

PHYSIOLOGICAL REACTION OF COMMON OSIER (*SALIX VIMINALIS* L.) VAR. JORR TO THE PRESENCE OF LEAD IN THE SUBSOIL

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ABSTRACT

The effect of lead ions of the concentrations within the range of 15–1000 mg·dm⁻³ on the physiological reaction of common osier var. Jorr was examined. The content of assimilation pigments, the rate the CO₂ assimilation, transpiration, the indices of relative water content and the deficit of water saturation and the content of lead in the nutrient solution. The studied physiological parameters in common osier var. Jorr were differentiated by the rate of lead ions in the nutrient solution. The Jorr variety of common osier was characterised by good values of the determined physiological parameters under stressful conditions at a large accumulation of lead. This suggests that it shows quite a high tolerance to the stress caused by contamination of the subsoil with lead.

Keywords: lead, photosynthetic pigments, CO₂ assimilation, transpiration, common osier.

INTRODUCTION

Pollution of the natural environment, resulted from the industrial activity of man, needs intensive procedures in order to restore the original state of soil and air. One of the ways of reclamation of soil chemically contaminated is the use of phytoremediation abilities of some plants [Baran 2000]. The effectiveness and the duration time of land reclamation with the use of phytoremediation depend not only on the concentration of impurities in the soil but also on a proper selection of plant species for a given type of pollution. Despite the fact that a series of plant species show increased resistance to given toxic compounds, not all, however, can be used in land reclamation [Baran 2000, Zemleduch and Tomaszewska 2007]. The studies within the range of chemistry, physiology and biochemistry allowed for more accurate acquaintance with mechanisms and processes responsible for the taking and the accumulation of toxins by plants. They also made

it possible to use physiological and biochemical parameters for the assessment of the usability of plants for reclamation of degraded terrains. Owing to this it is possible to use deliberately natural abilities of plants and plan optimal methods of remediation of pollution from degraded land [Zemleduch and Tomaszewska 2007].

Shrub willows (*Salix* sp.) are used for many purposes including land reclamation and biological restoration of chemically degraded areas. The broad application of phytoremediation and phytoextraction to contaminated soils result mainly from their abilities to remove hard to degrade biological impurities as well as from their high tolerance to pollution and their pioneering character [Eltop et al. 1991, Šottniková. et al. 2003, Deng et al. 2006, Hermle et al. 2006, Wrzosek et al. 2008].

The aim of the studies was to determine the physiological reaction of common osier (*Salix viminalis*) var. Jorr under the condition of the subsoil contaminated with lead and to determine the

usefulness of this form for the development of the polluted soils.

MATERIAL AND RESEARCH METHODS

Common osier (*Salix viminalis* L.) var. Jorr was used as the experimental material. 60 cuttings of willow obtained from the last year's shoots were used for the studies. Laboratory studies were carried out as a hydroponic culture with different doses of lead. Willow cuttings were divided into four groups of the same number and placed in containers filled with Hoagland full nutrient solution. Lead nitrate (III) was added to the nutrient solutions 14 days after the cuttings were placed in the hydroponics, when the plants had taken roots and had shot. Three levels of contamination of the nutrient solutions with lead ions were applied: 15, 100 and 1000 mg·dm⁻³. The plants placed in Hoagland full nutrient solution were the control object. The determination of physiological parameters was carried out four times: on the 7th, 14th, 21st and 28th day after the day the rates of lead ions were applied. The measurements of the parameters of gas exchange ($A - \text{CO}_2$ assimilation, $E -$ transpiration, $c_i -$ concentration of CO_2 , $g_s -$ stomatal conductance for water vapor, $g_c -$ stomatal conductance for CO_2) were made (the measurement was replicated three times) on leaves using a gas analyser TPS-2, working in an open system with a chamber of PLC-4 type. The measurement of the concentration of CO_2 (c_i) was carried out on the basis of its absorption in infrared radiation. The rate of photosynthesis was calculated as a quotient of the concentration of CO_2 absorbed by a leaf, in a defined time in the measurement chamber, and the value of the surface of leaves. The rate of transpiration was determined on the basis of the volumetric humidity sensor readings. In the analyser cuvette the following conditions were established: a permanent inflow of carbon dioxide, humidity equal to the humidity of environment and lighting equal to 2053 PAR ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), supplied by means of a light unit attached to the cuvette. On the basis of the obtained results of the assimilation and transpiration rates, photosynthetic effectiveness of the use of water (ω_p) was calculated. The determination of assimilation pigments (of chlorophyll "a" and "b" and carotenoids) were carried out on the same leaves on which the parameters of gas exchange were determined. The content of chlorophyll was

