differences were only evident in control treatments between *P. deltoides* cl. S 1-8 and PE 4/68 that had higher values than *S. alba* cl. 380

Considering E, there were no significant differences between treatments within clones except for clone 79/64-2 for which E was significantly lower on Belgrade treatment than in control treatment. In Belgrade treatment, the E value was the highest for poplar clone S1-8, and willow clones 380 and 79/64-2. In the control treatment, the most notable differences were observed among clones S1-8,

PE4/68, and 79/64-2, which had higher E values than clones 107/65-9 and 380. Similarly, in the Novi Sad treatment, clones S1-8 and PE4/68 exhibited higher E values compared to clones 107/65-9 and 380 (Figure 2). Regarding  $g_s$ , there were no significant differences between treatments within clone. However, in the Belgrade treatment, clone S1-8 exhibited higher  $g_s$  values compared to clone 380. In the control treatment, clones S1-8 and PE4/68 demonstrated higher  $g_s$  values than clones 107/65-9 and 380. Similarly, in the Novi Sad treatment, clones S1-8 and PE4/68 had higher  $g_s$  values than all other examined willow clones (Figure 2).



**Figure 2.** Interactions between clone and treatments based on leaf gas exchange (net photosynthesis (A), rate of transpiration (E), stomatal conductance ( $g_s$ ), and water use efficiency (WUE)) of three poplar clones (*P. deltoides* cl. PE 19/66, S 1-8, and PE 4/68) and three willows clones (*S. alba* cl. 380, 107/65-9 and 79/64-2). Bars with the same letters were not different according to Tukey's test at  $p < 0.05$ .

#### 4. Discussion

A significant topic of the 21<sup>st</sup> century is the use of poplars and willows in various environmental applications, such as phytoremediation, landscape rehabilitation, carbon sequestration, enhancing biodiversity, and improving urban amenities (McCutcheon and Schnoor, 2003; Nissim and Labrecque, 2021; Guidi Nissim et al. 2023). Given the proven effectiveness of poplar and willow clones in phytoremediation, this study aimed to test the physiological responses of three poplar clones (*P. deltoides* W.Bartram ex Marshall cl. PE 19/66; cl. PE 4/68; cl. S 1-8) and three willow clones (*S. alba* L. cl. 380; cl. 107/65/9; cl. 79/64/2) grown in soil obtained from landfills in the vicinity of Novi Sad and Belgrade.

Additionally, this study highlighted the interdependence and complexity of the examined physiological parameters. Among these, A exhibited its highest intensity in the clones of *P. deltoides* (cl.

S 1-8 and PE 4/68). Similar trends were observed across other physiological parameters, indicating the superiority of these particular clones over the others. These findings emphasize the significant impact of proper clone selection on photosynthetic processes, as it influences factors like transpiration and water-use efficiency, as evidenced in prior research on black locust (*Robinia pseudoacacia* L.) (Župunski et al. 2016; Ábriet al. 2023), poplars, and willows (Polle et al. 2013; Borišev et al. 2016; Pilipović et al. 2022). The elevated values of physiological parameters can be attributed to various factors. It is plausible that superior poplar clones harbor genetic variations that augment their photosynthetic efficacy. Variations in genes governing photosynthetic pathways, chlorophyll content, and stomatal conductance among different clones may result in disparities in net photosynthesis (Li and Xu, 2017; An et al. 2022). These superior poplar clones could possess a more efficient photosynthetic apparatus, characterized by heightened activity of photosynthetic enzymes like Rubisco, improved chloroplast structure, and more effective mechanisms for light absorption and energy conversion (Parry et al. 2013; Muhammad et al. 2021). Collectively, these factors contribute to the observed higher net photosynthesis in *P. deltoides* clones compared to others.

The analysis of variance results indicated a strong significance ( $p < 0.05$ ) regarding the interaction between treatment and clone for all examined parameters ( $A$ ,  $E$  and  $g_s$ ), with the WUE. Understanding the intricate clone  $\times$  treatment interactions is crucial for optimizing plant growth and productivity in various environments (Ferrari et al. 2014; Zalesny et al. 2021). Numerous studies have investigated the effects of different treatments on the physiological parameters of poplar and willow clones, shedding light on the complexities of these interactions. For instance, Wu et al. 2018 demonstrated that specific physiological parameters, such as net photosynthesis rate, stomatal conductance, and water use efficiency, varied significantly among different poplar and willow clones when subjected to different soil conditions. These variations were attributed to genetic differences among the clones, as well as the influence of soil factors such as nutrient availability, pH, and moisture content (DesRochers et al. 2007; Heilig et al. 2021). In our study, when examining soil pH, it becomes evident that the pH is higher in the Belgrade landfill compared to Novi Sad. This discrepancy in soil pH levels could potentially be a contributing factor to the observed variations in physiological parameters among the studied clones. This combined analysis simplifies the selection process, allowing researchers to select the most suitable clones based on the aggregated data from all treatments, instead of having to assess each treatment individually. This approach has been effectively utilized in various studies for selecting clones under uniform conditions, which streamlines the decision-making process in clone selection (Mueller et al. 2020).

Furthermore, studies have shown that certain poplar clones exhibit a higher degree of adaptability to specific soil conditions, resulting in superior performance in terms of physiological parameters compared to others. For example, Moreira et al. 2021 found that certain poplar clones exhibited enhanced photosynthetic efficiency and water use efficiency when grown in soils with higher nutrient content, highlighting the importance of genetic variability in mediating plant responses to soil environments.

## 5. Conclusion

Our research findings suggest variations among the investigated clones concerning all measured physiological parameters. Based on these findings, it appears that poplar clones S 1-8 and PE 4/68 demonstrate promising potential for growth in the examined soils. All examined parameters could serve as indicators for selecting suitable poplar clones for phytoremediation. The proper selection of clones also plays a significant role because it ensures the cultivation of plants with desirable traits such as enhanced drought resistance, contamination tolerance, and the ability to sequester more carbon dioxide. This not only improves the plants' survival and productivity under changing environmental conditions but also contributes to climate change mitigation efforts. Overall, these findings underscore the complex interplay between treatment and clone in shaping physiological responses in poplar and willow clones, emphasizing the need for tailored management strategies to maximize productivity and

sustainability in agroforestry systems. Future research should incorporate the chlorophyll fluorescence parameter, which has proven effective in selection processes.

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