assessed using the method of Arnon et al. [1956] modified by Lichtenthaler [1987], whereas the content of carotenoids was determined by means of the Hager and Meyer-Bethenrath method [1966]. The indices of the relative water content (RWC) and the deficit of water saturation (WSD) were determined according to Bandurska [1991]. The content of lead in leaves was determined after previous wet mineralization in a mixture (2:1) of nitric (V) and chloric (VII) acids with a spectrometer of atomic absorption Unicam Solaar 929. The statistical analysis of the studies was carried out using Duncan's test at the level of significance $\alpha_{0.05}$. Pearson linear correlation coefficients between the analysed variables characteristic of gas exchange and between the concentration of lead in leaves and the rate of assimilation of CO_2 and transpiration and the content of assimilation pigments in leaves were calculated.

RESULTS AND DISCUSSION

The obtained results of the measurements of gas exchange, the analysis of the content of assimilation pigments and the indices determining water balance of a plant showed a differentiated effect of lead on the determined physiological parameters.

It was observed that the increasing concentration of lead ions in the nutrient solution resulted in a decrease in the content of photosynthetic pigments in the leaves of common osier var. Jorr. The largest rate of lead ions caused a decrease in the concentration of chlorophyll "a" by about a little over 56%, chlorophyll "b" by 60%, and carotenoids by about 50%. Whereas the rates of lead ions in the amount of 15 and 100 mg·dm⁻³ caused a decrease in the assessed pigments from 20% (carotenoids at the rates of lead ions 15 mg·dm⁻³) to 37.5% (chlorophyll "a" at the rate of Pb^{2+} 100 mg·dm⁻³) – Table 1. Malinowska et al. [2010a] observed a significant decrease in the content of chlorophyll "a", "b" and carotenoids in the leaves of common osier, clone Bjor and Tora, cultured in the nutrient solution with different rates of lead ions. A significant decrease in the concentration of assimilation pigments in common osier, Jorr, was observed under the influence of cadmium ions [13]. A decreased amount of chlorophyll "a" and carotenoids by 62% and chlorophyll "b" by 53% were also obtained by Malinowska et al. [2010b] in common osier, Bjor, after the ap-

Table 1. The toxic impact of lead on the content of photosynthetic pigments [$\text{mg}\cdot\text{g}^{-1}$ FW], CO_2 assimilation [$\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$], transpiration [$\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$] and water use photosynthetic efficiency (ω_p) of common osier clone Jorr

Dose of Pb^{2+} [$\text{mg}\cdot\text{dm}^{-3}$]	Chlorophyll a (% of control)	Chlorophyll b (% of control)	Carotenoids (% of control)	CO_2 assimilation (% of control)	Transpiration (% of control)	Water use photosynthetic efficiency (ω_p)
Clone Jorr						
0	2.95±0.12 (100)	1.11±0.11 (100)	1.15±0.15 (100)	7.77±0.26 (100)	0.81±0.09 (100)	9.59
15	1.94±0.15 (65.9)	0.82±0.09 (74.0)	0.92±0.11 (79.9)	3.93±0.18 (50.6)	0.61±0.04 (75.3)	6.44
100	1.84±0.14 (62.5)	0.72±0.09 (65.0)	0.83±0.14 (72.1)	3.14±0.16 (40.4)	0.48±0.10 (59.3)	6.54
1000	1.29±0.10 (43.7)	0.44±0.08 (39.5)	0.58±0.10 (50.3)	1.86±0.11 (23.9)	0.24±0.07 (29.6)	7.75
LSD _{0.05} dose:	0.203	0.096	0.112	0.693	0.234	

plication of cadmium ions in the amount of 280 $\text{mg}\cdot\text{dm}^{-3}$. Similar reactions to heavy metals were observed by Malinowska and Smolik [2011], Becerril et al. [1988].

A significant effect of lead on the rate of assimilation and transpiration in clone Jorr of common osier was observed. In the case of both processes a significant decrease in the rate was shown along with the increase in the rate of the rate of lead ions in the nutrient solution. The intensity of the process of photosynthesis and transpiration in clone Jorr at the presence of the highest rate of lead ions constituted 24% and 30%, respectively, in relation to the control plant (Table 1).

The decrease in the intensity of photosynthesis in common osier, var. Bjor and Tora, cultivated in the solution with the addition of lead ions was shown by Malinowska et al. [2010a], Malinowska et al. [2010b]. In our previous studies that cadmium caused a significant decrease in these processes in basket willow, clone Jorr. Photosynthetic effectiveness of water use is often a decisive indicator of plant productivity under the stressful conditions [Górny and Garczyński 2002]. The calculated index was differentiated depending on the applied rates of lead ions. A decrease in this parameter by about 33% in relation to the control plants was observed when the rates of 15 and 100 $\text{mg}\cdot\text{dm}^{-3}$ were applied. Whereas the application of the highest rate of lead ions caused an increase in the effectiveness of use of water by 14% in relation to the other rates applied. An increase in the value of this parameter results, first of all, from a low rate of transpiration of the studied variety of willow (Table 1). Toxic effect of ions of heavy metals concerns different types of blockades of key metabolic reactions by destabilization of the functional groups and struc-

tural changes of the enzymes and transportation proteins [Schützendübel et al. 2001, Verma and Dubey 2003] and it influences the lipid composition of membranes [Hall 2002]. This results, first of all, in disturbances to the processes of gas exchange, transpiration and stomatal conductance. There is a decrease in the effectiveness of the light-dependent reactions and light-independent („dark“) biochemical reactions [Zenk 1996, Panda et al. 2003].

On the basis of the values of the correlation coefficient a negative significant relation between the content of total chlorophyll and the concentration of lead in the leaves of common osier, clone Jorr. The correlation coefficient (r) of this relation was $r = -0.5706$. The significant linear correlation negative between the CO_2 assimilation and the content of Pb^{2+} in the leaves took place in common osier Jorr. The value of the correlation coefficient (r) for this relationship was $r = -0.6766$ (Figure 1).

The indicators of the changes in water balance in a plant are RWC and WSD indices. Increasing rates of lead ions caused a decrease in the content of water in the leaves of the studied variety of common osier. The largest decrease in the index of relative water content by 23.6% was observed when the largest rate of lead ions in relation to the control plants was applied (Figure 2). The observed changes in the intensity of the studied physiological parameters could result, under unfavourable conditions, in both stress and repair mechanisms [Starck 2002].

On the basis of the parameters of the gas exchange the analysis of the linear correlation was carried out between the assimilation of CO_2 (A) and transpiration (E) and the concentration of carbon dioxide in intercellular spaces (c_i), stomatal

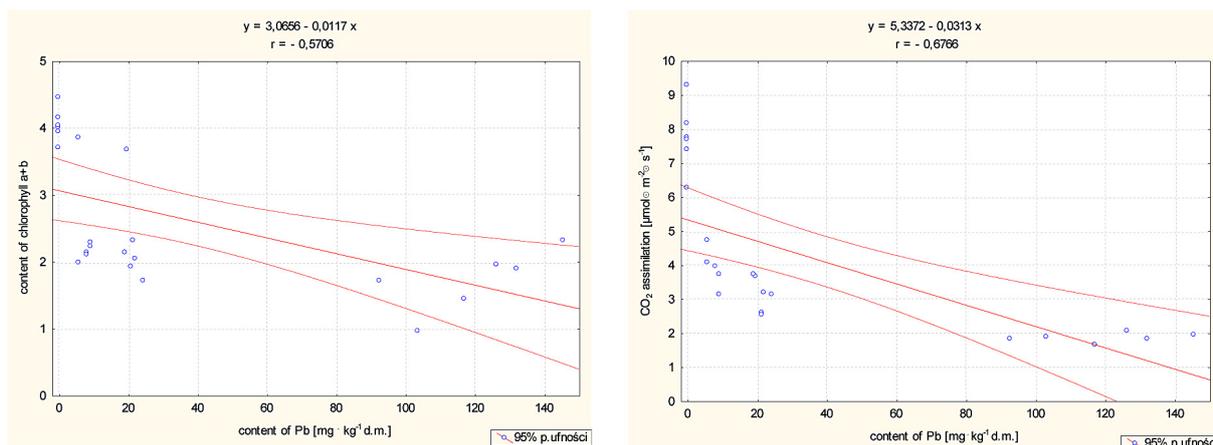


Figure 1. Correlation between the content of photosynthetic pigments [$\text{mg}\cdot\text{g}^{-1}$ FW], CO_2 assimilation [$\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$] and the content of lead in the leaves of common osier

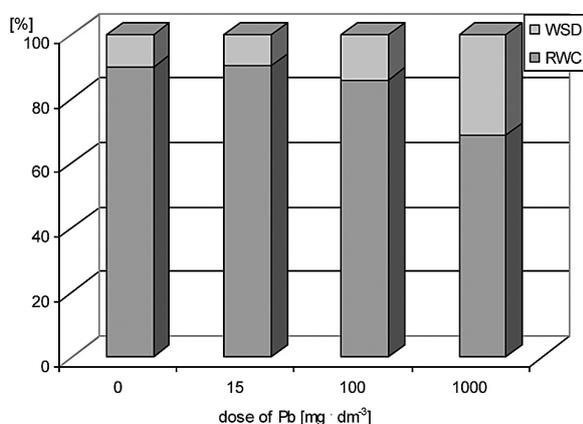


Figure 2. Water indices (%) of common osier – clone Jorr in relation to a dose of lead

conductance for CO_2 (g_c) stomatal conductance for water vapor (g_s).

In the carried out studies the CO_2 assimilation and transpiration of common osier var. Jorr was limited to a large extent. Significant linear positive relationships between the parameters of gas exchange occurred in the studied variety

of common osier both under control conditions and under conditions of contamination with lead ions. The relationship between these parameters increased along with the increase in the rate of Pb^{2+} (Table 2). The analysis of correlation coefficients for the relationship between transpiration and stomatal conductance for the water vapor showed the largest significant coefficient of correlation in Jorr when the highest rate of lead ions was applied (Table 2). Straight line relationship between transpiration and conductance of stomatal apparatuses in common osier under stressful conditions were shown by Wróbel et al. [2006]. The closing of stomatal apparatuses is a response of plants to many stress factors. It runs according to a complicated mechanism related to the phosphorylation of some proteins and the increase in the amount of abscisic acid and it leads to accumulation of H_2O_2 and activation of the calcium channel in membranes of the stomatal cells. This results in a decrease in the rate of transpiration [Mott and Parkhurst 1991, Hinckly et al. 1994, Laloi et al. 2004].

Table 2. Correlation between the parameters of gaseous exchange processes of common osier clone Jorr

Dose of Pb^{2+} [$\text{mg}\cdot\text{dm}^{-3}$]	Variables		Correlation coefficients (r)
	y	x	
0	CO_2 assimilation (A)	Stomatal conductance for CO_2 (g_c)	$r = 0.434^*$
	Transpiration (E)	Stomatal conductance for water vapor (g_s)	$r = 0.496^*$
15	CO_2 assimilation (A)	Stomatal conductance for CO_2 (g_c)	$r = 0.422^*$
	Transpiration (E)	Stomatal conductance for water vapor (g_s)	$r = 0.564^*$
100	CO_2 assimilation (A)	Stomatal conductance for CO_2 (g_c)	$r = 0.685^*$
	Transpiration (E)	Stomatal conductance for water vapor (g_s)	$r = 0.703^*$
1000	CO_2 assimilation (A)	Stomatal conductance for CO_2 (g_c)	$r = 0.733^*$
	Transpiration (E)	Stomatal conductance for water vapor (g_s)	$r = 0.794^*$

The obtained results of the studied physiological parameters can be useful for the evaluation of the resistance of the studied clone of willow to the stress caused by lead, and their usefulness for reclamation of the antropogenically degraded land. The Jorr variety of common osier was characterised by good values of the determined physiological parameters under stressful conditions at a large accumulation of lead. This suggests that it shows quite a high tolerance to the stress caused by contamination of the subsoil with lead.

CONCLUSIONS

1. The rate of the assimilation CO₂, transpiration, and the content of assimilation pigments in common osier was differentiated by the rate of lead ions in the nutrient solution.
2. The CO₂ assimilation and transpiration of common osier var. Jorr were significantly limited stomatally.
3. A significant correlations were dependence was shown between the assimilation of CO₂, the content of total chlorophyll and the concentration of lead in the leaves of common osier, clone Jorr.
4. The addition of lead ions to the nutrient solution caused a decrease in the index of relative water content (RWC) and a deficit of water saturation in the leaves of the studied variety of common osier.
5. The obtained results suggest that the determined physiological parameters can be used for a thorough evaluation of the influence of common osier var. Jorr on the stress caused by the presence of lead ions in the subsoil.

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